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# Prologue

This Abstracts Book contains the summaries of 839 original works accepted by the Scientific Committee of the 17th European Congress of Pathology and the XIX Spanish Congress of Pathology. These works have been selected from the 1084 abstracts initially submitted.

Even though the Abstracts were selected following strict criteria of quality, the number remaining is the largest ever presented at a European Congress of Pathology. This fact shows the increasing role of the Congress of the European Society of Pathology as an international forum for scientific activities, as well as the synergism developed by the simultaneous organization of the European Congress with the Congress of the National Society of the host country, in this case the Spanish Society of Pathology.

For organizational reasons, most abstracts were categorized as posters, and only 106 scheduled as oral presentations. Therefore, we would like to emphasize the similar scientific value of both types of presentations. The Presidential Papers were selected only among those abstracts whose authors had chosen platform presentation.

We would like to thank all the authors for their scientific contribution and active participation in this joint Congress. Their attendance helps to enhance the role of pathology in European medicine.

Finally, we would like to record our appreciation of Springer-Verlag for its support in making the publication of this special issue of *Virchows Archiv* possible.

Prof. Dr. G. Klöppel
President of the European Society of Pathology

Prof. Dr. A. Cardesa
President of the Spanish Society of Pathology

## O-001

MIXED MEDULLARY-FOLLICULAR THYROID CARCINOMA: MOLECULAR EVIDENCE FOR A DUAL ORIGIN OF TUMOR COMPONENTS.

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**Aims**: To clarify the histogenetical origin and possible molecular mechanisms of thyroid mixed medullary-follicular (MMFC) development. To this purpose the two histological components were separated and tested with different molecular markers to ascertain whether they are derived from the same cell clone or whether they arise independently.

**Methods:** Laser-based microdissection was used to study separately the follicular and medullary component of 12 cases of MMFC, diagnosed according to the WHO classification. Both primary tumours and mixed lymph node metastases have been analyzed for mutations of the *RET* and gsα gene by means of non-isotopic PCR-SSCP and HDE mutation analysis and direct sequencing, for allelic losses at 9 different loci in 6 chromosomes using a PCR-based approach and for the clonal composition of the four female patients by the HUMan Androgen Receptor Assay.

**Results:** The two components consistently exhibited a different pattern of mutation and allelic losses as well as clonal composition in 7 tumours, which were suitable for clonal analysis or had detectable molecular aberrations at the genes or loci investigated. Furthermore, the follicular structures in most cases showed a oligo/polyclonal pattern exhibiting more frequently hyperplastic than neoplastic histological features.

**Conclusions:** Our data provide strong evidence that the two components in MMFC are not derived from a common stem cell and that at least a subset of cases are medullary carcinomas containing hyperplastic follicles.

## O-002

A LARGE 6q DELETION IS A COMMON CYTOGENETIC ALTERATION IN FIBROADENOMAS, PRE-MALIGNANT LESIONS AND CARCINOMAS OF THE BREAST

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**Aim:** In order to assess if some breast proliferative lesions are genetically connected with invasive carcinoma we cytogenetically characterized 80 breast lesions including hyperplastic lesions and benign and malignant tumors. Three classes of breast lesions, namely benign tumors (including fibroadenomas) (BBT), putative pre-malignant lesions (including cases of atypical hyperplasia) (PPL) and invasive carcinomas (CA), were compared both at cytogenetic and molecular level. **Methods:** Each breast sample was studied using both conventional and molecular cytogenetic approach (FISH). Chromosome analysis was performed on direct preparations using conventional banding technique. FISH was carried out using simultaneously alpha-satellite and YACs as probes. The YACs clones mapped in 6q26-27, 6q25, 6q24, 6q21, and 6q13 regions. **Results:** A genetic relationship between the three classes of breast lesions was clearly demonstrated by the sharing of several anomalies, among which 6q deletions outnumbered all other alterations detected. Indeed, deletions of the long arm of chromosome 6, most likely occuring in epithelial cells, were present in 83% of BBT, 64% of PPL and 77% of analyzable CA. Noteworthy 6q deletions were identified in 26 out of 31 fibroadenomas showing a high proliferative activity of epithelial cells, identified by Ki67 immunohistochemical staining. Furthermore the interval comprised between 6q24-qter appeared to be the common region of deletion in all three classes of breast lesions, whereas the minimal common region of deletion was 6q27-qter. **Conclusions:** The data collected in this study raise an interesting point concerning the temporal aspects of breast tumorigenesis which implies the occurrence of 6q alterations as one of the earliest events in the pathogenetic process leading to carcinoma.

## O-003

**THE LEPTIN RECEPTOR (OB-Rb) IN THE HUMAN BRAIN.**

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**Aims** The specific aim of this study was to further investigate the role that the brain may be playing in the pathogenesis of obesity in humans. We studied the expression of OB-R mRNA (both, common and long isoforms) in the brains of obese (n=5), lean (n=5) and diabetic subjects (n=4), by means of *in situ* hybridization, Northern blot and RT-PCR analysis. We used two alternative probes; one that recognizes all known splice variants and a second that just recognizes the long form. Several brain regions were evaluated.

**Results** *In situ* hybridization studies revealed that both, common and OB-Rb mRNAs are widely distributed in the human brain. The specific hybridization signal was detected in the cytoplasm of the cell body, dendrites and proximal axonal regions of neurons from the hypothalamic nuclei, Purkinje cells and dentate nuclei of the cerebellum, inferior olivary and cranial nerves nuclei, amygdala and neurons from both the neo and entorrhinal cortex.. No significant differences were identified neither among regions nor among the three groups studied. These results, match those previously obtained by us where the distribution of the OB-R protein in the human brain was first described. Northern blots and RT-PCR indicate that the OB-Rb is expressed at a relatively high level in the hypothalamus and cerebellum, with lower expression in the choroid plexus. Control experiments including the omission of reverse transcriptase allow to exclude genomic DNA amplification from mRNA.

**Conclusion** The original hypothesis that the OB-Rb was only present in the hypothalamus needs to be reconsider. This OB-Rb isoform seems to be widely expressed in the human brain. Obesity and hyperleptinemia seems not to be associated with an down-regulation of the OB-Rb in the human brain.

## O-004

MOLECULAR CYTOGENETIC COMPARISON OF APOCRINE METAPLASIA AND APOCRINE DUCTAL CARCINOMA IN SITU OF THE BREAST

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**Aims:** To compare the genetic alterations in benign apocrine hyperplasia with apocrine ductal carcinoma in situ (DCIS) of the breast using comparative genomic hybridisation (CGH).

**Methods:** Nine cases of apocrine hyperplasia and nine cases of apocrine DCIS were studied. The lesions were microdissected from paraffin-embedded sections by Laser Capture Microdissection. Genomic DNA was amplified using degenerate oligonucleotide primed (DOP)-PCR. Differentially-labelled 'lesional' and normal DNA was cohybridised to normal metaphase spreads and captured using a cooled CCD camera. DNA copy number changes were detected using Vysis software.

**Results:** Both types of proliferations exhibited copy number changes. The average number of alterations in apocrine hyperplasia was 4.5 compared to 10.9 in apocrine DCIS. In order of decreasing frequency, the most common alterations in apocrine hyperplasia were gains of 2q, 13q, and 1p and losses of 1p, 17q, 22q, 2p, 10q and 16q. Apocrine DCIS showed gains of 2q, 1p, 1q, 3q, 4q, 5q, 6q, 7q, 9p, 13q and losses of 1p, 17q, 22q, 12q, 16q, 2p, 9q, 2q, 3p, 6p, 8q, 11q, 12p and 13q.

**Conclusion:** Apocrine hyperplasia is considered to be a benign lesion without a subsequent risk of invasive carcinoma. Our data shows that it is a clonal disorder exhibiting gains and losses at a number of chromosomal arms. The genetic changes show considerable overlap with those identified in apocrine DCIS, for example losses at 1p, 16q and 17q. These alterations are also commonly seen in non-apocrine DCIS and invasive breast cancer. A number of novel alterations, e.g. gains at 2q, have also been identified. The data are consistent with apocrine hyperplasia as a putative non-obligate precursor of apocrine DCIS.

## O-005

**Transition from adenoma to carcinoma in colorectal tumours is marked by loss of chromosome 8p, gain of 13q and loss of 18q**

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Knowledge of genomic changes accompanying progression from colorectal adenoma to carcinoma within *individual* tumours is limited. The aim of the present study was to compare chromosome aberrations in pre-invasive (adenoma) and invasive (carcinoma) components within individual colorectal tumours.

Pairs of adenoma and carcinoma tissue were micro-dissected from 10 to 20 consecutive 10 micrometer sections of 25 colorectal tumours containing both components. With CGH analysis, chromosomal aberrations in adenomas appeared to be more randomly distributed in adenomas compared to carcinomas. In order to eliminate the influence of random genetic changes in the evaluation of chromosomal abnormalities, we focussed at those chromosomal aberrations that were previously shown to be frequently present in carcinomas: gain of 8q, 13q, 20q, and loss of 8p, 17p and 18q (J. Clin Pathol. 1998;51:901-909).

Among these events, loss of 17p was nearly always retained and sometimes acquired in the carcinoma (56% in adenoma, 76% in carcinoma), while loss of 8p (20% to 36%), gain of 13q (32% to 48%), and loss of 18q (36% to 52%) increased remarkably from adenoma to carcinoma. In this series, gain of 8q and 20q remained more or less constant at 28% in the tumours analysed.

K-ras mutation analysis (so far completed in 10 pairs) showed 7 pairs as wildtype in both components, one pair wildtype in the adenoma and mutated in the carcinoma, one pair mutated in the adenoma and wildtype in the carcinoma, and one case mutated in both components.

In conclusion, 8p loss, 13q gain and 18q loss marked the transition of adenoma to carcinoma in this series of colorectal tumours, while in most cases 17p loss was already present in the adenoma stage of these tumours. Conclusions on the role of k-ras mutation in the transition from adenoma to carcinoma await completion of the data.

## O-006

TELOMERASE ACTIVITY IN PULMONARY NEUROENDOCRINE TUMOURS. CORRELATION WITH HISTOLOGICAL SUBTYPE.

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**Aims:** To measure telomerase activity in a prospective study in 13 cases of neuroendocrine pulmonary neoplasms, and to correlate it with their different malignant potential.

**Methods**: Tissue samples from 13 neuroendocrine pulmonary neoplasms and non neoplastic lung were shock frozen in liquid nitrogen and stored at -80ºC. They corresponded to Typical carcinoid (TC), four cases; Atypical carcinoid (AC), three cases; Large cell neuroendocrine carcinoma (LCNEC), four cases, and Small cell carcinoma (SCLC), two cases. We performed TRAP-ELISA assay (Bochringer-Manheim) with an H&E stained control frozen section to guaranty the presence of normal or neoplastic tissue. We measured telomerase activity also in 52 non neoplastic lung samples obtained from surgical proceedings.

**Results**: The mean telomerase activity in non neoplastic lung controls was 182,44 (95% confidence interval -CI- 125-239,88). The mean activity in the neoplastic samples was 1244,23 (95% CI 683,31-1805,14). TCs showed a mean telomerase activity of 104,5 (95%CI 22,7-186,2). The rest of neoplasms showed a mean telomerase activity of 1750,78 (95%CI 1363,5-2138,1). There were no differences between ACs, LCNECs and SCLCs (1742,33; 1823,5 and 1618 respectively). The differences between TCs and ACs, LCNECs and SCLCs were significative in ANOVA test ($p<0,01$)

**Conclusions**: TC cells have no significant telomerase activity. AC, LCNEC and SCLC cells have very high levels of telomerase activity. TCs are composed by mortal cells, but AC, LCNEC and SCLC cells are immortal. A morphological control could be necessary in Telomerase Activity assay.

## O-007

**Simultaneous $p27^{KIP1}$ and Cyclin D3 over-expression in a subset of aggressive B-cell lymphomas is a consequence of $p27^{KIP1}$ sequestration by Cyclin D3.**

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**Aims:** Cell cycle progression is regulated by the combined action of cyclins, cyclin-dependent kinases (CDKs), and CDK-inhibitors (CDKI), which are negative cell-cycle regulators. $p27^{KIP1}$ is a CDKI involved in G1 arrest, whose down-regulation is essential for transition to S-phase of cell cycle in proliferating cells. In contrast with this observation, an anomalous high $p27^{KIP1}$ expression has been previously shown in cases of Diffuse Large B-cell Lymphoma (DLBCL) with high proliferation index and adverse clinical outcome, suggesting that the abnormally accumulated $p27^{KIP1}$ protein was functionally inactive. Our aim was to evaluate $p27^{KIP1}$ and Cyclin D3 expression in a group of aggressive B-cell lymphoma and to look for possible association.

**Methods:** We have analysed $p27^{KIP1}$ and Cyclin D3 expression in a group of aggressive B-cell lymphoma including 54 cases of DLBCL and 20 Burkitt lymphoma, using immunohistochemical techniques, laser confocal microscopy analysis and coimmunoprecipitation. Twenty samples of reactive lymphoid tissue were analysed as normal patterns of expression of these proteins.

**Results:** A group of cases of DLBCL and Burkitt lymphoma was characterised by simultaneous high $p27^{KIP1}$ and Cyclin D3 expression. Laser confocal studies showed that in these cases, both proteins were colocalized. Studies in the Raji cell line (Burkitt-derived) showed coimmunoprecipitation of both Cyclin D3 and $p27^{KIP1}$.

**Conclusions:** These results show the existence of complexes Cyclin D3-$p27^{KIP1}$ in a subset of aggressive B-cell lymphoma, where $p27^{KIP1}$ seems to lack the inhibitory activity. This interaction could lead to an abnormal nuclear accumulation, thus rendering both proteins detectable by immunohistochemical techniques.

## O-008

SIGNIFICANCE OF APOPTOTIC AND PROLIFERATION MARKERS IN THE BIOLOGICAL BEHAVIOUR AND RECURRENCE OF MENINGIOMAS

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**Aims:** We aimed to investigate the significance of apoptotic and proliferation markers in the elucidation of biological behaviour and the prediction of recurrence in meningiomas.

**Methods:** A series of 52 intracranial, totally excised meningiomas were immunohistochemically analysed for the expression of bcl-2 and p53 proteins, in parallel with the assessment of the proliferating cell nuclear antigen labelling index (PCNA LI) and the mitotic index (MI) All clinicopathological variables entered a statistical analysis; Kaplan-Meier's disease-free survival curves were drawn; predictors for recurrence were tested by using the Cox-Mantel test.

**Results:** bcl-2 was expressed in 26,8% and p53 in 32,6% of the tumours, with low levels of immunoreactive cells. The bcl-2-positive/p53 negative subgroup showed a significant association with a bemign histological pattern. Expression of bcl-2 appeared to have no influence on the rate of recurrence; p53 expression was shown to be the only factor with marginal prognostic significance for recurrence ($p=0,10$). There was no interaction between bcl-2 and p53 expression. The PCNA LI was correlated to the MI and to the grade of malignancy. Proliferation indices appeared to have no relation with the recurrence rate of totally resected tumours. Meningiomas which expressed the bcl-2 protein presented a high proportion of proliferating cells in S phase. In contrast, all the tumours which recurred had a minimal S fraction of proliferating nuclei.

**Conclusions:** These findings improve our understanding of interrelations between cell proliferation, bcl-2 and p53 expression and biological behaviour in meningiomas, but fail to constitute a clear set of criteria for the prediction of meningioma recurrence.

## O-009

DYSREGULATED EXPRESSION AND MUTATION OF b-CATENIN IN HIGH GRADE THYROID CARCINOMA.

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**Aims:** b-Catenin (b-cat) is an ubiquitously expressed protein which has a crucial role in both E-cadherin-mediated cell-cell adhesion and also as a downstream signaling molecule in the wnt/wg pathway. Stabilization of b-cat followed by nuclear translocation and subsequent Tcf/LEF mediated transcriptional activation is emerging as an important step in oncogenesis. Stabilization and nuclear translocation of b-cat may occur through mutations in the *CTNNB1* coding region of exon-3 which contains the Serine/Threonine phosphorylation sites for ubiquitination and degradation of the protein. To assess whether the wnt/wg pathway is important in thyroid tumor progression, subcellular localization of b-cat and mutations at exon-3 were investigated on 28 patients with Poorly Differentiated Carcinoma (PDC) and 31 patients with Undifferentiated (Anaplastic) Carcinoma (UC).
**Methods:** Immunostaining for b-cat was carried out with antigen retrieval, using Cy3-conjugated secondary antibodies. Mutational analysis was performed by PCR-SSCP and DNA sequencing of mobility shifts.
**Results:** Downregulation of b-cat and discontinuous membranous pattern was seen in all patients with PDC. Immunoreactivity was markedly decreased in 14/28 (50%) PDCs, with half of these cases (7/14) (25%) exhibiting in addition focal nuclear staining. Sequencing of mobility shifts observed in PDC demonstrated somatic mutations in 3/28 (11%) of cases. Immunofluorescent staining of UCs showed discontinuous antibody binding to the cell membrane in 6/31 (20%) of the tumors while nuclear localization was seen in 14/31 (45%) cases. Twelve of thirty-one (39%) UCs featured total lack of b-cat expression. Nucleotide sequencing of mobility shifts revealed somatic mutations in 19/31 (61%) UCs and Serine/Threonine phosphorylation target residues were mutated in 15/19 (79%) of the UCs with somatic mutations. The remaining 4 UCs were mutated at codons 22,32,36,43,44,49,58 adjacent to or within a few residues of the Serine/Threonine phosphorylation sites. Mutations at codons 22,32,36,43,44,49,58 may therefore similarly affect ubiquitination and degradation of b-cat. Nuclear staining was detected in 10/19 (53%) UCs with mutated b-cat while 3/31 (10%) UCs showed nuclear staining but no b-cat mutations.
**Conclusions:** b-cat is downregulated in high grade (PDC and UC) thyroid carcinomas indicating that this pathway is important for thyroid tumor progression. Mutations of the *CTNNB1* coding region of b-cat are very common in UC and the majority of them seem to activate transcription since they are associated with nuclear translocation of b-cat. The frequent dysregulation of b-cat in UC is likely to contribute to the highly aggressive behavior of this tumor type.

## O-010

**CK7-POSITIVE OROPHARYNGEAL SQUAMOUS CELL CARCINOMAS AND CYSTIC METASTASES.**

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**Background:** Oropharyngeal SCC often present with cystic LN metastases (Regauer et al, Br. J. Cancer, 1999, 79:1437). The majority represents cystically degenerated SCC. A small percentage, however, contains clear fluid. We presently search for an explanation of the genesis of these fluid-filled cystic metastases.
**Methods:** We evaluated the association of 80 primary oropharyngeal SCC with minor submucosal salivary glands on H&E stained sections. We further analyzed immunohistochemically the CK-profile of 40 primary SCC and their metastases. Special emphasis was placed on the expression of CK7, which is expressed by salivary glands. CK7 is considered a marker for glandular differentiation and is expressed in some mucosal SCC, such as uterine cervical cancer, and large cell carcinomas of lung.
**Results:** Primary oropharyngeal SCC were mostly non-keratinizing with strong expression of "simple" CK8 and 19, and "stratification"-related CK14. "Simple" CK7 was strongly expressed in serous acini, secretory and excretory ducts of the salivary glands, but was negative in 33/40 primary SCC. The CK7-positive SCC were characterized by large polygonal cells reminiscent of salivary gland duct epithelium, demonstrated abortive glandular differentiation and appeared to arise from excretory salivary gland ducts. Other ducts surrounding these SCC showed prominent intraductal epithelial hyperplasia and squamous metaplasia. Cystic LN metastases contained either necrotic debris or macrophage-rich fluid and clear fluid. All cystic metastases were strongly CK8 and 19 positive, but only 7 fluid-filled cystic LN metastases (along with their corresponding primary SCC) expressed CK7. Solid metastases were negative for CK7, while CK8 was uniformly and CK14 focally expressed.
**Conclusion:** A minority of primary oropharyngeal SCC are CK7-positive, characterized by an intimate association with salivary glands and a large cell morphology. These CK7-positive large-cell SCC arise from excretory salivary gland ducts and produce CK7-positive fluid-filled cystic metastases. All this suggests that a subtype of oropharyngeal SCC is of salivary gland duct (glandular) origin with a distinct morphology, and that pluripotent salivary gland cells capable of fluid secretion and/or possibly fluid production participate in the genesis of these SCC.

## O-011

**ANALYSIS OF THE 27-KILODALTONS HEAT SHOCK PROTEIN EXPRESSION IN HUMAN OESOPHAGEAL SQUAMOUS CELL CARCINOMA.**

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**Background and aims:** Various stress conditions such as heat, chemical and mechanical stresses are known to play a major role in oesophageal squamous cell carcinoma development. Our goal was to evaluate whether changes in stress-induced 27-kilodaltons-heat-shock-protein (HSP27) expression could be demonstrated during oesophageal carcinogenesis.
**Material and methods:** HSP27 expression was studied using immunohistochemistry on formalin-fixed paraffin-embedded tissue sections from twenty-one oesophageal squamous cell carcinoma occurring in smokers and/or alcohol abusers patients. Oesophagus from healthy patients (controls) (5), chemical (8) and infectious oesophagitis (6) were also included in the study.
**Results:** In normal oesophagus, the protein is present only in the upper epithelial layers. In contrast in chemical or infectious oesophagitis its expression is strong and occurs in all the epithelial layers including the basal one. In non tumoral oesophageal mucosa from smoking and/or drinking patients adjacent to invasive component, the distribution of the protein is patchy and irregular. In neoplasic area, HSP27 expression increases drastically form dysplasic lesions to invasive carcinoma, being highest in the less differentiated areas.
**Conclusions:** In human oesophagus, HSP27 expression is induced by various stresses but alcohol and tobacco generate focal perturbations in the stress response. The tumour immunoreactivity for this protein increases with the anaplasia of the tumour, as it has already been demonstrated in some other tumours where it is considered to play a role in the drug resistance.

## O-012

**SPINDLE CELL SQUAMOUS CARCINOMA OF THE OESOPHAGUS : MORPHOLOGIC ANALYSIS OF 17 CASES**

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**Aims**: To analyse morphological and immunohistochemical characteristics of 17 cases of spindle cell squamous carcinoma of the oesophagus
**Methods**: 17 cases of spindle cell squamous carcinoma of the oesophagus surgically resected at Beaujon hospital(1979-1998) were analysed. Immunohistochemical stains for cytokeratin, vimentin, smooth muscle actin, desmin, p53 protein, E-cadherin and β catenin were performed.
**Results**: The tumour was polypoid in 13 cases and ulcerated and infiltrative in 4 cases. In 11 cases the tumour was superficial. Histologically two types of tumour cells were present, i.e. differentiated squamous cells and spindle cells. The squamous cells were present both at the base of the polyp and in the adjacent mucosa and in the polypoid mass intermingled with the spindle cells. On immunohistochemistry, the squamous cells were positive for cytokeratin, and the spindle cells showed variable expression of vimentin and smooth muscle actin. None of the tumours was desmin positive. The p53 protein was overexpressed in 10 cases, in both types of tumour cells showing strong nuclear positivity. In most tumours, E-cadherin was expressed in the squamous cells and absent in the spindle cells. In contrast, β catenin was expressed in both cell types, strongly in the squamous cells and weakly in the spindle cells.
**Conclusion** : Spindle cell squamous carcinoma of the oesophagus usually presents as a large polypoid tumour, showing superficial invasion of the oesophageal wall. The similar pattern of p53 protein expression in the two tumour cells types, suggests their common origin. The pattern of expression of E-cadherin and β catenin suggests that the loss of E-cadherin is not only associated to the acquisition of increased invasiveness of the tumour cells as previously shown in most carcinomas, but may also be associated to the acquisition of spindle cell morphology.

## O-013

**ABERRANT SPASMOLYTIC PEPTIDE (TFF2)-EXPRESSING CELL LINEAGE (SPEM), PSEUDOPYLORIC METAPLASIA AND THE RESPONSE TO GASTRIC MUCOSAL DAMAGE**

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Recent studies have demonstrated a possible association between *H pylori* and gastric cancer. The presence of an aberrant cell lineage expressing the trefoil peptide hSP/TFF2 in the fundic mucosa of mice infected with *H felis* as well as patients with *H pylori* fundic gastritis has been claimed (*Gastroenterology* 1997; 112: A163, 1997), and a recent study (*Gastroenterology* 1998; 114: A673) has suggested a strong association between this lineage, called SPEM, and gastric carcinoma, providing the bridge between *H pylori* infection and carcinoma. Pseudopyloric metaplasia in the stomach is a rather vague entity variously described as encroachment of the pyloric mucosa into the fundus or as the conversion of acid-secreting fundic gland tubules to those of pyloric type. Because of the possible association between pseudopyloric metaplasia and the aberrant SPEM lineage we explored the secretory phenotype of pseudopyloric metaplasia. We obtained a series of 27 lesions in the fundus of the human stomach from archival material including peptic ulcers, carcinomas and stromal tumours, all of which showed apparent pyloric-type glands entirely surrounded by acid-secreting mucosa. The most reliable marker of the process was its abundant expression of hSP/TFF2 and of the chromosome 11 mucin gene MUC6. Occasional cells expressed pS2/TFF1 and PSTI was also positive in this lineage. Thus, in several aspects, the secretory phenotype of pseudopyloric metaplasia in the fundus resembles that of the mucous neck cell; analysis of fundic glands immediately adjacent to areas of pseudopyloric metaplasia indeed show a marked increase in the content of mucous neck cells. We conclude that pseudopyloric metaplasia occurs in fundic glands as a result of hyperplasia of mucous neck cells, cells which have a wide secretory repertoire of peptides which have an important role in mucosal defence.

## O-014

**CLINICOPATHOLOGICAL PROFILE AND P-53, EBV, CEA, MIB1 EXPRESSION, IN ADVANCED GASTRIC CARCINOMA PATIENTS WITH LONG-TERM SURVIVAL:**

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**Aims:** patients with advanced gastric carcinoma often have an unfavorable outcome. The proposed classifications of gastric carcinoma, the immuno-histochemical and molecular genetic markers has lead to conflicting data. The TNM staging system provides useful information in determining clinical outcome. Recently, a new Grading (Goseki) was proposed, and the number of involved lymph nodes (Roder & al) was proved to be related to the prognosis independent of their anatomic localization.

**Methods:** we have reviewed the clinicopathological profiles of 17 patients with advanced gastric cancer, who survived more than 5 years. Alcian blue stain was used to detect mucin production. Antibody against LMP1 as well as In Situ Hybridization were done in order to detect EBV. Antibodies against ACE, p53, Ki67 were also studied.

**Results:** the 17 patients, 9 men and 8 women, aged from 31 to 72, were operated of subtotal or total gastrectomy ; lymph node dissection was limited in 12 cases. Tumor size varies from 1,5cm to 12cm (mean: 5,9). Serosa was involved in 12 cases, Muscularis propria in 3, and Adipose tissue in 2. The number of lymph node metastasis was 0, 1, 2, 3, 4, 5 and 6 in 2, 7, 2, 0, 2, 3 and 1 case, respectively. Recurrence and death occurred in 2 cases 6 years after the diagnosis (liver metastasis); one died 10 years later (bone metastasis), and 14 are still alive 9 to 20 years, free of disease. Mucin secretion was detected in 14 cases. P53 was strongly positive in 14/17 cases. Ki67 was highly elevated in 10 cases. ACE was positive in 15 cases. LMP1 and EBER were positive in only 1 case.

**Conclusions:** the TNM staging is still the most important guide to prognosis in patients with gastric carcinoma. The number of lymph node metastasis can be an independent risk factor. P53 positivity suggests an advanced progression of neoplasm rather than a poor prognosis. ACE, Goseki grading and Mib1 do not predict the survival of patients.

## O-015

**SPORADIC AND FAMILIAL ADENOMATOUS POLYPOSIS-ASSOCIATED FUNDIC GLAND POLYPS: AN IMMUNOHISTOCHEMICAL STUDY WITH ONCOFETAL AND MIB1 ANTIGENS**

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**Aims-** Fundic gland polyps (FGPs) are small sessile (2-5 mm) usually multiple polyps arising in the gastric, acid-secreting mucosa of disputed histogenesis. They had been described, with identical histology, in a sporadic form, prevalently in middle aged females, or associated with familial adenomatosis coli (FAP)-Gardner's syndrome and their genetic variants (syndromic FGPs).

**Methods-** We performed an immunohistochemical study on 2 syndromic and 22 sporadic FGPs, using monoclonal antibodies (MoAbs) against Ck 7 and 20, EMA, chromogranin A, oncofetal and proliferation antigens, aimed to show any possible difference in the polyps immunophenotype.

**Results:** Ck 20 diffusely stained controls, sporadic and syndromic FGPs; EMA was strongly postive on parietal cells. Chromogranin-positive cells in FGPs were alike controls, except for a case of nodular hyperplasia. Ck 7, as expected, was negative in controls, whereas the 2 syndromic FGPs and 20 on 22 sporadic FGPs showed a diffuse neo-expression. *Hp* antiseum gave negative results on all 24 FGPs. Sporadic and syndromic FGPs showed a neoexpression of CEA, sylial-Tn, Ca19.9 and CA50 mucin associated oncofetal epitopes. MIB1-labelling index of surface and deep compartments of syndromic FGPs was enhanced, with high statistical significance, either in comparison to controls or sporadic FGPs.

**Conclusions:** Sporadic and syndromic FGPs showed a neoexpression of CK7, CEA, and mucin epitopes. As CK7 and mucin epitopes are normally expressed by fetal stomach, FGPs showed an "immature" immunophenotype. No differences between syndromic and sporadic polyps were seen, apart a statistically significant enhanced MIB1-labelling index expression by syndromic FGPs when compared to Sporadic polyps and controls.

## O-016

**SAMPLING STRATEGIES FOR ENTEROCHROMAFFIN-LIKE (ECL) CELL CHANGES IN ZOLLINGER-ELLISON SYNDROME**

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**Aims:** To ascertain the optimum number of gastric biopsies that should be taken and the potential regional variations that may influence the sampling for evaluation of qualitative ECL cell changes in patients at risk of developing ECL cell gastric carcinoids.

**Methods:** A total of 1176 endoscopic biopsies were taken from 8 different sites (4 of the lesser (LC) and 4 of the greater curvature (GC)) of the body mucosa in 149 consecutive patients with the Zollinger-Ellison syndrome (ZES). ECL changes were assessed in Chromogranin-A stained sections using the Solcia's classification. The significance of the expression of human chorionic gonadotropin-α (hCG-α) was also assessed.

**Results:** There was an excellent correlation ($r>0.93$-$0.99$, $p<0.01$) between biopsies from different sites within the GC or the LC. In contrast, a normal ECL cell pattern, found in 12.4 % of 1101 evaluable specimens, was more frequent in LC than in GC biopsies ($p<0.008$). Diffuse (DH), linear (LH) and micronodular (MH) ECL cell hyperplasia were found in 61.2%, 18.5% and 7.8% of biopsies, respectively, with the LH pattern significantly more frequent in GC ($p<0.04$). Dysplastic ECL cell changes were found in 37 biopsies (3.4%) from 17 patients and endoscopically unremarkable carcinoid tumors were diagnosed in 13 biopsies (1.2%) from 9 patients, both equally divided between the LC and the GC. The chance of the dysplastic and carcinoid lesions of being diagnosed was found to be strictly related to the number of biopsies examined. Marked ECL cell expression of hCG-α was more frequent in GC than in LC biopsies ($p<0.03$), being mostly associated with LH and MH. hCG-α was more expressed in patients with ECL cell dysplastic changes or carcinoid independently of the ECL cell pattern.

**Conclusions:** Sampling of a limited number of biopsies in the GC mucosa appears to be adequate for ECL cell monitoring in patients with no risk of carcinoid development or for evaluation of hCG-α expression. In contrast, extensive sampling of both the LC and GC regions is required if early diagnosis of dysplastic and/or neoplastic ECL cell lesions is looked for in patients at risk. Extensive hCG-α expression may indicate an increased risk of ECL cell dysplasia or carcinoid.

## O-017

ALTERED EXPRESSION OF THE E-CADHERIN/CATENIN COMPLEX IN MALT LYMPHOMAS OF THE STOMACH

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**Aims :** MALT-lymphomas predominantly occur in the G-I tract and are typically characterized by the presence of lympho-epithelial lesions (LELs). LELs are defined as infiltration of neoplastic lymphocytes with concommitant destruction of glandular epithelium. As normal E-cadherin/catenin expression is essential for the formation of stable epithelial cell-cell adhesion, we wanted to investigate the expression of the E-cadherin/catenin complex in these destructed glands.

**Methods :** 12 biopsies of histologically and immunohistochemically proven MALT-lymphomas of the stomach were stained with monoclonal antibodies against E-cadherin, β- and γ- catenin, and a polyclonal antibody against α-catenin.

**Results :** In all cases the expression of E-cadherin was highest at the surface of the mucosa and gradually decreased towards the base of the glands. Although the staining for γ- catenin was rather weak, a similar expression pattern was found for the studied catenins.
In LELs a downregulation of membrane bound expression of all the constituents of the E-cadherin/catenin complex was observed whereas a cytoplasmic staining became more apparent.

**Discussion :** MALT-lymphomas, unlike other types of lymphomas, are typically associated with the presence of LELs. Downregulated membrane bound expression of the E-cadherin/catenin complex and an accompanied increase of cytoplasmic staining may be due to an internalisation of the complex thereby facilitating the migration of neoplastic lymphocytes into the lumina of the glands. However, mechanisms evolving these changes in expression remain to be elucidated.

## O-018

**THE DIAGNOSTIC VALUE OF IMMUNOHISTOCHEMISTRY IN GASTROINTESTINAL STROMAL TUMORS (GIST).**

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**Introduction:** GISTs are subclassified by using immunohistochemistry (IHC) as; smooth muscle tumors (SMT), neural tumors (NT), tumors showing dual differentiation and dedifferentiated tumors according to the recent classification.

**Aim:** The aim of the present study is to determine the immune profiles and probable biological behaviour of 30 cases of GISTs.

**Methods:** Cases diagnosed as GIST in the Department of Pathology, University of Ankara Medical School were examined by both light microscopy (LM) and IHC. Tumors were diagnosed as benign, borderline and malignant with respect to tumor size and number of mitoses per 50 HPF. A panel of antibodies including Desmin, SM Actin, S-100 protein and CD34 were used by means of Streptavidin-Biotin preoxidase technique.

**Results:** According to light microscopical examination 18 cases showed evidences of smooth muscle differentiation whereas 5 cases demonstrated neural differentiation, three cases lacked features for either. Among the remaining cases 2 were diagnosed as lipoma, 2 as inflammatory fibroid polyp, thus were excluded from immunohistochemical analysis. When the results of both light microscopical and immunohistochemical analysis were considered together, 4 tumors with SM differentiation showed CD34 expression as well as SM markers. Also 2 tumors in which LM failed to identify the differentiation pattern, IHC showed SM profile in one and neural in the other. One SMT and 1 NT(light microscopical diagnosis) showed negative immunmicroscopy except CD34 positivity while one tumor with SM differentiation at LM showed neural by IHC.

**Conclusion:** The results of the present study showed that IHC is helpful in determining the differentiation pattern and also that CD34 positivity and/or neural differetiation seem to corralate with features of malignancy observed at LM.

## O-019

**GENETIC ALTERATIONS OF MALIGNANT GIST BY CGH**

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**Aims**: Gastrointestinal stromal tumors (GIST) are uncommon neoplasms for which current criteria for the diagnosis of malignancy (location, size and mitotic index) do not always reliably predict patient outcome. Comparative genomic hybridization (CGH) provides an opportunity to screen the whole genome for chromosomal aberrations which may be associated with poor clinical outcome.

**Methods**: DNA was obtained from fresh frozen tissue of 17 tumors from 13 patients. From three patients tissue from primary and metastasic tumor was available, one sample was from liver metastases, three from peritoneal recurrence and the other eight from primary tumors. One microgram each of tumor and of reference DNA were labeled by nick translation and hybridization and detection were performed as previously described. Deviations from normal were interpreted as gains or losses. An event was defined as gain or loss of (part of) a chromosomal arm. Clinicopathological data (size, location, mitotic index etc.) were recorded from the pathological chart and/or review of the microscopic slides.

**Results**: Seven tumors were located in small bowel, two in stomach and one in rectum. All the metastatic tumors were liver metastases. All the primary tumors were considered malignant based on conventional criteria. Eight cases showed chromosomal aberrations ranged from 1 to 10 events (mean 4,3). There were most frequent losses than gains, with chr 14 (6 cases), 1p (4 cases) and 15 (3 cases) being the most frequent losses. Chromosome 5 (5 cases) were the most frequent gain. From the metastatic or recurrence tumours, all cases had alterations, ranged from 4 to 10 events (mean 5,8). All these cases showed loss of chr 1p and 4 of 7 gain of (part of) chr 5. All the three cases from which primary and metastatic tumor were available showed gain of chr 5 and loss of chr 1p.

**Conclusions**: GIST are tumors that could be genetically characterized by loss of chr 14 and 15. Additional loss of chr 1p and gain of chr 5 could define tumors with high tendency to recur or metastatize.

## O-020

SURGICAL RESECTION MARGINS IN COLORECTAL CARCINOMA: ARE THEY NECESSARY?

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Aims: Histological evaluation of surgical margins is currently a routine component of the pathological evaluation of colorectal carcinoma resection specimens. The real value of this exercise has not been critically evaluated. This study was undertaken to provide information on the frequency with which the surgical margins of colorectal carcinoma resections when grossly negative showed marginal involvement histologically.

Methods: Colorectal surgical resection specimens from the files of the pathology department of the University of Pittsburgh Medical Center Hospitals, Presbyterian and Shadyside for the eleven-year period 1988 to 1998 inclusive were reviewed. Those with identified adenocarcinoma constituted the material for this study.

Results: 875 cases were pathologically confirmed as colorectal carcinoma of which 11 (1.2%) showed histological evidence of colonic and/or perirectal margin involvement. Of these 7(0.8%) cases showed positive colonic margins: 2 involving proximal, 4 involving distal and 1 involving proximal, distal, and perirectal margins. The remaining 4 cases showed tumor involving the circumferential perirectal margin only. All 11 cases involved the rectosigmoid region and were classified as either Dukes C or D. Seven cases showed angiolymphatic and/or perineural invasion.

Conclusion: These results provide evidence that surgical margins of colorectal carcinoma resection specimens are rarely involved by tumor on histological section in the absence of gross marginal involvement and show no relationship to the size or location of tumor within the specimen. Positive margins occurred predominantly in advanced stage rectosigmoid lesions with angiolymphatic and/or perineural invasion. These observations question the justification of routine histological evaluation of resection margins in colorectal carcinoma when there is no gross tumor involvement of the margins.

## O-021

### DISORGANIZATION OF INTESTINAL CELL APICAL STRUCTURE IN COLON CANCER IS NOT RELATED TO E-CADHERIN FUNCTION

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**Background**: E-cadherin through its association with β-catenin, α−catenin, p-120, and actin regulates cell adhesion. Normal function requires the presence of this protein in the membrane and its association with intracellular actin. The aim of this study is to investigate the relationship between structural changes at the apical domain of cells in tissue samples of colon cancer and the pattern of E-cadherin distribution.

**Methods**: Samples of colorectal adenocarcinomas (n=30) were fixed in glutaraldehyde and osmium tetroxide and embedded in epoxy resin. Sections from neoplastic glands were selected for EM. Presence and development of cell junctions and microvilli and quantity and distribution of actin were recorded. In addition paired samples of the same areas were frozen for immunohistochemical assays with anti-E-cadherin HECD-1 Ab (Zymed, San Francisco, CA, USA) after Triton pre-treatment, using streptavidin-biotin-alkaline phosphatase and Fast Red as chromogen. Intensity and percentage of positive cells were combined as an index.

**Results**: Cases of group I had a well developed microvilli, group III a disorganized apical structure, and Group II an intermediate phenotype. E-cadherin showed a membrane (M), cytoplasmic (C), or mixed (Mx) pattern.

| EM vs E-cad | n | E-cad M | E-cad C | Ecad Mx |
|---|---|---|---|---|
| I | 6 | 1 | 0 | 5 |
| II | 14 | 4 | 1 | 9 |
| III | 10 | 3 | 1 | 6 |

**Conclusions**: Apical domain structure is not related to E-cadherin distribution . Furthermore, the structural differences in the apical zone of neoplastic glands do not appear to be associated with changes in E-cad expression.

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## O-022

### PATHOLOGICAL ASSESSMENT OF ENDOSCOPICALLY REMOVED MALIGNANT COLORECTAL POLYPS

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**Aims:** To evaluate the significance of histological parameters in predicting adverse outcome after endoscopic polypectomy for colorectal adenomas with invasive cancer.

**Methods:** We reviewed the specimen slides of 178 adenomas with invasive cancer fulfilling the following criteria: 1) carcinomas invading through the muscularis mucosae into the submucosa; 2) polypectomies considered to be complete by the endoscopist; 3) patients undergoing large bowel resection after polypectomy. Patients with familial adenomatous polyposis, inflammatory bowel disease, previous or synchronous colorectal cancer were excluded from the study.

In each case the following parameters were evaluated: 1) distance of cancer cells from polyp resection margin (negative ≥ 2 mm., positive < 2 mm.), 2) presence or absence of lymphatic or venous invasion, 3) histological grade of the cancer (I, II, or III).

Adverse outcome was defined as the finding of residual cancer or lymph node metastases in large bowel resection specimens.

**Results:** None of the 70 cases with favourable histology had an adverse outcome. When unfavourable histology was present, an adverse outcome was observed in 20 of 108 cases (18.5%): 11 with lymph node metastases alone, 3 with lymph node metastases and residual cancer, and 6 with residual cancer alone.

**Conclusions:** Our data provide evidence that colorectal adenomas with invasive cancer and favourable histology require no further therapy following colonoscopic removal.

## O-023

### PROGNOSTIC VALUE OF NEOVASCULARIZATION IN COLORECTAL CARCINOMA

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**Objective:** To determine the prognostic influence of angiogenesis in colorectal carcinoma and to stablish its relation with several usual histological, clinical and immunohistochemical prognostic factors. We also review the literature on this matter.

**Methods:** We selected 126 consecutive patients with classic colorectal adenocarcinoma (Dukes A, B and C) in whom a curative surgery was performed, and that did not receive any further chemotherapy or radiotherapy. We collected the usual histological and clinical prognostic factors. As a vascular marker, we chose CD34, detecting it with immunohistochemistry with the avidine biotine-antibiotine technique. The vascular counts were performed by two independent investigators following Gasparini's criteria. To test the prognostic influence of these factors in our patients, we used the disease free survival (DFS) and the overall survival (OS) and compared the Kaplan-Meier curves with Breslow's test.

**Results**: The tumours were 11.9 % stage A, 48.4 % stage B and 39.7 % stage C. The histologic grade was well differentiated in 47.6 %, moderately differentiated in 45.2 % and undifferentiated in 7.2 %. We found vascular invasion in 25.4 % of the cases and perineural invasion in 10.3 %. Nuclear immunoreactivity for p53 was not found in 32.5 % of the cases, the remaining cases being positive. Those patients with vascular counts over 77 vessels/200 x field showed an statistically significant worse prognosis, both for DFS (p = 0.0143) and for OS (p = 0.0395). The univariate analysis only showed a significant association between histological grade and angiogenesis

**Conclusions:** As in other tumours, the neovascularization is an indicator of bad prognosis in colorectal carcinoma, although this prognostic effect shown in the univariate analysis is lost in the multivariate analysis (it is not independent from the stage). This prognostic influence appears both for DFS and OS and shows no significant interactions with other classical prognosticators.

## O-024

### EXPRESSION OF TRANSCRIPTION FACTOR AP-2 IN COLORECTAL ADENOMAS AND ADENOCARCINOMAS

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**Aims:** To investigate the role of transcription factor AP-2 in colorectal adenomas and adenocarcinomas.

**Methods:** The expressions of AP-2α, AP-2β and AP-2γ were analyzed in 30 human colorectal carcinomas and 13 adenomas by immunohistochemistry (IHC) and the mRNA status of AP-2α was determined by in situ hybridisation (ISH). The expression patterns of AP-2 were correlated with clinicopathological variables.

**Results:** In adenomas AP-2 expression was not related to degree of dysplasia. AP-2α expression was reduced (<10%) in advanced Dukes stages (p=0.02). In high grade tumours both AP-2α and AP-2γ were diminished (p=0.04 and p=0.01, respectively). By ISH an increased AP-2α level was found in high grade carcinomas (p=0.04).

**Conclusions:** Our results show that the low expressions of AP-2α and AP-2γ are inversely correlated with tumour malignancy. The ISH data suggest that the loss of AP-2α expression may be due to post-transcriptional regulation.

## O-025

### MSH2- AND MLH1-MUTATIONS IN HEREDITARY NONPOLYPOSIS COLORECTAL CANCER: CORRELATION OF MORPHOLOGY, IMMUNOHISTOCHEMISTRY, AND GENETIC ANALYSIS

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Hereditary nonpolyposis colorectal cancer (HNPCC) results from an autosomal dominantly inherited mutation in mismatch repair genes. The diagnosis is mainly based on clinical criteria (Amsterdam-, Kopenhagen-, and Bethesda-Criteria). Since the discovery of the most relevant germline mutations (MSH2 and MLH1) accounting for this syndrome, in approximately 70% of all cases the diagnosis can be confirmed via sequencing. Recently, antibodies for immunohistochemical detection of the mismatch repair gene products were made available.
52 primary colorectal adenocarcinomas were selected for morphologic and immunohistochemical analysis. Of these, 20 microsatellite stable carcinomas served as control. All the other 32 carcinomas were microsatellite unstable including 22 carcinomas where the underlying germline mutation had been sequenced (14 MLH1 and 8 MSH2 mutations).
The histomorphological analysis already allowed a possible distinction of sporadic carcinoma and HNPCC. Additonally, loss of hMSH2 or hMLH1 immunostaining could be demonstrated in 86 % of the genetically proven HNPCC in contrast to internal control tissue and to all microsatellite stable carcinomas.
Hence, the combination of morphology, immunohistochemistry and clinical data (such as patient's age) can serve as a valid screening method in the diagnosis of these hereditary carcinomas.

## O-026

### SMALL ROUND CELL TUMOUR OF THE PAROTID GLAND

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The category of small round cell tumours comprises eg. small cell carcinoma, small cell neuroendocrine carcinoma, PNET, Merkel cell carcinoma, etc. In the head & neck region small round cell tumours are usually found in the larynx, the nose, and on rare occasions in the salivary glands. We here describe a tumour that presented as a non-ulcerated mass in the left parotid region and upper neck in a 70 years old man. Two years previously a skin basal cell carcinoma of the ipsilateral pinna had been excised. A Tru-cut biopsy from the mass in the neck showed a small cell carcinoma with certain basaloid features. A total parotidectomy and radical neck dissection were performed. The tumour measured 5x5x4 cm and consisted of small, undifferentiated cells with scanty cytoplasm and uniformly dense nuclear chromatin. The tumour merged with normal parotid glandular tissue, and mimicked in certain areas a primary salivary gland small cell carcinoma. Immunohistochemistry revealed a strong positivity for synaptophysin, chromogranin A and NSE. Cytokeratins were positive, and cytokeratin 20 and cytokeratin 34BE12 showed a single punctate zone of cytoplasmic immunoreactivity, being a rather characteristic feature of Merkel cell carcinoma. The previously excised basal cell carcinoma was thus reviewed, and showed an immunoreactivity identical to that of the parotid tumour, and a morphology compatible with a Merkel cell carcinoma.

The lesion was thus interpreted as a regionally recurrent Merkel cell carcinoma with direct invasion into the parotid gland, rather than a primary small cell carcinoma of the parotid. The origin of Merkel cell carcinoma, or cutaneous small cell undifferentiated carcinoma, or neuroendocrine carcinoma, is controversial. The Merkel cells are present within the basal layer of the epidermis, in the oral mucosa, and in the bulge area of the hair follicles. The Merkel cells are regarded as epithelial neuroendocrine cells and may possess a neurosecretory function.

## O-027

### ADENOID CYSTIC CARCINOMA OF SALIVARY GLANDS: A COMPARISON OF GENETIC ALTERATIONS IN TUMOR TISSUE OF DIFFERENT HISTOLOGIC GROWTH PATTERNS

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**Aims:** The prognosis of salivary gland adenoid cystic carcinoma (ACC) depends on the clinical stage and the histologic grade. Although in general ACCs with a cribriform growth pattern have a better prognosis than those with solid growth, no generally accepted histological grading criteria are available yet. The aim of the study was to separately analyze and compare the frequency and localization of loss of heterozygosity (LOH) in major salivary gland ACCs of different histologic growth patterns.

**Methods:** 23 tumor samples including primary tumors and metastases of 5 patients with ACCs were analyzed. Areas with cribriform and solid growth patterns were separated by microdissection. Six microsatellite markers at chromosome 6q, 2 markers at chromosome 19q and one marker at chromosome 20q were used for PCR-based analysis.

**Results:** LOH was most frequently observed at D6S310 (10/12 informative samples), which is located on 6q. This aberration is present in primary tumors and metastatic tissue with both cribriform (4/5) and solid (6/7) growth pattern. The overall LOH rate for all the other microsatellite markers was 28% and for these markers LOH was much more frequent in solid than in cribriform tumor tissue (44% vs. 6%; $p<0.01$). There was no increase in LOH rates in metastases versus primary tumors.

**Conclusions:** These findings indicate that LOH at D6S310 is an early event in the tumorigenesis of ACC and that this locus may harbor a tumor-suppressor gene involved in the development of these tumors. The data also show that tumor dedifferentiation from cribriform to a solid growth pattern goes along with an accumulation of LOH at multiple other locations.

## O-028

### CRIBRIFORM ADENOCARCINOMA OF THE TONGUE.

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**Introduction:** We describe eight cases of a distinctive type of adenocarcinoma of the tongue, for which we propose the name cribriform adenocarcinoma of the tongue (CAT). The tumours were morphologically distinctive, bearing similarity both to polymorphous low grade adenocarcinoma of minor salivary glands by growth patterns, and to papillary carcinoma of thyroid by cytological features.
**Methods:** Immunohistochemically, the tumours expressed cytokeratin and S-100 protein. Actin was patchily positive, staining for thyroglobulin was negative. Ultrastructurally, the cells had clefted nuclei, and the cytoplasm contained a few mitochondria, lysosomes, Golgi apparatus, and bundles of microfilaments. Many tumour cells displayed well formed microvilli on their apical surface, thus the cells revealed features of both myoepithelial and secretory differentiation.
**Results:** All the tumours were unencapsulated and were divided by fibrous septa into lobules. Major parts of each lesion were composed of areas with solid, microcystic, and cribriform growth patterns. The most striking cytological feature was that tumour nuclei were pale-staining with a "ground glass" quality, and they often appear to overlap one another. Mitoses were very sparse.
**Conclusions**: All eight patients had at the time of presentation metastases in the regional neck lymph nodes, but all are alive two to five years after the excision and irradiation.

## O-029

POSSIBLE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN DISEASE PROGRESSION OF THE ORAL MUCOSA

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**Aims:** Previous studies indicate that tumourigenesis is accompanied by angiogenesis. The aims of this study were to determine the possible contribution of the angiogenic factor VEGF to disease progression and angiogenesis in the oral mucosa.

**Methods:** Sections of normal oral mucosa (n=12), dysplastic lesions (n=10) and squamous cell carcinoma (n=15) were stained with a specific antibody to VEGF (R & D). The expression of VEGF in the epithelium was graded according to three indices: (i) percentage of area stained, (ii) intensity of staining, and (iii) product of area and intensity (final score). Blood vessels were visualised by staining with antibody to vWF (Dako) and quantified by the sterological method of point counting (vascularity). Statistical analyses were done by non-parametric tests.

**Results:** VEGF was localised in the epithelium of all normal and dysplastic tissues and 80 % of the tumours. Its expression decreased significantly with the severity of the lesion. For example: indices (i) and (iii) were, respectively, 84 and 164 in the normal mucosa, 74 and 139 in dysplasias and 40 and 52 in tumours. In contrast, vascularity increased significantly ($p<0.001$) with disease progression. VEGF was also present in blood vessels; the percentage of total (vWF-positive) blood vessels that expressed VEGF was significantly lower in tumours (34%) than in normal (83%) and dysplastic (60%) tissues.

**Conclusions:** The expression of VEGF in oral tissues is inversely associated with severity of the lesion and vascularity. Our results suggest that tumourigenesis in the oral mucosa is accompanied by angiogenesis, and this is initiated and regulated by factors other than VEGF.

## O-030

CELL PROLIFERATION AND APOPTOSIS IN SQUAMOUS CARCINOMAS OF THE MOUTH AND OROPHARYNX

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**Aims:** This study examines the expression of molecular markers of cell proliferation and apoptosis in oral and oropharyngeal squamous carcinomas, two sites with different responses to radiotherapy.

**Methods**: Paraffin-embedded, formalin fixed tissue sections from resection specimens of 35 cases of squamous carcinoma of the mouth (24 males, age 49-76y), and 39 cases of carcinoma of the oropharynx (24 males, age 34-77y) were examined by immunohistochemical labelling for the Ki-67 antigen, for p53, mdm-2, p21, bax and bcl-2 proteins, and by in situ DNA end-labelling for apoptosis.

**Results:** Cell proliferation was significantly higher in oropharyngeal carcinomas (oropharynx, mean 37% cells labelled; mouth mean 16% cells labelled), and the apoptotic index was significantly lower in oropharyngeal carcinomas (oropharynx 8%, mouth 12%). There was no significant difference in the proportion of cases showing p53 labelling (mouth 60%, oropharynx 64%). Mdm-2 expression was seen in 4 oral and 9 oropharyngeal carcinomas. p21 expression was present in 68% oral carcinomas compared with 94% of oropharyngeal carcinomas. p53 expression was not related to expression of either p21 or mdm-2. Expression of bcl-2 was present in most cells of most oropharyngeal carcinomas but was more variable in oral carcinomas. Bax expression was closely related to squamous differentiation.

**Conclusions:** Apoptosis is more frequent and MIB-1 expression is less frequent in carcinomas of the mouth than in those of the oropharynx. This is associated with abnormalities of expression of several proteins involved in apoptosis. p21 expression appears to be induced independently of p53 expression. Further work is required to elucidate how these differences are related to different responses to treatment.

## O-031

**THE SIGNIFICANCE OF PATTERN OF MANDIBULAR INVASION, CYCLIN-D1 AND KI-67 STAINING IN ORAL SQUAMOUS CELL CARCINOMA**

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**Aims:** To determine if the histologic pattern of mandibular invasion by oral squamous cell carcinoma and the staining pattern of cyclin D1 and Ki-67 are predictive of outcome.

**Methods:** Pts with oral squamous cell carcinoma treated at Mass. Eye and Ear Infirmary between 1990 and 1996 were reviewed to determine the histologic pttn of bone invasion using previously published criteria; Erosive pttn: broad pushing front, sharp interface between tumor and bone, osteoclastic bone resorption, fibrosis along tumor front, absence of bone islands within tumor. Infiltrative pttn: nests and cords of tumor along an irregular front, haversian system penetration, residual bone islands within the tumor. Immuno stains for cell cycle markers cyclin D1 and Ki-67 were performed on paraffin embedded tissue. F/U was from pt charts.

**Results:** 68 patients were included, 43M:25F, aged 38-86 (mean 62)yrs. Tumor size range 0.4-7.0 (mean 3.3) cm. 10 tumors were well differentiated, 39 moderately diff, 19 poorly diff. 41 tumors showed an erosive pattern, 23 infiltrative pattern, and 4 mixed. 59 underwent immuno staining; % of tumor + for cyclin D1: 0-5%=33, 6-25%=11, 26-50%=7; 51-75%=3; 76-100%=8. From 1-286 (ave 95) cells of 300 were + for Ki-67. Multivariate analysis revealed the pattern of invasion and the tumor grade are prognostically significant ($p=.0045, .0391$). 3 yr disease free survival for the erosive pattern= 73%, infiltrative= 30%. Cyclin-D1 and Ki-67 staining did not correlate with outcome or the pattern of invasion.

**Conclusion:** Patients with the erosive pattern of mandibular invasion by oral squamous cell carcinoma have a significantly better prognosis than those with the infiltrative pattern, therefore the pattern of invasion should be commented on in the pathology report. Cell cycle markers cyclin D1 and Ki-67 do not predict prognosis.

## O-032

**P-53 AND P-GLYCOPROTEIN (MDR1-gene-related) EXPRESSION IN PRIMARY OROPHARYNGEAL SCC.**

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**Background**: Presently, we lack reliable prognostic markers predicting the clinical course, such as recurrence rate and survival, of patients with oropharyngeal squamous cell carcinoma (SCC). Furthermore, treatment modalities are not standardized and include surgery, radiation and chemotherapy. Our goal was to identify further possible prognostic markers - other than LN-status and tumor stage - which can be assessed by routine methods in formalin-fixed, paraffin-embedded material. We focused on expression of p53, a tumor supressor gene, and p-glycoprotein, a MDR-1 gene product, which is considered a prognostic factor for chemotherapy failure and associated poor survival in leukemias.

**Method:** We analyzed the expression of p53 and p-glycoprotein by immunohistochemistry on archival formalin-fixed, paraffin-embedded material of SCC. Staining was divided into positive and negative, and survival was calculated for the still alive patient group (A) and the deceased patient group (D).

**Results**: Staining results of the SCC were as follows:

Any positive Staining

| | p 53+ | p53- |
|---|---|---|
| MDR + | 20 | 16 |
| MDR - | 20 | 38 |

Survival in months seperated into deceased (D) and still alive (A)

| | p 53+ | p53- |
|---|---|---|
| MDR + | 25 D<br>37 A | 22 D<br>45 A |
| MDR - | 24 D<br>28 A | 31 D<br>42 A |

**Conclusion:** Our preliminary data indicate that p53 negativity is associated with longer mean survival among the still living patients. Among the patients who died of disease, the p53 and MDR negative SCC group demonstrated the best mean survival. In the p53 negative group, MDR positivity was associated with lower mean survival (22 mo) than MDR negativity (31 mo) in the deceased patient group.

## O-033

**BASALOID SQUAMOUS CELL CARCINOMA OF HEAD AND NECK : CLINICOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY OF 10 CASES**

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Basaloid squamous cell carcinoma (BSCC) is a rare variant of squamous cell carcinoma (SCC) of head and neck, with distinct histologic features and biological behavior. It is often misdiagnosed in this localization, despite its characteristic bimorphic pattern, consisting of differentiated SCC in the overlying epithelium in brutal transition with an infiltrative basaloid indifferentiated carcinoma. The aim of this study is to allow best knowledge of this tumor in terms of diagnosis, prognosis and treatment.

Immunohistochemistry (IHC) was performed on 10 cases with keratins (KL1, Cam 5.2, AE1/AE3, CK19), EMA, ACE, vimentine, α smooth-actin (1A4), NSE, chromogranin and synaptophysin.

M/F sex ratio was 9:1. All men were smokers and alcohol drinkers. The mean age was 54.2 years (range 40-70 years). The site of the tumor was buccal mucosa (4 cases), hypopharynx (4 cases) and larynx (2 cases). At presentation, 8 patients had high-stage (T3 and T4) ; 8 had metastatic cervical lymph nodes ; none had distant metastasis. Two patients had additional malignancy. Treatment consisted of surgery followed by radiotherapy in 6 cases, one of which with neo-adjuvant chemotherapy ; and of chemotherapy followed by radiotherapy in 4 cases. The mean of follow-up was 14 months (range 3-36 months). Four patients died (2 of them with hematogenous metastases). Six are alive : 5 without disease and 1 with cutaneous metastases. Epithelial markers were positive. Neuro-endocrine markers, PS100 and 1A4 were negative.

IHC can be useful in small biopsies of ulcerated lesions, for distinguishing BSCC from adenoid cystic carcinoma and small cell indifferentiated carcinoma, whose prognosis and therapy stringly differ. Poor prognosis of BSCC seems to be almost due to its frequent high-stage presentation. Its propensity to hematogenous metastasis justifies its recognization as a distinctive entity among SCC. The combination of chemotherapy-surgery-radiotherapy seems to be advisable.

## O-034

REDUCTION OF TRANSFORMING GROWTH FACTOR-β TYPE II RECEPTOR (TGF-β RII) EXPRESSION IN LARYNGEAL CARCINOGENESIS

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**Aims**: Expression of TGF-β RII is required for the growth inhibitory effect of TGF-β on epithelial cells. Abnormal expression and genetic alterations of this receptor result in a resistance to TGF-β mediated growth suppression and have been implicated in the development of carcinomas from several sites. To assess the potential role of the loss of TGF-β RII expression in laryngeal carcinogenesis, we undertook a retrospective immunohistochemical study using a series of biopsy specimens from patients with precancerous conditions of the laryngeal mucosa.

**Methods**: Fifteen consecutive patients with precancerous laryngeal lesions who experienced a malignant progression of the disease during the follow-up period (median 6.9 years) and a control group of 30 patients with hyperplastic/dysplastic laryngeal lesions, risk factor exposure and therapy comparable to those of the study population and who had no progression of the disease, were selected. The immunohistochemical study was performed on formalin-fixed, paraffin embedded sections using a rabbit polyclonal antibody against TGF-β RII (Santacruz Biotechnology, Santa Cruz, CA).

**Results**: A complete loss or a marked decrease (<20% of cells) of TGF-β RII immunoreactivity in the squamous epithelium was observed in 11 of the 15 premalignant lesions that progressed to squamous cell carcinoma. Conversely, only 5 of the 30 laryngeal lesions of the control group showed a loss of expression of the receptor to a similar degree, and this difference was statistically significant ($p<0.001$, two tailed Fisher exact test).

**Conclusions**: These data indicate that a reduction of TGF-β RII expression may play an important role in the progression of laryngeal precancerous lesions to squamous cell carcinoma.

## O-035

**EXPRESSION OF TENASCIN AND FIBRONECTIN IN EPITHELIAL HYPERPLASTIC LESIONS AND SQUAMOUS CARCINOMA OF THE LARYNX**

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**Aims:** Tenascin (T) and fibronectin (FN) are glycoprotein components of extracellular matrix and are presumably involved in cancer progression. The aim of our study was to analyze the expression of T and FN in epithelial hyperplastic lesions (EHL) and squamous carcinoma (SC) of the larynx and to reveal their possible diagnostic significance.

**Methods:** Samples of surgical specimens of 20 laryngectomies containing transition from normal and/or hyperplastic epithelium to SC, and 5 autopsy samples of normal laryngeal mucosa were included. EHL were classified according to the criteria of the Ljubljana classification (Histopathology 34: 226-34, 1999). Immunohistochemistry was performed with antibodies against T and FN.

**Results:** T and FN were either absent or stained weakly along the basement membrane in normal epithelium and in simple and abnormal hyperplasias. In atypical hyperplasia and carcinoma in situ, T and FN were slightly increased in the stroma beneath the epithelium. In 18/20 cases of SC, T and FN were markedly increased in the tumor stroma, whereas in 8/20 cases staining tended to be more intense at the invasive front. In 2/20 cases, we found areas of atrophic fibroproliferative sialadenitis deeply in the stroma which were also positive.

**Conclusions:** Both T and FN gradually increase with the severity of EHL and are markedly increased in the stroma of the invasive SC. More intense staining at the invasive front is consistent with the presumed role of T and FN in cancer progression. We conclude that T and FN immunostaining provides useful information on epithelial-stromal interaction in EHL and SC of the larynx and could be therefore regarded as a useful adjunct to traditional histology.

## O-036

**DIRECTIONAL ANGIOGENESIS IN SQUAMOUS CELL CARCINOMA OF THE LARYNX.**

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The role of angiogenesis in the analysis of development and progression of cancer of several organs, notably the breast and prostate, has been shown in several studies. The results have, however, been disputed. The aim of the present study was to assess the participation of the vascular system in neoplasia of the larynx.

In the present study vessel location and amount was analyzed in 60 specimens of squamous cell carcinoma of the larynx using computer-based morphometry of FVIII and laminin stained specimens. The total amount of positive staining of vascular structures as well as size, structure and direction of vessels in relation to the basement membrane was determined using a CAS200 system and software developed for the purpose.

The results showed dysplasia to be associated with increased vascularity seen as increased total optical density of FVIII staining. In well-differentiated squamous cell carcinomas (grade 1) directional angiogenesis was observed by comparing vessel length in relation to the basement membrane. In these specimens vessels were directed in the direction of invading neoplasms. In moderately differentiated neoplasms (grade 2) vessels were prominent, associated with tumor islets. In less differentiated neoplasms (grade 3), irregular tumor sheets and cords were intermixed with irregular vascular structures.

The results show the usefulness of computer-based morphometry and the role of angiogenesis in tumor development. Increased vessel formation was an early event, directional angiogenesis occurred in invading neoplasms, in undifferentiated lesions a close association with tumor formation was seen. The clinical significance of directional angiogenesis is not yet settled.

## O-037

METASTASES IN SMALL LYMPH NODES IN HEAD AND NECK CANCER: DOES IT MATTER IF WE MISS THEM?.

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**Aims**:The finding of small positive lymph nodes in head and neck cancer resections is not uncommon. Small positive nodes (less than 1cm max.) which often appear clinically and grossly negative, may be overlooked at dissection, particularly in the absence of a consensus on how many nodes constitutes an adequate yield. We set out to examine what proportion of positive nodes were small, and if their identification was of clinical significance.
**Methods**: Neck dissections for head and neck cancer were dissected while fresh, and the numbers and size of all nodes was documented.
**Results**: Of 129 neck dissections (92 patients), 64 (34 patients) were tumour free. 30 cases included paratracheal nodes. The mean yield for radical/ modified radical necks was 33 (range 10-62), and for selective necks 15 (range 3-27). Paratracheal node yield was 9.8 (range 2-16). Of a total of 206 positive nodes, 61(44.1%) were 0.5-1cm and 30 (14.5%) were less than 0.5 cm. The finding of a small positive node influenced either treatment or staging in 10 patients (10.8%) where the node was 0.5-1cm and 3 patients (3.2%) where the node was <0.5cm.
**Conclusions**: Small lymph nodes which may appear innocent grossly constitute a substantial proportion of positive nodes in head and neck cancer, and failure to identify them may result in inappropriate management.Methods to maximise node retrieval should be explored in head and neck cancer.

## O-038

P53 GENE STATUS PREDICTS CLINICAL RESPONSE OF HEAD AND NECK SQUAMOUS-CELL CARCINOMAS TO INDUCTION CHEMOTHERAPY.

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**Aims:** To determine whether p53 gene status predicts tumor responses to platin- and fluorouracil-based induction chemotherapy in locoregionally advanced squamous-cell carcinomas of the head and neck.
**Methods:** Tumor responses of 105 patients were measured at the primary site. All coding parts of p53 gene were directly sequenced. P53 expression in tumor cells was determined by immunohistochemistry. Odd ratios were adjusted by stepwise logistic-regression analysis and Kaplan-Meier life-tables were compared by log-rank test.
**Results:** P53 mutations, p53 expression and tumor stage were sufficient to explain the variation in responses to chemotherapy in multivariate models. P53 mutation was the only variable to significantly predict objective response (odds ratio 0.23, 95 percent confidence interval 0.10-0.57; P=0.002) and was the strongest predictor of major response (odds ratio 0.29, 95 percent confidence interval 0.11-0.74; P=0.006). Specific mutations (contact mutations) accounted for much of the reduction in the risk of major response associated with overall mutations, and were significantly associated with a shorter overall survival than noncontact mutations. P53 expression (odds ratio 0.39, 95 percent confidence interval 0.16-0.98), tumor stage (adjusted odds ratio 0.31, 95 percent confidence interval 0.10-0.96) also predicted major response. P53 expression was weakly predictive of major response in the subgroup with wild-type p53.
**Conclusion:** P53 gene mutations are strongly associated with a poor risk of responses to chemotherapy. Contact mutations are associated with the lowest risk of major response and a worse prognosis.

## O-039

**MOLECULAR CHANGES IN SQUAMOUS CARCINOMA OF THE HEAD AND NECK IN SLOVENIAN PATIENTS**

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**Aims:** The major mechanisms involved in genomic instability during tumor progression are loss of heterozygosity (LOH) and microsatellite instability (MSI). Molecular genetic analysis of 67 squamous head and neck carcinoma (SCHN) was performed in order to determine the role and the nature of the most frequent genetic changes in the development of this cancer.
**Methods:** DNA was isolated from tumor and adjacent normal tissue using standard procedure. Non isotopic MSI and LOH analysis was performed with 8 microsatellite markers on chromosomes 1, 2, 5, 11, and 17, including BAT26, the most informative marker in assessment of high MSI colorectal tumors. All tumors have been tested on the presence of mutations in p53 gene and MSI positive tumors have been tested on the presence of mutations in *h*MLH1 and *h*MSH2 mismatch repair protein genes. PCR - non-isotopic conformation analysis was used for mutation analysis.
**Results:** 7/67 (10,5%) tumors showed MSI at at least one locus tested and only two tumors exhibited MSI at multiple loci. BAT 26 was altered only in one tumor. In the *h*MLH1 and *h*MSH2 genes only benign polymorphisms have been detected. Most frequently observed region analysed involved in LOH was 17p chromosome arm (35%). p53 mutations were detected in 15% of tumors.
**Conclusions**: The used microsatellite markers showed relatively low incidence of MSI in SCHN suggesting another pathway of carcinogenesis compared to hereditary non polyposis colorectal cancer (HNPCC) with high informativity of the same markers. The finding of only benign polymorphisms in *h*HLM1 and *h*HSH2 genes suggests a different mechanism of MSI in SCHN comparing to HNPCC where mutations are very frequently found.

## O-040

**DISTRIBUTION OF ENDOTHELIAL MARKERS IN NASO-PHARYNGEAL ANGIOFIBROMAS (NA)**

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**Aims**: NA are typically composed of innumerable blood vessels set in a fibrous stroma. The vessels differ considerably in size and architecture, which may result in a different phenotype and biology of the endothelium. Therefore, we initiated a study on the distribution of classical endothelial markers in NA.
**Methods**: Paraffin-embedded material of 32 cases of NA (25 primary tumors and 7 recurrences) was studied immunohistochemically by the ABC-method using antibodies to CD31, CD34, FVIII-RAg and thrombomodulin. The incubations were performed on strictly consecutive sections, thus being able to judge the immunoreactions of nearly identical vessel-segments.
**Results**: With antibodies to CD31, FVIII-Rag and thrombomodulin the endothelium of all vessels, irrespective of their size and architecture, was evenly stained. However, with antibodies to CD34 the endothelium of smaller vessels (capillary- and sinusoidal-type) was decorated more intensly than endothelial cells of larger vessels. Pericytes, vascular smooth muscle cells and stromal fibroblasts displayed no immunoreaction with any of the antibodies applied.
**Conclusions**: Despite the irregularity in size and architecture of the tumor vessels in NA, their endothelium shows qualitatively a typical immuno-phenotype of endothelial cells. However, the striking difference in staining for CD34 with emphasis on smaller vessels indicates its active role in tumor-angiogenesis. The staining pattern in conjunction with the irregularity of vascular architecture, especially with regard to the vascular smooth muscle layers, is highly suggestive of NA's representing vasoproliferative malformations.

## O-041

HOW TO EVALUATE LYMPHOID INFILTRATES IN BONE MARROW BIOPSIES?

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**Aim:** Morphologic criteria including phenotyping, to distinguish reactive from neoplastic lymphocytosis in trephines, have been defined but are not always reliable. PCR analysis of the CDR-3 region of the IgH gene can aid in this respect by demonstrating monoclonality and is now frequently used for the detection and follow-up of B-cell lymphoma (NHL). The aim of our study was to compare morphologic findings with data obtained by CDR-3 PCR, using consensus FRW3 and JH primers, starting from trephines of patients with and without proven B-cell NHL.

**Methods:** Based on morphology and phenotyping, 89 consecutive trephines with lymphoid infiltrates were diagnosed as positive (33), negative (17) and suggestive (39) for bone marrow involvement by B-cell NHL. CDR-3 PCR analysis was performed on all samples.

**Results:** Presence of a clonal B-cell proliferation was found in 45 biopsies, including 21 samples considered to be positive, 17 to be suggestive and 7 to be negative by morphology. In the remaining 44 trephines no clonal proliferation was detected although 12 of these trephines were considered to be positive by morphology. This group of 44 PCR negative trephines comprises 17 cases with a clonal proliferation demonstrable with the same PCR technique in the original diagnostic tissue excluding the possibility of non-binding of the primers as cause of a false negative result.

**Conclusion:** These results indicate that CDR3 PCR may be of greater sensitivity and specificity than morphology but that negative PCR findings do occur as a result of poor DNA preservation. In addition, these results emphasize the need to combine morphology with PCR analysis as complementary investigations in the evaluation of lymphoid infiltrates in the trephine.

## O-042

**THROMBOPOIESIS IN CHRONIC MYELOID LEUKAEMIA AND THE POSSIBLE INFLUENCES OF B2A2 AND B3A2 BCR/ABL PROTEIN STRUCTURE**

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**Aims:** To investigate possible relations between the structure of b2a2 and b3a2 type of BCR/ABL fused protein and thrombopoiesis in patients with Chronic Myeloid Leukaemia in chronic phase (CML-CP).

**Methods**: Seventyfour (74) consecutive unselected patients with CML in CP were studied at presentation. Haematological, morphological, cytogenetic and molecular data at time of diagnosis were obtained. RT-PCR on leukaemic transcripts and semi-automated morphometric analysis of megakaryocytes (MK) were performed. The secondary structure-prediction of b2a2 and b3a2 type BCR/ABL protein has been obtained with several methods.

**Results**: Thirtyone (31) out of 74 patients had b2a2 and 43 b3a2 transcript; platelet count was significantly higher ( $P< 0.001$) in b3a2- than in b2a2-patients ; size and density of MK in the bone marrow as well as the other haematological parameters studied did not show any significant difference; the secondary structure was almost identical for the two transcripts, as presence or absence of the 25 aminoacids-b3 exon did not influence the overall molecular structure.

**Conclusions**: It is well known BCR/ABL protein could influence the process of platelet formation by interacting with cytoskeleton, integrins and extracellular matrix molecules. We suggest differences between the two types of BCR/ABL transcripts should be responsible of different efficiency in the platelet production process and, consequently, of the time course of platelet formation. Nevertheless, the type of rearranged protein is not sufficient alone to explain the increment of thrombopoiesis.

## O-043

**EFFECTS OF CHEMOTHERAPY AND INTERFERON ON BONE MARROW PATHOLOGY IN PH$^{+}$-CML**

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**Aims:** A retrospective clinicopathological study was performed on sequential bone marrow biopsies (intervals 8 to 17 months) in 128 patients with Ph$^{+}$-CML to elucidate therapy-specific effects.

**Method:** According to applied regimens 28 patients received busulfan (BU), 38 hydroxyurea (HU), 39 interferon-α2b (IFN-α) as single agents and 23 patients a combination of IFN and HU. Using monoclonal antibodies for the identification of erythroid precursors (Ret40f), megakaryocytes (CD61), macrophages (CD68, BSA-1) and a reticulin stain, specimens were evaluated by morphometry. To compute dynamics of histopathology implicating alterations in time, relevant indices have been established.

**Results:** Clinical criteria for complete hematological response were easily determinable by the regeneration of erythropoiesis. Megakaryopoiesis including its precursors showed a significant increase in the IFN-α and BU-treated groups opposed to patients receiving HU. Although increase in fiber density was detectable in the IFN-α and the BU group as well, the progression index proved to be twice as high after IFN-α therapy. In contrast, a considerable number of patients displayed a regression of myelofibrosis following HU treatment. The resident macrophage population revealed a slight (BU group) to marked (IFN-α group) proliferation.

**Conclusions:** The close association of the megakaryocyte lineage with myelofibrosis is in line with experimental findings suggesting a functional impact. IFN-α exerts a fibrogenetic and HU a fibrolytic capacity. Growth and activation of macrophages following IFN-α administration may be compatible with a stimulation of their cytocidal and phagocytic properties (scavenger macrophages).

## O-044

**A MULTICENTER STUDY ON THE ASSOCIATION OF MORPHOLOGICAL AND CLINICAL FINDINGS IN 530 PATIENTS WITH PH$^{+}$-CML PREDICTING SURVIVAL**

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**Aims:** Clinical findings and bone marrow features in Ph$^{+}$-CML are characterized by a striking heterogeneity which should be appreciated by proper means. Prognostic classification is generally based on risk models consisting of clinical and hematological parameters. In this context, the prognostic property of histological parameters is discussed controversially.

**Methods:** A multicenter, immunohistochemical and morphometric study was performed on pretreatment bone marrow specimens in 530 patients with chronic phase Ph$^{+}$-CML to compare histological features with clinical findings and to calculate corresponding variables predicting survival.

**Results:** In about 26% of patients early (reticulin) to advanced (collagen) fibrosis was detectable. Significant correlations were calculated between reticulin stain-measured fiber density and the number of megakaryocytes including their precursors. There was an association of erythroid precursors with the number of mature (resident) macrophages including their activated subpopulation. Other relationships were observable between reduction in the amount of erythroid precursors or increase in fibers with clinical features like anemia, peripheral myelo- and erythroblasts or spleen size. Indicators of myeloid metaplasia like occurrence of erythroblasts and/or splenomegaly as well as anemia were the most important clinical parameters for prognosis. Inclusion of morphological features like degree of bone marrow fibrosis and erythroid precursors in risk classification resulted in a substantial improvement of prognostic efficiency compared to relevant clinical scores.

**Conclusions:** A number of clinical and morphological variables are in keeping with more advanced stages of CML which indicate a transition to myeloid metaplasia and thus exert a significant impact on survival.

## O-045

AgNOR PATTERN IS AN INDEPENDENT PROGNOSTC PARAMETER FOR THE EVOLUTION OF CHRONIC LYMPHOCYTIC LEUKEMIA

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**Aims:** to compare the AgNOR pattern of peripheral blood lymphocytes with other prognostic factors regarding the duration of the stable phase in chronic lymphocytic leukemia (CLL).

**Methods:** age, peripheral blood counts, total tumour mass score (TTM) and the AgNOR pattern (cells with one cluster, cells with compact nucleoli) in circulating lymphocytes were assessed in 57 newly diagnosed patients with CLL and compared with the period between diagnosis and the time where patients fullfilled the criteria for start of chemotherapy (stable phase).

**Results:** Cox regression showed that TTM and the percentage of lymphocytes containing one AgNOR cluster (circulating proliferative fraction) were the only independent factors predicting the duration of the stable phase. This model, which showed to be stable after the bootstrap resampling procedure permitted to create a prognostic score, summing up both parameters.

**Conclusions:** the study of the AgNOR pattern in circulating lymphocytes provides additional and independent information regarding the duration of the stable phase in CLL.

## O-046

HETEROGENEITY OF BONE MARROW LYMPHOID NODULES IN MYELOYSPLASTIC SYNDROMES

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**Aims:** lymphoid aggregates are found in bone marrow (BM) biopsies of up to 20% of the patients with myelodysplastic syndromes (MDS). Its significance is unknown. We characterize the composition of these aggregates using immunohistochemical techniques.

**Methods:** diagnostic BM biopsy sections of patients with MDS seen at our Institutions and showing lymphoid nodules in HE were immunostained for CD20, CD45RO, CD3 and bcl-2 using standard techniques. The distributions of the stained cells was analysed.

**Results:** In 14 cases studied, aggregates showed 3 patterns with CD20: diffuse strong staining (4 cases), perinodal positive cells (3 cases), and dispersed cells throughout the nodule (7 cases). In 2 patients, CD20 positive cells were found also dispersed outside the aggregates. CD45RO showed scattered positive cells in 6 cases, and CD3 had this pattern in only 3 cases. Cells showing expression of bcl-2 were seen in only one case, that also had a strong diffuse positivity for CD20. Although a small number of cases was analysed, no correlation was found between the pattern of the lymphoid aggregates and FAB type, or peripheral blood counts.

**Conclusions:** the composition of lymphoid aggregates in BM of MDS is heterogeneous and contains mostly B cells.

## O-047

**THE ROLE OF BCL-2 AND FAS/APO-1 EXPRESSION IN THE APOPTOTIC PATHWAY OF NON-HODGKIN'S LYMPHOMA**

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**Aims:** To evaluate the possible role of Bcl-2 and Fas/APO-1 in tumor progression of non-Hodgkin's lymphoma

**Methods:** A total of 90 cases from 80 patients with nodal and extranodal non-Hodgkin's lymphoma were selected from archival tissue samples. Immunohistochemical techniques were utilized against antibodies to Bcl-2 oncoprotein (DAKO clone 124) and Fas/APO-1 (Oncogene Research).

**Results:** Bcl-2 expression was observed in 82% of small cell lymphomas (SCL), 81% of large cell lymphomas (LCL), 72% of small/large cell lymphomas (SLCL), 80% immunoblastic (IB) and 100% of lymphoblastic lymphomas (LBL). Burkitt's lymphoma did not express Bcl-2. All of the follicular lymphomas expressed Bcl-2.

Fas/APO-1 was observed in 75% SCL, 56% LCL, 43% SLCL, 40% IB, 40% LB and 75% Burkitt's lymphomas. Fas/APO-1 was detected in 43% of diffuse lymphomas, 93% of follicular lymphomas and 89% of follicular/diffuse pattern lymphomas.

**Conclusion:** Our study indicates that Fas/APO-1 was expressed in 75% of low grade B cell lymphomas and to a lesser proportion in large cell lymphomas 43% and immunoblastic and lymphoblastic lymphomas 40%.

Epression of Bcl-2 was observed in a larger percentage of the small cell and large cell lymphomas showing expression in 82% and 81%, respectively and in the high grade lymphomas, such as immunoblastic 80% and lymphoblastic 100%.

The co-expression of Bcl-2 and Fas/APO-1 was observed in 100% of follicular lymphomas 42% of diffuse lymphomas and 77% of follicular/diffuse lymphomas. The loss of expression of Bcl-2 and the upregulation of Fas/APO-1 may suggest a more aggressive behavior in non-Hodgkin's lymphomas. The co-expression of these proteins may interact in the apoptotic process in a way to protect cell mediated programmed cell death.

## O-048

APOPTOSIS REGULATION IN LYMPHOMA CELLS

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**Aims**: To correlate the *bcl-2* family expression profile, the *p53* status and the Fas/Fas-L pathway with features of drug-induced apoptosis in Burkitt vs follicular lymphoma cell lines.

**Methods**: *bcl-2* family gene expression (*bcl-2*, *bax*, *bcl-XL* and *bcl-XS*, *bad* and *mcl-1*) was studied by northern and/or western blotting techniques. *p53* status was evaluated by immunocytochemistry and/or DNA sequencing. Fas and Fas-L expression was analysed by flow cytometry and western blot. Drug (hydroxiurea and etoposide) effects were confirmed by cell cycle analysis and apoptosis was addressed by TUNEL and western blot analysis of PARP fragmentation. Caspase activation was followed using an affinity-labelling method.

**Results**: In addition to the known low vs high *bcl-2* levels in, respectively, Burkitt vs follicular lymphoma cells, the different cell lines expressed similar levels of *bcl-XL*, *bax*, and *mcl-1*; both at mRNA and protein level, differences in *bcl-XS* were observed and no *bad* expression was detected. All cell lines were similarly sensitive to apoptosis under serum deprivation but behaved differently under drug treatment, concerning both level and time course of apoptosis; those differences did not correlate with the expression pattern of *bcl-2* family genes. Fas expression was very low in untreated cells and no significant changes occurred under drug treatment, either in *p53*- wt or mutated cells. No caspase 8 activation was detected but caspases 3 and 6 were activated by both drugs studied.

**Conclusions**:1) "physiologic" levels of Bcl-2 *per se*, or even an extended pro- vs anti-apoptotic ratio do not predict the actual threshold to apoptosis; 2) in contrast with other models, etoposide results in caspase 3-dependent apoptosis without a significant role of the recently described *p53*-mediated autocrine Fas/Fas-L pathway.

## O-049

INTRAVASCULAR LYMPHOMATOSIS IS A CLONAL LYMPHOMA THAT DOES NOT ASSOCIATE WITH EPSTEIN-BARR VIRUS IN IMMUNOCOMPETENT PATIENTS. MOLECULAR REARRANGEMENT AND IN SITU HYBRYDIZATION ON PARAFFIN EMBEDDED MATERIAL.

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**Aims**: Intravascular lymphomatosis (IL) is a very rare B non-Hodgkin lymphoma specially affecting brain and skin. Anatomoclinical characteristics remain to be elucidated. Association between IL and Epstein-Barr virus (EBV) infection has been described. There are no reports on clonality in a series of IL. The aim of the study was to test clonality and EBV status in an IL affected immunocompetent series.

**Methods**: Retrospective analysis with chart review of five patients was done. This included 4 complete autopsies and 2 diagnostic biopsies, one of which was a skeletal muscle biopsy and the other was a skin biopsy. Immunophenotypic characterization of tumoral cells and latent membrane protein (LMP-1) were carried out by immunohistochemistry. Immunoglobulin gene rearrangement by polymerase chain reaction (PCR) and fluorescein-labelled antisense EBV (EBER-RNA) by *in-situ* hybridization were performed from selected paraffin-embedded tissue.

**Results**: The mean age was 71 years (67-80). Vasculitis-like neurological disturbances, progressive dyspnea, recurrent inflammatory panniculitis and telangiectasia were the usual symptomatology. Two anatomoclinical forms, acute and chronic, were observed in these patients. All lymphomas were of B-cell type. Mono or oliclonality was observed in all cases. LMP-1 protein and EBV genome were not identified in any case.

**Conclusions**: Two anatomoclinical forms of IL, acute and chronic, were observed. IL seems to be a clonal neoplasia that is not associated with EBV in a series of non-immunocompromised patients.

## O-050

TGFβ SIGNALING IN HUMAN LYMPHOID CELLS

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**Aims:** TGFβ is usually an anti-proliferative regulator in lymphoid cells and this action is frequently lost after transformation. Aim of this study is to identify TGFβ activity in human malignant lymphoid cells.

**Methods:** lymphoma cells of human origin (e.g. HT58, Raji, BL41); immunocytochemistry; flow cytometry; RTPCR

**Results:** Response of the lymphoma cell lines to TGFβ was different: TGFβ induced cell cycle arrest in Raji cells, apoptosis in HT58 cells and both in BL41 cells. The percentage of apoptotic HT58 cells increased with time (about 60% at 72 hr), and combination with anti-μ had a stimulatory effect (although anti-μ itself caused no apoptosis). Neither TGFβ nor the combination with anti-μ induced p53 or FasR expression. Using Z-VAD it was shown that at least part of TGFβ activity is caspase-dependent.

**Conclusions:** Lymphoma cells respond differently to TGFβ. HT58 cells produce but not respond to endogenous TGFβ, but exogenous activated form induced apoptosis in a percentage of cells using a caspase directed pathway.

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## O-051

**GENETIC ALTERATIONS DISRUPTING THE NUCLEAR LOCALIZATION OF THE RETINOBLASTOMA RELATED GENE RB2/p130 IDENTIFY DIFFERENT PATHOGENETIC MECHANISMS IN AND AMONG BURKITT'S LYMPHOMAS**

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Burkitt's Lymphoma (BL) occurs as an Epstein-Barr virus (EBV) associated highly aggressive B-cell lymphoma with high incidence among children in the malaria belt of equatorial Africa (endemic BL,eBL) and sporadically in other geographical areas where it can also occur among adults (sporadic BL, sBL). In the REAL classification of lymphoid neoplasm, eBL and sBL are collectively reported as BL because of their isomorphism, although differences are acknowledged between endemic and sporadic forms in terms of EBV incidence, type of translocation involving the c-myc proto-oncogene and cell kinetic characteristics. Furthermore, in the past years with the spread of the AIDS epidemic, BL has frequently been reported as a common neoplasm occurring in HIV infected patients. In addition to the c-myc gene rearrangement, several other chromosomal translocations involving putative oncogenes occur in association with BL. p53 mutations have been found in 30-40 percent of BL samples, while the majority of these lymphomas with wild-type p53 might have lesions on other genes, e.g., of the growth suppressor type. Genetic alterations disrupting the nuclear localization of the retinoblastoma related gene *RB2/p130* have recently been documented in BL cell lines and primary tumors. Therefore, we have tested our BL cases, AIDS-related or not, for the expression and genomic organization of the *RB2/p130* gene. Given the importance of *RB2/p130* gene in controlling cell growth, mutations of this gene may result in different cell kinetic characteristics. Our results suggest that BL is composed of a mixture of molecular types with distinct genetic and phenotypic patterns, probably resulting in different pathogenetic mechanisms. It seems conceivable that different genetic lesions may be able to cooperate with c-myc deregulation in the lymphomagenesis of BL.

## O-052

**Cell cycle -regulatory proteins in malignant lymphomas. Additional prognostic markers?**

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The cell cycle is a highly organized and complex process , which ensures that there is a complete and accurate replication of the cell before division. There has been rapid progess in our understanding of the biochemical and molecular details of cell cycle control. Still, the benefits for clinical medicine have so far been moderate, perhaps disappointingly so, given the extensive progress in basic research. More recently antibodies have been produced against cell cycle related proteins which are present during specific phases of the cell cycle. Indeed, pathologists have an important role in bettering our understanding of these proteins and they may be in a position to utilize this information for clinical practice. In this study, we revaluated proliferative features of 98 lymphoma cases in view of our increasing knowledge of cell cycle control. The combined use of proliferation associated variables (mitotic index and percentages of Ki-67+, p34+, cyclinA+, and cyclinB+ cells) and their entry into a multivariate cluster analysis, separated the entire cohort into 3 groups with distinct proliferative characteristics without overlaps. Furthermore cyclin A+ and cyclin B+ cells could distinguish a subgroup of highly aggressive malignant lymphomas with a particular favourable clinical outcome after chemotherapy. Yet, low immunohistochemical levels of the retinoblastoma related pRb2(p130) protein, detected in 83 untreated patients with malignant lymphomas, shows an inverse correlation with a large fraction of cells expressing high levels of p107 and proliferation associated proteins. Down regulation of pRb2(p130) in our cases of malignant lymphomas also resulted in a better response to therapy.

## O-053

HIGH FREQUENCY OF A 30-bp DELETION IN THE EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN-1 GENE AND TYPE B EBV IN MEXICAN HODGKIN'S DISEASE AND REACTIVE LYMPHOID TISSUE

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**Aims:** Depending on geographic location and patient age Hodgkin's (HD) disease is associated with Epstein-Barr virus (EBV) in 20-100%. HD from developing countries shows a higher rate of EBV-positivity, but little is known about EBV subtype distribution and the prevalence of the 30 bp deletion of the LMP-1 gene which initially was reported to be associated with more aggressive disease.
**Methods:** We investigated the presence of EBV in a series of mexican HD (n=57) and reactive lymphoid tissues (n=20) with special emphasis on the prevalence of the 30-bp deletion and the type of EBV. EBER in situ hybridization, LMP-1 protein immunhistochemistry and polymerase chain reaction (PCR) specific for the LMP-1 deletion and the EBV subtype was applied for EBV analysis.
**Results:** EBV was detected in H-RS in 35/57 (61%) mexican HD. Surprisingly, the LMP-1 gene deletion was found in 28/35 (80%) EBV-positive HD and in 73% of the reactive lymph nodes. 10/26 HD cases were infected by type B EBV (38%) as compared to 10/19 (53%) in the reactive lymphoid tissue. All the 20 cases associated with type B, whether neoplastic or reactive, displayed the LMP-1 del gene configuration.
**Conclusion:** EBV-positive HD in Mexico shows a very high incidence of both the LMP-1 deletion as well as type B EBV as compared to reports from other regions. The similar frequency of these genotypes in reactive lesions, however, indicates that this reflects their prevalence in the Mexican population rather than a tumor-specific phenomen.

## O-054

NEW MONOCLONAL ANTIBODIES REACTIVE AGAINST HODGKIN'S CELL LINE KMH2.

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**Aims:** To develop and characterize new murine monoclonal antibodies against a Hodgkin's disease cell line KMH2. To determine whether these antibodies recognize antigens other than CD30.
**Methods:** Four new monoclonal antibodies of IgG type (R9.14, R23.1, R24.1 and R26.9) were developed against cloned KMH2 cells by standard hybridoma techniques. The purified antibodies were tested against Hodgkin's disease (HD) cell lines, anaplastic large cell lymphoma (ALCL) cell lines, several leukaemia and lymphoma lines and PHA-activated T cells.
**Results:** All 4 antibodies label Hodgkin's cell lines KMH2, L428 and anaplastic large cell lymphoma cell lines DEL and JB. R9.14 also labels U937 cells, which are CD30 negative. None of the antibodies label other CD30 negative cell lines. All four label Reed-Sternberg (RS) cells in frozen sections of Hodgkin's disease biopsies. Only R24.1 and R26.8 label RS cells in formalin-fixed tissue. None of the antibodies label PHA-activated T cells even during the peak CD30 expression of PHA-activated blasts.
**Conclusions:** The four new antibodies recognize cell surface antigens, which are probably distinct from CD30. Two of the antibodies recognize formalin-resistant epitopes. The antigens they recognize are not expressed by activated T cells, unlike CD30. Despite the distinction from CD30, the antigens recognized by the four antibodies show limited distribution in HD, ALCL and acute monocytic leukaemia cell line U937.

## O-055

**Pathological and clinical characteristics of 76 Hodgkin's disease (HD) patients misincluded in a lymphoma protocol ( LNH87)**

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The REAL classification defines clear criteria to distinguish NHL and HD and identifies a provisionnal entity placed on the border between the two diseases, the anaplastic large-cell lymphoma-hodgkin-like (ALCL-HL). However, the reality of the ALCL-HL entity is now debated. Between 1987 and 1993, 76 out of 2855 lymphomas included in the LNH87 protocol as NHL or ALCL-HL, had diagnosis rectified to HD after revisions and complete immunohistochemistry (applied on paraffin-embedded sections with CD30, CD15, CD20, CD3, CD45, CD43, TIA1, BNH9, LMP, EMA, ALK1 antibodies). The purpose of our study was (i) to analyze the various histological pitfalls encountered, and (ii) to follow the survival of these 76 patients affected with HD and treated initially with NHL regimens.

Among the 76 lymphomas, 46 were easily recognized as HD after one revision (Group A). The diagnoses of the 17 ALCL-HL (Group B) and the 13 T-cell NHL (Group C: AILD: 5, lymphoepithelioid: 4, others types: 4) were rectified to HD after a second revision and complete immunohistochemistry. None was ALK1 positive. These 76 HD were classsified according to Lukes classification and BNLI grading as follow :

| Group | A | B | C |
|---|---|---|---|
| Type 1 | 2 | 0 | 0 |
| Type 2, interfollicular | 6 | 0 | 4 |
| Type 2, grade I | 13 | 0 | 2 |
| Type 2, grade II | 15 | 15 | 1 |
| Type 3 | 5 | 0 | 5 |
| Type 4 | 0 | 0 | 0 |
| Unclassified | 5 | 2 | 1 |

The main clinical characteristics were : median age : 37 (range : 16-83), stage I : 7%, II : 49%, III : 9%, and IV : 35%, B symptoms : 52% and bulky mediastinum : 21%. According to EORTC index for stage I and II, 35% were favorable and 65% unfavorable. For advanced stages, the International Prognostic Score was ≤2 : 21%, 3 : 30%, 4 : 49% and ≥5 : 9%. Fifty-five percent of the patients completed the full NHL treatment according to the LNH87 protocol : the 5-years DFS was 53% and the 5-years OS was 76%.

Mains pitfalls focused on difficulties to differentiate HD (type 2 interfollicular and type 3) from T-cell NHL on one hand, and HD (type 2 grade 2) from ALCL-HL on the other hand. ALCL-HL subtype was overdiagnosed, because of unclear and subjective criteria.

## O-056

GRADING OF INVASIVE BREAST CANCER IN FROZEN TISSUE SAMPLES

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**Aims:** Due to recent developments in diagnostics of breast cancer tumors are today detected earlier and smaller in size. Therefore, there may not be enough material left for formalin-fixation after peroperative diagnosis on frozen sections. Frozen section diagnosis is generally considered very reliable but more detailed interpretation of frozen sections may not be sufficiently reliable and reproducible. We set out to evaluate the morphological changes caused by freezing during tissue processing in histological malignancy grading of invasive breast cancer.
**Methods:** We studied frozen and non-frozen formalin fixed paraffin embedded samples of 18 cases of invasive breast cancer. Features associated with breast cancer grading, i.e. nuclear area, mitotic activity, and tubular differentiation, were assessed by quantitative morphometrical methods.
**Results:** Frozen samples had consistently a smaller mean nuclear profile area (NPA) than non-frozen samples (mean difference 32.18 %). The frozen nuclei were also less symmetrical and uniform in shape than the non-frozen nuclei. Moreover, frozen samples had consistently higher mitotic indices than non-frozen samples (mean difference in standardized mitotic index (SMI) 65.74 %). Tubular differentiation as expressed in fraction of fields with tubular differentiation (FTD) increased by 16.04 % in association with freezing.
**Conclusions:** Feature values from frozen samples can be corrected to correspond to those of non-frozen samples by multiplying with the following factors: 1.475 (NPA), 0.603 (SMI), and 0.862 (FTD). Our results suggest that special caution should be taken when subjective or morphometric grading is practiced on frozen tissue samples.

## O-057

**DUCTAL CARCINOMA IN SITU (DCIS) - RESIDUAL TUMOR IN BREAST CONSERVING THERAPY**

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**Aims:** The complete excision of the DCIS as breast conserving therapy (BCT) should cure this non-invasive non-metastazing lesion definitely. Nevertheless local recurrences do appear. 92% of them result from residual DCIS in the breast. We examined the influence of a standardized pathological examination with regard to extension, status of the margins and grade of differentiation of DCIS on the choice of treatment.

**Methods:** 1096 women were operated because of unilateral breast carcinoma from January 1993 till December 1997 in the Berlin-Buch Medical Centre. 217 (19.7%) of the carcinomas were DCIS. Whereas 12 of these patients primarily were treated by mastectomy (ME), in the remaining 205 patients an excision was performed to attempt BCT.The specimens were examined pathologically according to the „Berlin-Buch practice protocol" developed in 1992. Based on the standardised sampling with consideration of the ductal orientation we estimated the diameter of the DCIS by combination of direct measuring and reconstruction.

**Results:** Free resection lines (RL) including margins of more than 10 mm were found in 60 of the 205 patients with an option of BCT. Because of involved margins in the remaining 145 cases re-operations were necessary. The following DCIS sizes were determined from the primary excisional specimens and re-excisional specimens (RE), respectively: A.) < 11 mm: 26 cases (21.7%) B. ) 11-25 mm: 32 cases (15.6%) C. ) 26-40 mm: 11 cases (5.4%); D. ) > 40 mm: 136 cases (66.3%). REs were necessary only in DCIS with diameters over 16 mm. In 9 cases of the groups B and C free RLs (including a 10 mm margin) could be reached by RE. In all 136 patients of the group D this could not be reached. Therefore ME was recommended to them. In the ME specimens of all these cases an involvement of at least one additional quadrant and/or the central gland has been proved. There was no significant statistical relationship between the grade of differentiation and the size of DCIS. Finally 136 (66,3%)women out of the primary BCT group of 205 patients were advised to undergo mastectomy. On the other hand 69 (33,7%) of the 217 patients with DCIS were treated by definitive BCT.

**Conclusions:** Involved margins do indicate residual DCIS in the breast and so they imply an indication for RE. The extension of a DCIS area over 40mm in diameter means a high risk of involvement of more than one quadrant of the breast and represents a contraindication for BCT. Prerequisite for such therapeutic decision making is the standardized examination of the excisional specimens by the pathologist as a member of the breast team.

## O-058

INTRADUCTAL CARCINOMA AND INVASIVE CARCINOMA WITH EXTENSIVE INTRADUCTAL COMPONENT OF THE BREAST.

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**Aims:** Nuclear pleomorphism is a fundamental feature for evaluating the aggressiveness grade of intraductal carcinoma (IDC) of the breast. In this study we performed a comparison between nuclei of pure IDC and infiltrating carcinoma with extensive in situ component (ISC) in order to understand if these are different entities or the same entity observed at different time of its evolution.

**Materials:** 10 cases of pure IDC and 10 of invasive carcinoma with ISC were selected. The case selection was independent of a grade preclassification. For each tumor we studied 30 nuclei in the first group and 30 nuclei of ISC and 30 nuclei of invasive component (IC) in the second group. A total of 900 nuclei was submitted to SAM (Shape Analytical Morphometry) procedure, wich allows to express numerically not only dimentions, (area, perimeter, diameter) but also nuclear contour irregularities and nuclear shape distorsions. Statistical comparisons were carried out between nuclei of: (1) IDC and ISC and (2) IC of infiltrating carcinoma, and (3) between ISC and IC of this last.

**Results:** Mean values of area, perimeter and diameter of nuclei were statistically higher ($p<0.001$) in pure IDC than in ISC and in IC of infiltrating carcinoma. Parameters related to nuclear contour irregularities and to shape asymmetry showed statistically higher values ($p<0.001$) in nuclei of infiltrating carcinoma, both in ISC and in IC. No significant differences between nuclei of ISC and IC of infiltrating carcinoma were observed. The same results were observed when the comparison was restricted to a subset of cases considered "poorly differentiated" according to Holland classification (4 cases among IDC and 5 cases among infiltrating carcinomas)

**Conclusion**: these results show the existence of morphologic differences between IDC and ISC of infiltrating carcinoma which can be expression of their different biological behaviour

## O-059

**HYALURONAN IN PERITUMOURAL STROMA AND MALIGNANT CELLS ASSOCIATES WITH BREAST CANCER SPREADING AND PREDICTS SURVIVAL**

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**Aims:** The purpose of the study was to evaluate the expression of hyaluronan (HA) in breast cancer specimens.

**Methods:** The localization and signal intensities of HA in peritumoural stroma and carcinoma cells were analyzed in 143 paraffin-embedded tumour samples of human breast carcinoma using a biotinylated affinity probe containing the HA binding domain of cartilage aggrecan and link protein.

**Results:** In the immediate peritumoural stroma, the HA signal was moderately or strongly increased in 39% and 56% of the cases, respectively. Normal ductal epithelium showed no HA, while in 57% of the tumours at least some of the carcinoma cells were HA positive. The intensity of stromal HA signal and the presence of cell-associated HA were both significantly correlated with poor differentiation of the tumours, axillary lymph node positivity and short overall survival of the patients. In Cox's multivariate analysis both the intensity of the stromal HA signal alone and that combined with the HA positivity in tumour cells were independent prognostic factors for overall survival. The latter achieved the same predictive power as tumour size, nodal status and age, and was clearly higher than other commonly used indicators e.g. the presence of estrogen and progesterone receptors.

**Conclusions:** The accumulation of HA in malignant cells and adjacent stroma was strongly associated with the invasive potential of tumours and poor outcome of patients, predicting that HA is directly involved in the spreading of breast cancer.

## O-060

**LOSS OF PTEN EXPRESSION IN DUCTAL CARCINOMAS OF THE BREAST**

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**Aims:** Germline mutations of the recently identified tumor suppressor gene PTEN (10q23.3) are found in Cowden syndrome which is associated with an increased risk for breast cancer. Loss of heterozygosity (LOH) of the PTEN region is frequent in sporadic endometrial cancer, glioblastoma and breast cancer. Unlike in endometrial carcinoma and glioblastoma, only a minute fraction of breast carcinomas have intragenic mutations in the re-maining PTEN allele. The aim of the study was to examine if PTEN expression is altered in sporadic breast carcinomas and if there is a correlation with LOH on 10q23.3 .

**Methods:** LOH analysis of polymorphic markers flanking and inside PTEN of 33 sporadic ductal breast carcinomas was performed. Intragenic mutations have been excluded by SSCP and direct sequencing. PTEN expression of these tumors was examined by immunohistochemistry.

**Results:** 11 tumors (33%) had complete or partial loss of one PTEN allele, 22 (66%) were heterozygous. Immunohistochemistry showed that 5 tumors had no PTEN protein detectable, 6 had a decreased immunoreactivity while 22 showed strong expression of PTEN. All the 5 tumors without detectable PTEN protein had LOH of one PTEN allele, 83% (5/6) tumors with reduced PTEN staining had LOH of PTEN while only 5% (1/22) tumors with strong PTEN expression had LOH of one allele.

**Conclusions:** Loss or reduction of expression of the tumor suppressor PTEN is a relatively frequent event (33%) in sporadic ductal breast cancer. LOH seems to be an important mechanism of PTEN-inactivation. However further mechanisms as hypermethylation of the remaining PTEN allele or it's promotor, decreased protein synthesis, increased protein turn-over or other must be responsible for the observed complete loss of PTEN expression.

## O-061

**Underexpression of p27 protein is associate with more aggressive phenotype in mammary carcinoma treated with adyuvant chemotherapy.**

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**AIMS**: P27 is a CDK inhibitor protein of cell cicle which loss of expression has been related with worst clinical outcome in breast carcinoma, specially in lymph node negative patients.
Our aim is to study this protein in a group of patients with high recurrence risk treated with chemotherapy before and after surgery.
**METHODS**: One hundred patients were evaluated. Thirty four patiens were treated with neoadyuvant quimiotherapy ( before surgery) and the rest with coadyuvant therapy (after surgery). We use a monoclonal antibody to p27 (Dako, clon SXx53g8) and n biotin- avidin complex immunohistochemistry technique. Immunohistochemical results were compared with classical clinical and mofphologic parameters and with overall and disease free survival (median follow-up: 72 months)
**RESULTS**: 23 tumors (23%) showed strong p27 expressión (100% of positive cells) and 26 (26%) moderate expression. In these patients, p27 expressión (strong and moderate) was correlated with RE positivity ($p<0.001$) . Althoug we observed a higher expression in low histologic grade ($p=0.6$) and smaller tumor there was no statistical significance. Only strong expression of p27 was related with better overall survival.
**CONCLUSIONS**: Underexpression of p27 protein was observed in more aggressive phenotype of chemotheray treated breast carcinoma and may have potencial use as a new predictive factor in the management of breast cancer.

## O-062

EXPRESSION OF CYCLIN DEPENDENT KINASE INHIBITOR PROTEINS $P27^{KIP}$ AND $P21^{WAF}$ IN LOCALIZED INVASIVE DUCTAL CARCINOMA OF THE BREAST

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Introduction: The KIP family of cyclin-dependent kinase inhibitors (CKIs) includes $P27^{KIP}$ and $P21^{WAF}$. These proteins are negative cell cycle regulators by inactivating the complexes of cyclins-cyclin dependent kinases 2, 4 and 6, which function in the G1 and S phases of the cell cycle. We investigated the concomitant patterns of expression of $P27^{KIP}$ and $P21^{WAF}$ in a homogeneous series of localized invasive ductal breast carcinoma and adjacent normal breast tissues and correlated these findings with the standard clinicopathologic parameters and outcome..
Methods: Seventy T1 and T2, N0, M0 invasive ductal breast carcinomas were reviewed. Normal and neoplastic formalin-fixed, paraffin-embedded tissue from each case was studied by immunohistochemistry, using antibodies to $p27^{KIP}$ and $p21^{WAF}$; the results were estimated as positive if +/- of 50% or 5% of tumor nuclei showed immunoreactivity for $P27^{KIP}$ and $P21^{WAF}$, respectively. Statistical analysis investigated the difference in $p21^{WAF}$ and $p27^{KIP}$ expression between benign and neoplastic tissue and their correlation with tumor size, grade and outcome.
Results: $p27^{KIP}$ expression in tumor and normal tissue was 44% and 54% respectively. The difference was statistically significant ($p=0.02$). $p21^{WAF}$ was expressed in 69% of tumors but was lacking from the benign epithelial tissues. The two proteins were simultaneously expressed in 30% of the tumors. Although loss of $p27^{KIP}$ expression was significantly associated with higher tumor grade ($p=0.002$), there was a trend for longer disease free survival for tumors lacking $p27^{KIP}$ ($p=0.054$). $p21^{WAF}$ expression was not associated significantly with tumor size, grade or outcome or with $p27^{KIP}$ expression.
Conclusions: Although invasive ductal carcinomas tend to lose $p27^{KIP}$ and acquire $p21^{WAF}$ expression compared to benign breast epithelium, no significant association between the expression of these proteins is identified in the neoplastic tissues. In localized, early stage ductal breast carcinomas, loss of $p27^{KIP}$ does not appear to adversely affect prognosis, unlike previous reports of series including neoplasms of all stages. Loss of $p27^{KIP}$ is correlated with higher tumor grade in early stage breast carcinomas. $p21^{WAF}$ expression is not associated with standard pathologic parameters and outcome in this tumor stage.

## O-063

**EXPRESSION PATTERN OF AP-1 (ACTIVATING PROTEIN-1) PROTEINS IN BREAST CANCER: CORRELATION OF FOSB AND FRA-1 EXPRESSION WITH HORMONE RECPTOR STATUS AND DIFFERENTIATION**

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**Aims**: In the present study, we investigated the expression of members of the AP-1 family of transcription factors (c-Jun, JunB, JunD and c-Fos, FosB, Fra1 and Fra2) in breast tumors (n=53).
**Methods**: Western blot analysis using antibodies specific for each of the AP-1 family members was employed. The tumors were also characterized with regard to grading, staging, histology, steroid receptor expression status and c-ErbB2/neu expression. For comparison, normal breast tissue samples and human breast cancer cell lines (T47D and MDA-MB231) and the transformed human breast epithelial cell line HBL100 have also been analyzed.
**Results:** For c-Jun, JunB, c-Fos and Fra2, a relatively uniform expression pattern without significant differences between the tumors was observed. JunD protein amounts varied strongly in the tumor specimens. FosB expression levels also varied strongly in the tumors showing weak/absent expression in 47% of the tumors, while 45% exhibited strong/very strong levels of expression. While none of the other AP-1 family members showed significant correlations with clinico-pathological tumor parameters and receptor status, expression of FosB was found to significantly correlate with positive steroid hormone receptor status (in the tumors and the cell lines) and a more differentiated tumor phenotype. Expression of two Fra-1-specific bands of molecular weight 33 and 36.5 kDa showed a significant negative correlation with FosB expression, as well as with estrogen receptor status and differentiation.
**Conclusions:** We conclude that strong differences in the expression pattern of AP-1 family members are present in breast tumors and certain members of this family, such as FosB and Fra-1, might be involved in the pathogenesis of these tumors.

## O-064

**SELECTION OF LYMPH NODE NEGATIVE UNFAVOURABLE PREMENOPAUSAL BREAST CANCER PATIENTS FOR ADJUVANT SYSTEMIC THERAPY CAN BE DONE BEST BY THE MITOTIC ACTIVITY INDEX (MAI)**

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Breast cancer is an increasingly important health care problem. Prognosis can be improved by means of adjuvant therapy but the over-aggressive NCI advice to treat all lymph node (=LN) negative patients with adjuvant chemotherapy (ACT) is not well accepted. Accurate prognostic factors should be used to identify LN-negative, yet high risk breast cancer patients for systemic adjuvant treatment.
Since 1975, a large number of potential prognosticators has been analysed, like oestrogen and progesterone receptor (OR, PR), DNA ploidy, p53, neu, p21, apoptosis, neovascularisation, Rb, PAI-1, cathepsin-D and many others, next to classical factors such as tumor diameter and grade.
It is important to note, however, that Good Laboratory Practice (GLP) sets certain conditions to a prognostic laboratory test, before it can be used routinely in clinical practice. Only very few new prognostic factors that ever have been proposed, fulfil all these criteria.
It has been shown by many investigators that features associated with proliferation are strong independent prognostic factors, also in LN-negative patients. This is especially true for the Mitotic Activity Index (MAI). Interobserver multicenter prospective reproducibility of the MAI in the national prospective Dutch Multicenter Morphometric Mammary Carcinoma Project (MMMCP) is very high (Kappa >0.90 for all centers), contrasting the low reproducibility of grade (Kappa <0.50). In the MMMCP, nearly 3,500 patients have been enrolled in 1988 and 1989 and of these, 600 patients were LN-negative and premenopausal. The 1998 interim 8 year survival of LN-negative MAI ≥10 patients is the same as patients with 1 or 2 positive lymph nodes (64%, contrasting 97% in LN-negative MAI <10 patients. Multiple regression also showed that the MAI is the strongest prognostic factor in the LN-negative patients, also in relevant subgroups. Thus, MAI <10 versus ≥10 "explains" all the prognostic information contained in other prognostic features (like tumour diameter, grade, type, OR).
Calculations learn that the expected gain in expected lives saved as a result of ACT, is highest if patients are selected on the basis of MAI ≥10. It therefore is best to select patients for adjuvant systemic therapy on the basis of MAI ≥10.

## O-065

SPORADIC AND FAMILIAL BREAST CANCER RELATED TO BRCA1. HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY.

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Aims: To examine the pathological differences between sporadic and familial breast cancer with and without known BRCA1 germline mutations previously studied by Protein Truncation Test (PTT), Conformational Sensitive Gel Electrophoresis (CSGE) and direct sequencing of genomic DNA.

Methods: 22 breast tumors (10 sporadic, 12 familial) with known BRCA1 gene status were studied. Histological type, grading and TNM were known in all cases. Standard immunohistochemical study on paraffin embedded tumoral samples was performed, using the following primary antibodies: Estrogen (ER), Progesterone (PR), P53, Cathepsin D, bcl-2 and c-erbB-2 (from DAKO, Denmark).

Results: Invasive ductal carcinoma was the most common type both in familial and non-familial cases. Four of the 7 familial cases with BRCA1 germline mutations were of the medullary type. High mitotic counts, great nuclear pleomorphism and low tubular differentiation were more frequent in familial cases with known BRCA1 germline mutations.

Immunohistochemical profile of familial cases was ER-, PR- and low bcl-2 staining. p53 and c-erbB-2 were positive in high grade sporadic and familial tumors.

Conclusions : Familial tumors with BRCA1 germline mutations are high-grade tumors and do not express positivity with ER, PR and bcl-2 antibodies.

## O-066

**IMMUNOHISTOCHEMICAL INDICATORS OF PROGNOSIS IN EARLY (T1N0M0) BREAST CARCINOMA**

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**Aims:** This study was undertaken to evaluate the prognostic utility of various immunohistochemical markers in early (T1N0M0) breast carcinoma and their relative value with regard to traditional prognostic factors (tumor size and grade).

**Methods:** The immunohistochemical (IHC) expression of carcinoembrionic antigen (CEA), c-erbB2, p53, bcl-2, estrogen (ER) and progesterone (PR) receptors and MIB-1 counts were assessed on paraffin-embedded tissue from 278 early breast carcinomas using commercially available antibodies. The results were correlated with cancer specific (CSS) and metastasis-free (MFS) survival in uni- and multivariate analysis. The median follow-up was 12 years.

**Results:** Whereas tumor size and patient age were not related to prognosis, histologic grade (assessed according to Nottingham scheme) showed a strong association with DFS (P=0.02) and CSS (P=0.0002). Among the IHC markers, p53, bcl-2, ER and PR were not related to prognosis. Patients with tumors having ≤10% MIB-1+ cells had significantly shorter MFS (P=0.005) and CSS (P=0.02). Expression of CEA and c-erbB-2 was associated with shorter MFS (P=0.005 and 0.009, respectively) but not with CSS. In multivariate analysis, MIB-1, CEA and c-erbB-2 were independent prognostic factors for MFS, whereas histologic grade and CEA were the only factors influencing CSS.

**Conclusions:** Our results suggest that in T1N0M0 breast carcinomas, IHC assessment of CEA and c-erbB-2 expression and MIB-1 counts provides important prognostic information in addition to that obtained by determination of histologic grade.

## O-067

BREAST CARCINOMA IN 108 YOUNG WOMEN. A NEW IMMUNOHISTOCHEMICAL PROGNOSTIC INDEX (IHPI) RELATED TO THE TUMOR SIZE.

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**Aims:** Breast Ductal Invasive Carcinoma in young women is classically related to a poor prognosis. Tumor size, histological grade and lymph node stage and four immunohistochemical factors (c-erbB-2, p53 and estrogen and progesterone receptors) are studied related to the survival and to the disease free relapse.

**Methods:** 108 cases of breast ductal carcinoma in women 35 years old or less, were studied, followed from one to 18 years. The immunohistochemical markers were: c-erbB-2 oncogene (positive: score 1, negative: 0); p-53 suppresor gene (positive: score 1; negative: 0); ER ( positive: score 0; negative: 1), and PR ( positive: score 0; negative: 1). To obtain the Immunohistochemical Prognostic Index (IHPI), the scores for each factor are added together, giving a possible total of 0-4 points. Three prognostic grades are allocated: 0-1 points: grade 1- good prognosis (n=32 cases); 2 points: grade 2- moderate prognosis (n=23 cases) and 3-4 points: grade 3-poor prognosis (n=53).

**Results:** The Kaplan-Meier survival analysis has demonstrated its prognostic usefulness for the overall survival and for the disease free survival, with a highly significant differences between the three groups of IHPI (log-rank: p<0.0001 in both). In the study of the influence of the IHPI in each group of tumor size(≤2 cm, >2 y ≤5cm and >5cm), it was observed that the IHPI vas very useful in the survival prediction of the tumors ≤ 5 cm (p=0.0001), but not in the >5cm. The best multivariate model in that series is the combination of tumor size (T) and IHPH. We established the following categories: **1**: T≤2cm and good-moderate IHPI; **2**: T 2-≤5cm and good-moderate IHPI; **3**: T≤5cm and poor IHPI and **4**: T>5cm with any IHPI. The four categories present high significant differences in the overall survival and in the disease free relapse(log-rank p<0.0001).

**Conclusions:** The study of the Immunohistochemical Prognostic Index in young patients with breast Ductal Invasive Carcinoma has a high prognostic value. It is very useful in tumors sized ≤ 5 cm and especially in those ≤ 2cm . In tumors > 5 cm, the IHPI has no prognostic influence, being the size itself the best prognostic value.

## O-068

PREDICTORS OF AXILLARY NODE STATUS IN T1 BREAST CANCER: A MULTIVARIATE ANALYSIS OF 1075 PATIENTS.

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**Aim:** The value of axillary dissection in the management of T1 breast cancer is controversial. To evaluate which of the routinely used morphological, clinical and immunophenotypical parameter can be useful to select patients with high risk of node metastases we studied 1075 invasive breast carcinomas smaller than 2 cm (pT1) with known lymph node status.

**Method:** The parameters considered were tumor size, grade, vascular invasion (VI), multicentricity, patient's age, hormone receptor status, immunocytochemical expression of p53 and of c-erbB2 and angiogenesis (CD31 positive vascular spaces).

**Results:** 374 patients had positive lymph nodes (N+) (34.8%). For the entire population, univariate analysis showed that N+ was significantly associated with VI (RR= 4.1 (3.1-5.5)), grade 2-3 (RR= 2.2 (1.6-3.0)), multicentricity (RR= 2.3 (1.4-3.8)), angiogenesis >100 microvessels/sqmm (RR= 1.4 (1.1-1.9)), age under 50 years (RR= 1.5 (1.1-2.0)). In multivariate analysis the parameters independently correlated with nodal status were: VI (p<0.0001), multicentricity (p<0.0001) and age under 50 years (p<0.0001). Under 50 years of age 40.4% of patients were N+ whereas from 50 to 70 years 30.8% of patients were N+. In both group, tumor size, VI and multicentricity were the most important paramenters correlated with N+.

**Conclusions:** These data showed that tumor size measured on histological section, vascular invasion morphologically searched on standard haematoxylin eosin stained slides and um or multicentricity are the best predictors of lymph node metastases. Age is an important independent factor and suggests that in patients over 50 year old with unicentric small tumors without VI, the node status is likely to be negative.

## O-069

EXPRESSION OF aFGF AND FGFR-4 IN BREAST FIBROADENOMAS

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**Aim:** Fibroadenomas (Fas) are breast lesions, more commonly found in young females. They are classified as a benign neoplasm but a hyperplastic state or an abnormal lobule development has been suggested because of hormonal dependency, regression in older age and the observation of polyclonality in their epithelial and stromal component. aFGF is a well known fibroblast activator, acting through FGFR-4. The aim of this study was to compare the aFGF-protein expression with the presence of aFGF-mRNA and FGFR-4 in order to understand their role in the growth of Fas.

**Methods:** Using immunohistochemistry (IHC) and in situ hybridization (ISH), formalin fixed and paraffin embedded tissues from 18 breast fibroadenomas have been investigated for FGFR-4, aFGF-proteins and aFGF-mRNA detection. For IHC the ABC-method was employed using specific anti-aFGF antibodies (UBI, Lake Placid). For aFGF-mRNA detection by ISH aspecific antisense oligonucleotide biotin-labeled probe (Biognostik) was used and revealed by tyramide amplification method (Gen Point Dako).

**Results:** Immunoreactivity (IR) for aFGF was observed in the epithelial component of 18 Fas. IR for FGFR-4 was observed in epithelial and stromal component of all cases under study. aFGF-mRNA by ISH was found in epithelial component of all 5 cases investigated. Myoepithelial cells found in Fas were negative for aFGF, FGFR-4 and for aFGF-mRNA.

**Conclusions**: The expression of the aFGF in epithelial cells and FGFR-4 in both epithelial and stromal cells suggests a paracrine/autocrine function of these two proteins in the growth of breast Fas. aFGF-mRNA detection by ISH only in epithelial cells confirms that aFGF is synthetised by epithelial component of breast Fas.

## O-070

HISTOSTEREOMETRY AND ULTRA LOW ANGLE X-RAY SCATTERING: NEW NONINVASIVE MORPHOLOGICAL DIAGNOSTIC METHOD OF BREAST CANCER.

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Ultra Low Angle X-Ray Scattering (ULAX) was successfully applied to structural molecular analysis of mucus, muscle and cornea in experiments. Histostereometry in complex with ULAX provides the opportunity to in vivo reveal pathological structural changes in the organs.

Aim of present study is comparative pathomorphological and ULAX structural analysis of breast benign and malignant diseases.

**Methods:** 1018 samples (8x2x2 mm fragments) of breast tissue were obtained during the surgical treatment from 509 patients. Samples were studied by ULAX (X-ray diffractometer), histologically (standard technique), and histostereometrically. Statistical methods were used to correlate the scattering and tissue data.

**Results:** The scattering data of epithelium, connective and adipose tissue of the samples with "normal" mammary gland tissue were different from the samples with benign and malignant breast diseases, for example range of periods of molecular structure for adipose tissue for "normal" tissue, fibrocystic disease and fibroadenomas was 46-83 nm, and 241-275 nm for cancer. Mean value of integral scattering intensity decreases in the series: fibrocystic disease, fibroadenoma, noninvasive carcinomas, invasive lobular carcinoma, and invasive ductal carcinoma. Scattering pattern recognition code was produced with diagnostic sensitivity better than 92% - close to biopsy method.

**Conclusions:** Present data led us to believe that different tissue components have distinct diffraction patterns. Each component's diffraction pattern changes differently under pathological process. The study suggests that ULAX is promising noninvasive morphological (structural) diagnostic method for breast cancer.

## O-071

**CHARACTERIZATION OF PLACENTAL INFLAMMATORY INFILTRATES IN MALARIAL INFECTION.**

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**Background:** A bias towards Th2 responses with suppression of cell-mediated responses induced by Th1 type cytokines during pregnancy has been proposed as the cause of the high susceptibility to several infections, such as malaria, observed in pregnant women, specially in primigravidas. Morphological studies of malarial placentas have demonstrated an increased inflammatory infiltrate in the maternal space but it has not been immunologically characterized.

**Aim:** To characterize the inflammatory infiltrate in placental malaria.

**Material and Methods:** Monoclonal antibodies against T-cells (CD3), T-cytotoxic cells (CD8, TIA-1), B-cells (CD20), granulocytes (G) (CD15), monocyte/macrophages (M/M) (CD68) and natural killer (NK) cells (CD56) were evaluated in 41 placentas from Tanzania (32 infected placentas, 4 acute, 18 chronic, 10 past, and 9 non-infected). 8 normal placentas from a non-endemic area (Barcelona, Spain) were also studied.

**Results:** In control placentas mean total inflammatory cells per $mm^2$ in the intervillous space was 42.62±16.37 (12% T-cells with 95% T8, 9% B-cells, 67% G, 12% M/M, 0% NK cells). All placentas from Tanzania showed a mild increase in inflammatory cells (60.67±10.74, with 16% T-cells, 18% B-cells, 52% G, 12% M/M in the non-infected group). A subgroup of placentas with chronic malarial infection showed a marked inflammatory infiltrate (401±169.7/$mm^2$) with a main component of M/M (45%), and T-cells (27%, with 98% cytotoxic lymphocytes), and absence of NK-cells. Intravillous inflammation was absent, and no differences were detected in any group. A significant reduction in birthweight (-454 gr., $p<0.001$) was observed in cases with marked inflammation in the intervillous space.

**Conclusion:** A marked inflammation mainly involving a Th1 response is detected in a significant proportion of placental chronic malarial infections. This response is associated with poor pregnancy outcomes.

## O-072

Withdrawn

## O-073

**Association between the HLA DQB1*0301 gene and Human Papillomavirus Infection in High-Grade Cervical Intraepithelial Neoplasia**

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**Aim**. The evidence for a link between HLA specificities and Human Papilloma virus (HPV) associated disease of cervix uteri is controversial. We report the distribution of DQB1 genes in Norwegian women treated for high-grade cervical intraepithelial neoplasia (HG CIN). These women have also been followed for recurrent disease.

**Methods**. Formalin-fixed, paraffin-embedded tissue sections from 170 biopsy specimens with diagnoses of CIN II (n=54) or CIN III(n=116), were DQB1 typed using allele specific PCR. The follow-up period for cases was 13-15 years. Papanicolaou smear was the only screening method used to identify women with residual and recurrent disease, even though the resection margins were not free. The control material comprised blood samples and endocervical brushes from 213 women without CIN. Both cases and controls had previously been HPV typed.

**Results**. We found an overrepresentation of the DQB1*0301 allele among cases compared with controls (OR=1.8). Presence of CIN was related to HPV infection, and HPV16 positivity was significantly associated with the presence of DQB1*0301(OR 1.8). The DQB1*0301 allele was significantly more prevalent in CIN III than in CIN II cases. The resection margins of the cone specimen were involved in 23% of the cases. However, only two cases of recurrent disease were diagnosed in the follow-up period. Both cases revealed CIN II by histology. One of these women was carrying the DQB1*0301 allele.

**Conclusions**. Our data indicate that women carrying the HLA-DQB1*0301 allele have an increased risk of developing CIN when infected by HPV16. However, in the 13-15 years follow-up period we have not registered any increased frequency of recurrent disease among women carrying this allele.

## O-074

**Correlation of Human Papillomavirus 16 and 18 with cervical neoplasia in histological typing and clinical stage: An In-Situ PCR Approach**

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**Aims:** *In situ* polymerase chain reaction (*In situ* PCR) is a new technique which promises to considerably enhance our ability to detect a few copies of target nucleic acid sequences in fixed tissues and cells. DNA-based analyses have consistently revealed a high prevalence of human papillomavirus (HPV) 16 and 18 DNA sequences in cervical carcinoma.

**Material and Method:** This study examined prevalence of HPV 16, and 18 DNA in biopsies from 63 cervical patients by *In Situ*-PCR, employing HPV 16, 18 consensus primers. There are forty-five patients with squamous cell carcinomas, thirteen with adenocarcinoma, two with adenosquamous carcinomaws and three with small cell carcinomas. The relation between the types of HPV detected, tumor type and clinical stage were analyzed.

**Result:** Fifty-two biopsies (82.5%) were HPV 16 or 18 -positive, HPV 16 being the most prevalent type. Squamous cell carcinoma had a high prevalence of HPV 16 (69.2%) and adenocarcinoma had a high prevalence of with HPV 18 (46.2%). HPV 18 was the predominant type among high clinical stage (III+IV) cases while HPV 16 and mixed HPV 16 with HPV18 were significantly correlated with low clinical stage (0+I+II).

**Conclusion:** Our results indicate that certain malignant cervical tumor phenotypes and stages correlate with specific HPV type, and that In situ PCR is a simple and fast method to detect HPV in these patients.

## O-075

**IMMUNOHISTOCHEMICAL DETECTION OF MATRIX METALLOPROTEINASES 1 AND 2, AND TISSUE INHIBITOR OF METALLOPROTEINASE 2 (TIMP-2) IN STAGE IB CERVICAL CANCER.**

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**Aims**: Matrix metalloproteinases (MMPs) are a family of zinc-dependent metalloendopeptidases which participate in the degradation of collagen and other extracellular matrix macromolecules. Expression of gelatonic MMPs, such as MMP-2 has been linked to enhanced tumor invasion and metastases in vitro and in vivo model systems. It was the aim of this study to determine whether the expression of MMP-1, MMP-2, and TIMP-2 correlates with survival in patients with surgically treated cervical cancer stage IB.

**Methods**: A sample of 154 paraffin-embedded tumor specimens of surgical treated FIGO stage IB cervical cancer was immunohistochemically investigated.

**Results**: MMP-1, MMP-2, and TIMP-2 were detected by immunohistochemistry in 74 % (113/154), 32 % (49/154), and 80 % (107/154) of the tumor samples, respectively. Correlation coefficients for MMP-1/MMP-2, MMP-1/TIMP-2, MMP-2/TIMP-2 were 0.14 (p=0.12), 0.37 (p=0.0001), and 0.17 (p:0.005), respectively. A significant correlation was found between MMP-1 and lymph node status (P<0.01) and lymphvascular space invasion (P<0.05). The expression of MMP-1 (log-rank test, p=0.6), MMP-2 (log-rank test, p=0.8), and TIMP-2 (log-rank test, p=0.15) were not correlated with overall survival.

**Conclusions**: MMP-1, detected by immunohistochemistry, seems to play a role in the development of lymphvascular space invasion and lymph node metastases, but is not helpful in predicting the prognosis of cervical cancer patients.

## O-076

**PROGNOSTIC AND THERAPEUTIC IMPACT OF QUANTITATIVE AND MOLECULAR PATHOLOGY IN ENDOMETRIAL HYPERPLASIA**

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Endometrial hyperplasia (EH) is a frequently occurring disease in the EC, with enormous economic impact for the health care system. The understanding of the development of EH and prediction of progression-or-not to cancer is hampered by lack of reproducibility of histologic classifications. As a result, considerable overtreatment occurs.

Over the past 20 years, we have developed and fine-tuned a computerized morphometric analysis (CMA) technique (the D-score), that consists of a measurement tool, and an expert system based tool to predict the outcome of endometrial hyperplasia cases. DNA ploidy assessed by FCM was of no value. With the multivariate D-score, specificity is equal to, or better than with the usual WHO classification, but the sensitivity of the D-score is much better[1,2]. This was confirmed in "the Philadelphia study", an independent blind multicenter collaboration[3] on 45 cases (sensitivity 100%, specificity 88.5%). In a second prospective routine clinical multicenter study, started in 1985, 96 cases with long follow-up have been evaluated, 11 of which progressed to cancer (11.5%). Again, sensitivity of the D-score is 100%, and specitivity 82%. Thirdly, it is estimated that routine nation-wide application of CMA in all EH cases in the Netherlands will save approximately 15 million U$ per year, due to prevention of unnecessary hysterectomies.

Fourthly, we have tested the ability of CMA to diagnose monoclonal putative endometrial precancers. 93 non-malignant areas of endometrium from 64 uteri of women with coexisting endometrial adenocarcinoma were scored as monoclonal (n=39) or polyclonal (n=54) by PCR analysis (monoclonal by either non-random X chromosome inactivation or clonal outgrowth of altered microsatellites). Lesions were blindly evaluated by 4 independent experienced gynaecopathologists, but their reproducibility was not optimal. In contrast, the D-scores were highly reproducible (inter-observer r>0.98), predicting monoclonal lesions with 95% sensitivity and 75% specificity, and polyclonal areas with 95% specificity and 77% sensitivity. Computerized image analysis is thus capable of recognizing monoclonal endometrial precancers with specificity and sensitivity at least comparable to (and usually higher than) that of experienced pathologists, with the additional advantage of excellent reproducibility.

References:
1 J Pathol. 154: 335-341, 1988.
2 Path Res Pract. 1992; 188: 856-859.
3 Am J Obstet Gynecol. 1996; 174: 1518-1521.

## O-077

MOLECULAR PROFILE OF THE HUMAN CHROMOSOME 10q25-q26 IN PATIENTS WITH ENDOMETRIAL CANCER

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**Aims**: Preliminary studies at molecular level seem to identify two regions of the human chromosome 10 involved in tumorigenesis of endometrial carcinoma (EC): one at 10q21-q24 and one at 10q25.2-q26.3. The purpose of this study was to evaluate microsatellite instability (MIN) and loss of heterozygosity (LOH) of the q25-q26 region and establish correlations with clinico-pathologic parameters.

**Methods**: Genomic DNA was extracted from 93 different EC tissue and normal pairs. All specimens were formalin-fixed and paraffin-embedded. By means of PCR analyses MIN and LOH was assessed at the following marker loci from the 10q25-q26 region (covering about 14 cM): D10S187, D10S221, D10S190, D10S1230, D10S1213, and D10S1656. All cases were reviewed histologically for confirmation of diagnosis, assignment of tumour grade and pathologic stage; disease stage was recorded according to FIGO classification.

**Results:** EC patients presented a median age of 61.5 (range 35 to 88); most of them (69/93, 74%) were at stage I. Twenty-eight (30.2%) cases were G1, 58 (62.3%) G2, and only 7 (7.5%) G3. Fifty-three (56%) tumour tissues presented MIN for at least one locus, whereas 29 (31%) for two or more loci. LOH for at least one marker was observed in 41 (44%) cases. The region encompassing D10S221 through D10S1213 has been demonstrated highly affected in EC patients. MIN was more frequent in patient with advanced disease (stage III and IV) and higher histopathologic grade (G2-G3). There was no correlation between LOH and progression of stage and grade.

**Conclusions:** Our results confirm the involvement of 10q25-26 in the molecular pathogenesis of EC and provides support for the existence of putative tumour suppressor gene(s) on chromosome 10q25-q26.

## O-078

PATTERNS OF THYMIDINE PHOSPHORYLASE EXPRESSION IN ENDOMETRIAL CARCINOMAS

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**Aims:** To investigate the role of thymidine phosphorylase (TP), a potent angiogenic factor, in endometrial cancer.

**Methods:** Paraffin sections from 122 cases of endometrial carcinoma was examined immunohistochemically for TP expression. Tumour cells were separately assessed for nuclear and cytoplasmic reactivity, while assessment of TP expression in stromal fibroblasts was simply nuclear. In all cases, a positive reaction in >50% of the component tumour or stromal cells was considered as being "high". The results were correlated with known histopathological parameters.

**Results:** Tumour cell nuclear and cytoplasmic reactivity was high in 12/122 and 39/122 endometrial carcinomas, respectively. Fibroblastic TP reactivity was high in 39/122 cases. A high nuclear TP reactivity in tumour cells was significantly associated with a similar reactivity in stromal cells (p=0.05). High nuclear TP reactivity of tumour cells was frequently seen in advanced FIGO stage, but this association did not reach a level of statistical significance (p=0.10). By contrast, a high fibroblastic reactivity was significantly associated with high histological grade (p=0.03) and with the non-endometrioid carcinomas (p=0.01). No association of any pattern of TP expression with the depth of myometrial or vascular invasion was noted. Similarly, the cytoplasmic reactivity of tumour cells was of no importance.

**Conclusions:** These preliminary results seem to indicate that TP expression is an adverse prognostic factor in endometrial carcer.

## O-079

STRONG PROGNOSTIC INFLUENCE OF REDUCED P16 PROTEIN EXPRESSION IN ENDOMETRIAL CARCINOMAS - A LARGE POPULATION BASED STUDY

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Aims: The CDKN2 ($p16^{INK4a}$) tumour suppressor gene is inactivated in several tumour types, but its prognostic significance in endometrial carcinoma is largely unknown. The purpose of this study was to assess the pattern and prognostic impact of p16 protein expression in a population based series of 286 endometrial carcinomas.

Methods: Clinico-pathological characteristics and follow-up data (median 9 years, range 4-15 years) from all patients diagnosed with endometrial carcinoma during 1981-1990 from Hordaland County, Norway, have been studied. Nuclear staining of p16 protein was also related to DNA ploidy, S-phase fraction, estradiol and progesterone receptor concentrations, microvessel density, Ki-67, p53 and p21 expression and patient survival.

Results: Absent/minimal nuclear p16 protein expression was associated with high patient age (p=0.02), high FIGO stage (p=0.01), serous papillary and clear cell histological types (p<0.001), high histological grade (p<0.001), aneuploidy (p=0.001), low estradiol and progesterone receptor concentrations (p≤0.001), high expression of Ki-67 (p<0.001), high microvessel density (p=0.03) and strong nuclear p53 protein expression (p<0.001). 5-year survival was 47% for patients with absent/minimal p16 expression (n=39) compared with 81% for patients with moderate/high expression of p16 (n=247) (p<0.0001). In Cox regression analysis, p16 expression showed an independent prognostic impact in addition to FIGO stage, patient age, Ki-67 expression and microvessel density, with an adjusted Hazard Ratio of 3.1 (95% CI 1.4-7.1). The other variables lost their prognostic impact when p16 expression was added to the Cox model.

Conclusions: Absent or minimal p16 protein expression was associated with aggressive tumours and high proliferative activity (Ki-67), and was found to represent a strong and independent prognostic indicator.

## O-080

MYXOID LEIOMYOSARCOMA: CORRELATION BETWEEN PROGNOSTIC MARKERS AND GENETIC CHANGES

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**Aims:** Myxoid leiomyosarcoma is an unusual but highly aggressive variant of smooth muscle neoplasms, whose diagnosis may be difficult due to its morphologic and phenotypic characteristics. We investigated the role of prognostic factors (MiB1, p53) as well as possible genetic changes in these tumors.

**Method:** Five cases of myxoid leiomyosarcoma presented clinically with irregular bleeding and abnormal Pap smears that led to the diagnosis of cervical (2 cases) or endocervical cancer (3 cases). Immunohistochemistry was performed for MiB1, p53, cytokeratin, EMA, SMA, and actin. Genomic DNA was extracted from microdissected tumor and normal cells, and LOH was investigated in chromosome 11q13 using 4 microsatellite markers near the MEN1 gene: D11S449, D11S4946, INT-2 and PYGM.

**Results:** Patients' ages ranged from 38 to 52 years (mean 46 years). Histologically the tumors were characterized by the presence of smooth muscle embedded in an exuberant myxoid matrix, infiltrating margins, and variable degrees of atypia and mitotic rates. The uterine cavity was distorted by large bulky masses in all cases that extended and ulcerated the cervix. All patients were DOD (8 ms to 11 y). Stains were positive for p53 in 4 cases, for MiB1 in 4 cases, for SMA and actin in one case. CK and EMA stains were negative. One case, known to have MEN1 syndrome, showed LOH at 11q13 at the (D11S449).

**Conclusions:** We conclude that myxoid leiomyosarcomas are rare variants of leiomyosarcoma that should be included in the differential diagnosis of unusual cervical lesions. Prognostic factors correlate well with poor outcome of these tumors. It is important to recognize that these neoplasms may occur as part of the MEN1 syndrome.

## O-081

**DETECTION OF POINT MUTATIONS OF THE c-K-*ras* GENE IN OVARIAN TUMORS**

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**Aims:** c-K-*ras* point mutations were analyzed in 239 ovarian epithelial tumors in order to asses their role in the pathogenesis, and their putative prognostic value in mucinous and non-mucinous ovarian tumors.

**Methods:** The tumors were divided in mucinous and non-mucinous ones. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue specimens. Point mutations were detected by the Polymerase Chain Reaction (PCR) using the Restriction Fragment Length Polymorphism (RFLP) analysis. A clinicopathologic study and a comparative analysis of c-K-*ras* mutations were also performed in six cases of synchronous ovarian and appendiceal mucinous tumors associated with pseudomyxoma peritonei.

**Results:** The overall frequency of codon 12/13 *ras* gene mutations was 66% in mucinous tumors (58% in benign, 86% in borderline and 85% in malignant) and 30.5% in non-mucinous ones (20% in benign, 25% in borderline and 35% in malignant tumors). The same point mutation was found independently in all cases of synchronic ovarian and appendiceal tumors.

**Conclusions:** We concluded that: a) c-K-*ras* mutations are related to mucinous differentiation, since they are significantly more frequent in mucinous than in non-mucinous ovarian epithelial tumors. b) c-K-*ras* mutational activation probably plays a role in the early steps of ovarian mucinous tumorigenesis; it occurs in benign and particularly in malignant mucinous ovarian tumors. c) c-K-*ras* mutations are not initial events in non-mucinous ovarian tumors but genetic alterations related to tumor progression. d) c-K-*ras* mutations were not related with prognosis, survival, or with any other clinicopathological parameter. e) The identical pattern of c-K-*ras* mutations in synchronic mucinous tumors of the ovary and appendix of all patients, supports a clonal nature of these neoplasms, and that they are not independent tumors but rather originate one from the other.

## O-082

SIMULTANEOUS VISUALIZATION OF TUMOR ASSOCIATED MACROPHAGES AND MICRO - VESSEL DENSITY IN OVARIAN CANCER

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**Aims**: The aim of the present study is to assess tumor associated macrophages (TAMs) both intraepithelial and stromal in relation to microvessel density (MVD) in ovarian cancer Results are correlated with patient's outcome ,tumor grade,stage, as well as p53 and cyclin D1 expression.

**Methods:** 48 malignant ovarian epithelial neoplasms were immunohistochemically double immunostained using Ki-M1p and anti- Factor IIIV related antigen antibodies with the two- step Envision kit from DAKO, Denmark. An image analysis system (CAS -200)was used to count both macrophages and blood vessels by x40 objective. Tumors were also stained with antibodies against p53and cyclin D1.

**Results:** Only intraepithelial macrophages were significantly correlated to tumor grade (P= 0.02) and to shorter survival (P=0.007). Stromal macrophages correlated with intraepithelial ones (P = 0.001)and to microvessel density (0.009) but not with other parameters. P53 expression was significantly correlated to high grade (P = 0.001 ),advanced stage (P = 0.0002)and shorter survival (P = 0.007).Cyclin D1was related with longer survival(P = 0.04).

**Conclusion:** Increased intraepithelial macrophage counts and P53 expression are indicative of aggressive behaviour and poor prognosis in ovarian cancer ,in contrast to cyclin D1 expression. Our results also indicate that macrophages may have a positive effect on angiogenesis in ovarian cancer.

## O-083

**Expression of carbohydrate antigens in ovarian epithelial carcinomas and their metastases- a clinicopathologic study.**

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**Aims:** Up-regulated expression or loss of expression of various carbohydrate antigens on the surface of cancer cells has been associated with a metastatic phenotype and poor survival in epithelial malignancies of different origins. The object of this study was to investigate the expression of carbohydrate antigens in ovarian carcinomas.

**Methods:** The study group comprised 53 patients diagnosed with ovarian carcinomas, all with disease spread outside the ovary (FIGO stages II-IV). The patients were segregated in two groups- long-term survivors (27 patients) and short-term survivors (26 patients). Sections from 83 paraffin-embedded blocks (45 primary ovarian carcinomas and 38 metastatic lesions) were immunohistochemically stained, using 5 monoclonal antibodies for Lewis Y, Sialyl Lewis X, TN and Sialyl TN antigens.

**Results:** A combined pattern of membranous and cytoplasmic staining was predominant. Staining for all four antigens was seen in the majority of cases (range= 80-99%), and tended to be comparable in primary tumors and their respective metastases. However, absence of immunoreactivity for Sialyl Tn was seen in 8/38 (21%) metastatic lesions and only 1/45 (2%) primary tumors. Diffuse staining (>25%) for Sialyl Lewis X and TN was more often seen in tumors of short-term survivors (15% and 5% vs. 34% and 20%, respectively). Enhanced staining for Lewis Y and Sialyl TN was detected in the invasive front of some tumors, while Sialyl Lewis X and TN immunoreactivity did not relate to cell location.

**Conclusions:** Carbohydrate antigens are widely expressed in both primary ovarian carcinomas and their metastases. The expression of membrane carbohydrate molecules in primary tumors may represent early acquisition of a metastatic carbohydrate phenotype, and an important step in tumor progression.

## O-084

MOLECULAR EVIDENCE FOR TWO TYPES OF SYNCHRONOUS ENDOMETRIOID CARCINOMAS OF THE UTERUS AND OVARY

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**Aims:** It is unclear weather synchronous endometrioid uterine and ovarian carcinomas araise independently or if they are caused by metastatic tumor disease. We investigated the prevalence of allelic loss at 10q23 (the location of the tumorsuppressorgene PTEN/MMAC1) and clonal composition of the two microdissected tumor components.

**Methods:** DNA was extracted from 6 formalin fixed and paraffin embedded synchronous tumors and corresponding nonneoplastic tissues. 7 microsatellite loci on 10q23 ( two of them intragenic) were used for LOH- analysis and the HUMARA- approach (J.Pathol.1998;186:3636-371) for analysis of clonal composition.

**Results:**

| Patients | #1 | #2 | #4 | #5 | #6 | #7 |
|---|---|---|---|---|---|---|
| LOH* | 1/7 | 5/7 | 2/7 | 1/7 | 4/7 | 3/7 |
| Clonality+ | mc | ni | mc | mc | x | nd |

*Differences in the LOH-patterns at 7 loci in both organs
+mc: monoclonal, x: LOH of X-chromosome, ni: non informative, nd: not done

**Conclusions:** Our results indicate that two different types of synchronous endometrioid carcinomas of the uterus and ovary might exist: one type representing a metastatic disease of one single primary tumor exhibiting the same clonality and a similar LOH- pattern in the primary as well as the metastasis, and a second type of tumor which represents two different but histologically identical carcinomas exhibiting a different clonality as well as LOH-pattern.

## O-085

EVALUATION OF DIFFERENT GRADING SYSTEMS FOR OVARIAN EPITHELIAL CARCINOMA IN A SERIES OF 100 PATIENTS WITH UNIFORM TREATMENT AND FOLLOW-UP

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**Aims**: Histological grade correlates with survival in most published series of ovarian carcinoma, but the grading system used commonly is not specified. Several grading systems exist. Moreover, some grading systems are dependent on the histologic type of the tumor being tested.

**Methods**: We studied a series of 100 patients (pts) treated for ovarian malignant invasive carcinoma from January 1983 to June 1998 at our institution. All the pts were managed uniformly with surgery and cisplatin-based chemotherapy. All the slides were reviewed in a double-blind manner by 3 pathologists, typed according to the WHO and graded with different grading systems ie, 1) FIGO (architectural), 2) BRODERS modified (cytological), 3) Dauplat and Nieberg (*Int J Gynecol Pathol 1989*) and 4) Shimizu and Kamoi (*Cancer 1998*) (combined grades). These data were matched with classical prognostic factors. Multivariate assessment of survival time was performed with the Cox model.

**Results:** Population parameters - mean age: 60 years, - stage (FIGO) I 9%, II 6%, III 66%, IV 19%. - survival OS: stage III & IV=2.2 years (22.5% 5 years). 60% pts died. Prognostic factors: clinical: age<60 ($p<0.001$), surgery ($p<0.01$), N+ ($p<0.02$), histopathological: *necrosis*>50% ($p<0.04$), *mitotic count<15MF/10HPF* ($p<0.03$) and *vascular invasion* ($p<0.03$). Those 3 parameters were assigned to a new grading system. Neither the histological types nor one the 4 grading systems tested correlated with OS or DFS in our series.

**Conclusion:** The new grading system (necrosis, mitotic count and vascular invasion) is simple, useful for all histologic types, non subjective and reproducible. Further studies are warranted to confirm its clinical utility.

## O-086

**MODIFICATION OF PROSTATIC STROMAL CELL PHENOTYPE BY NORADRENALINE, DOXAZOSIN AND SEX HORMONES**

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**Aims.** To determine whether the cytoskeletal filaments in prostatic stromal cells from patients with benign prostatic hyperplasia (BPH) are increased by exposure to noradrenaline (NA) and whether this is modified by doxazosin and/or androgen and estrogen.

**Methods.** Cultures of prostatic stromal cells were obtained by collagenase digestion of TURP chippings from patients with BPH. In the first experiment 3 cell lines were grown in medium containing NA bitartrate at 7 increments of concentration (2.5-50.0μM) for 10 days. In the second experiment 10 confluent cell lines were rendered quiescent with 1% stripped FCS and exposed to 20μM NA and/or the $\alpha_1$-adrenoceptor antagonist, doxazosin (0.001-10μM). Finally, 6 cell lines were exposed to the above and also β-estradiol or testosterone (0.1μM). In each case cells were labelled with FITC-conjugated antibodies against α-smooth muscle actin, myosin, desmin, vimentin and talin. Fluorescence intensity was measured by flow cytometry.

**Results.** After an initial fall in immunoexpression between 2.5-15μM NA, there was a progressive rise in all 5 cytoskeletal proteins up to 50μM. Actin and myosin were significantly higher than control in 10 cell lines incubated with 20μM NA. Doxazosin (1μM) significantly reversed this shift to a contractile phenotype, and this effect was greatest in confluent, quiescent cells. Although estradiol and testosterone greatly increased the effect of NA in some cell lines, the average increase was not significant.

**Conclusions.** The therapeutic use of doxazosin not only inhibits noradrenergic contraction of stromal cells in BPH but may also reduce their capacity to contract in the first place.

## O-087

**ALTERED GYLCOSYLATION IN PROSTATIC CARCINOMA IS RELATED TO TUMOUR GRADE PROGRESSION AND METASTASIS. A STUDY OF $P^{53}$ EXPRESSION AND LECTIN BINDING**

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**Aims:** Glycoconjugates confer recognition and stability to the cells. Malignant tumours continue to alter in ways which eventually lead to their escape from the biological controls. A few lectins have previously been used as histochemical probes to characterise the glycans of normal and non-neoplastic lesions of prostate, however little is known about the biological processes in maliginat prostate. We examined glycan and $P^{53}$ expression in Prostatic Carcinoma.

**Methods:** A panel of 26 biotinylated lectins were applied to formalin - fixed, paraffin embedded prostatic tissue, distinguished histologically as hyperplastic (7 cases) and carcinoma (40 cases) - well differentiated (13 cases), moderately differentiated (14 cases) and poorly differentiated (14 cases). Primary specific antibody against $P^{53}$ (DO-7, Novacastra Laboratories) was used. The site, pattern and intensity of lectin staining were recorded and compared. The specimens with nuclear immunoreactivity in more than 10 percent of the malignant cells were regarded as $P^{53}$ positive. Abnormal lectin binding patterns were correlated with $P^{53}$ expression and prognosis.

**Results:** Well differentiated tumours closely resembled the hyperplastic tissues and several lectins stained tumours of all grades. The low grade tumours showed predominately apical staining. A few lectins showed selective cytoplasmic staining of the moderately and poorly differentiated tumours. In poorly differentiated tumours glycan expression was noted on the cell membrane as well as within the cell, representing a "biochemical pleomorphism." $P^{53}$ overexpression strongly correlated with high grade tumours.

**Conclusions:** The lectins are valuable tools to study functional changes before morphological & clinical features become pronounced. The results imply that there is a marked difference in glycan expression between well differentiated and moderately and poorly differentiated groups. Since the lectins are extremely sensitive & specific sugar binding profeins it is concluded that there is increased diversity of sequences at non-reducing termini involving terminally-acting glycotransferases on the cell surface. These findings suggest that accelerated degradation or decreased synthesis of terminal cell surface carbohydrates may facilitate tumour progression and increase the metastatic potential. The results of $P^{53}$ suggest genetice instability in a subset of prostate carcinomas that leads to tumour progression. Hence altered glycosylation with altered $P^{53}$ expression were found to be associated with bad prognosis.

## O-088

**PROGNOSTIC IMPORTANCE OF MICROVESSEL DENSITY (MVD) IN CLINICALLY LOCALIZED PROSTATE CANCER.**

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**Aims:** The results of previous studies using microvessel density (MVD) as a measure of angiogenesis in prostate cancer have been contradictory, and the relationship to prognosis is not clear. The purpose of this study was to assess the prognostic value of MVD in clinically localized, moderately differentiated (WHO histologic grade) prostatic adenocarcinomas after radical prostatectomy.

**Methods:** From a series of 104 consecutive patients treated for presumed organ-confined cancer between 1988 and 1995, 66 patients (median age=62 years) with moderately differentiated (WHO histologic grade) prostatic adenocarcinomas were studied. The area of lowest differentiation was selected from the whole-mount sectioned prostatectomy specimens and used for microvessel quantitation. Vessels were high-lighted by staining for factor-VIII-related antigen and counted in 10 fields at x312.5 magnification (0.6148 $mm^2$) in the "hot-spot" area. The findings were related to biochemical failure (n=24) defined as s-PSA elevation ≥ 0,5 ng/ml, and clinical recurrence (n=7).

**Results:** MVD was associated with preoperative s-PSA ($p=0.011$) and positive surgical margins ($p=0.001$). In univariate analysis of the first five years, mean MVD ($p=0.0074$), s-PSA, maximum tumor diameter, capsular penetration, seminal vesicle invasion and positive surgical margins were all significant predictors of biochemical failure, while MVD ($p=0.0084$) was the only significant predictor of clinical recurrence. All patients with clinical recurrence had high MVD counts (above median). In multivariate Cox' analysis, mean MVD ($p=0.0006$), capsular penetration ($p=0.0105$) and maximum tumor diameter ($p=0.0186$) remained as independent predictors of biochemical failure.

**Conclusions:** Assessment of MVD in moderately differentiated prostatic adenocarcinomas may aid in stratifying patients into different risk groups after radical prostatectomy.

## O-089

Molecular Analysis of Multifocal Prostate Cancer Lesions

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**Aims:** To analyse the origin of multifocal prostate cancer lesions.
**Methods:** Radical prostatectomy specimens from 17 patients were examined. As a marker of genetic lineage, the allelotype based on 33 microsatellite loci was compared between the different tumours present in a given case.
**Results:** Some results provide evidence suggestive of a clonal origin of multiple tumours in a subset of the prostates. In 5 cases, for example, comparison of multifocal tumour lesions within a given case revealed at least 2 concordant changes in allelelic imbalance (AI) sequence dosages at different loci. In addition, considerable heterogeneity of allelotype was found within and among tumour foci of a given case. In 5 of the 6 tumours analyzed for intratumour heterogeneity, for example, more than 5 discordant AI changes were found in one tumour region but not in the other.
**Conclusions:** Conclusion regarding the clonality of such heterogeneous lesions are difficult to draw. A high frequency of AI changes in four lesions exhibiting prostatic intraepithelial neoplasia (mean 6.5 changes/lesion range 3-6) was found as compared to 8 primary tumors present in the same cases (mean 5.8 changes/lesion, range 3-6). The interpretation of AI associated with clinically detected prostate cancer remains a highly complex issue. The fact that no clear evidence was obtained for either a clonal or a non-clonal origin of multiple tumour lesions in a given prostate indicates that several mechanisms are likely to operate in establishing the allelotype and that additional evidence from unique mutations or selective gene inactivation may be necessary to obtain definitive results.

## O-090

**ENDOCERVICOSIS OF THE URINARY BLADDER. IMMUNOHISTOCHEMICAL STUDY IN COMPARISON TO 4 NORMAL ENDOCERVICAL MUCOSA.**

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We report the case of a 35-year-old woman, presenting with catamenial dysuria and urgency, with relation to a 2.3 cm mass situated in the posterior wall of the bladder. It corresponded to endocervicosis present on the transurethral resection and on the partial cystectomy performed. The pathogenesis of this rare condition is still on debate : could it be considered as a metaplastic urothelial event or as a disorder of the secondary müllerian system ? Therefore we conducted a comparative study between the lesion, the normal urothelial counterpart and 4 normal uterine cervix. Were tested antibodies classically present in normal female genital tract : progesterone and estrogen receptors (PR, ER), CA15-3 (DF3), HBME-1, Chromogranin A. A proliferation marker (MIB1) and the histiocytic reaction were also tested.

| | PR | ER | Chromogranin A | DF3 | HBME 1 | MIB1 (%) | CD68 |
|---|---|---|---|---|---|---|---|
| Lesion | ++ | ++ | ++ | ++ | ++ | 14,9 | + |
| Normal urothelium | - | - | + | + | | 7,4 | |
| 4 normal uterine cervix | +/- | + | + | ++ | + | 3 (+/- 0,5) | + |

These results are additional arguments in favor of the müllerian origin of the endocervicosis, already assessed by the usual clinical data : women of reproductive age, catamenial symptoms, posterior localization, possible association with endometriosis.
We must notice the significantly high indice of proliferation in the lesion and cannot assess the purely indolent behaviour of this lesion in the absence of surgical excision.

## O-091

CARCINOMA IN SITU AND DYSPLASIAS OF THE URINARY BLADDER SHOW FREQUENT DELETIONS OF CHROMOSOME 9 IN ADDITION TO P53 MUTATIONS.

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**Aims:** The recent hypothesis to describe bladder cancer development uses two different pathways for papillary tumors (associated with chromosome 9 aberation) and flat urothelial neoplasia (associated with p53 mutation). Within a study of genetic alterations of early bladder cancer lesions, we determined the frequency of deletions at Chromosome 9 and the frequency of p53 mutations in multifocal carcinomata in situ (CIS) and moderate urothelial dysplasia (DII).
**Methods:** 36 CIS and 17 DII from a total of 21 patients were investigated. Biopsies were obtained from photodynamic diagnosis with 5-aminolevulinic acid. The urothelial cells were seperated from stromal cells using laser or manual microdissection (PALM). From contigous sections, cells were either dissolved to a nuclear suspension or DNA was isolated. Dual colour fluorescence in situ-hybridization (FISH) was performed with digoxigenin labeled probes for the gene loci 9q22 (FACC), 9p21 (CDKI2/p16) and 17p13 (p53). DNA was preamplified using Primer Extension Preamplification (PEP)-PCR and subsequently specific microsatellite PCR using 8 markers on chromosome 9 and 17 and direct sequencing od Exons 5-9 of the p53 gene were performed.
**Results:** The frequency of deletions in CIS was 69.4% on both arms of chromosome 9 and 72.7% on chromosome 17. In 84% of the samples the LOH analysis was in complete concordance with the FISH. 18 of 25 investigated CIS showed p53 mutations. DII showed an overall lower deletion rate in comparison to CIS with a distribution of 9p=42.8%; 9q=29.4%; 17p=43.8%). In 4 of 7 dysplasias, p53 mutations were detected.
**Conclusion:** The data presented are the first indicator of CIS having chromosome 9 changes in numbers comparable to p53 deletions. Thus chromosome 9 can not seperate the two tumor entities. Urothelial dysplasias have the same genetic alterations as CIS, although in a lower frequency.

## O-092

IMMUNOHISTCHEMISTRY OF PAPILLARY NEOPLASMS OF THE URINARY BLADDER WITH DIFFERENT MALIGNANT POTENTIAL

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**Aims:** In urothelial low grade carcinomas of the bladder stage pT1, prognosis in general is good. In a subset of these tumors, infiltrating beyond the lamina muscularis mucosae, nevertheless prognosis clearly worsens. Unfortunately, evaluating of the lamina muscularis mucosae often is very difficult or even impossible due to its incomplete extension.
**Methods:** In an immunohistochemical study on 131 pTa and pT1 urothelial tumors without provable lamina muscularis mucosae, we evaluated the proliferative activity with the monoclonal antibody MIB-1 and the expression pattern of cytokeratins of high molecular weight with the monoclonal antibody 34ßE12.
**Results:** The highest proliferative indices were found in tumors with a diffuse expression pattern of MIB-1 and 34ßE12. A preliminary analysis of follow up data revealed, that 70.6% of the pT1 GIb-GIIa tumors which recurred showed a diffuse expression pattern for both markers.
**Conclusions:** Whether these patients are candidates for a closer follow up or even for a more radical therapy has to be subject of further follow up studies.

## O-093

THE IMPORTANCE OF DIAGNOSTIC TESTICULAR BIOPSY IN VIEW OF ASSISTED REPRODUCTION

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**Aims:** In order to promote the human reproduction more attention must be paid on male infertility. The spermogram and the testicular biopsy are the most informative examinations.

**Methods:** Testicular biopsies of 21 patients were evaluated histologically. According to the sperm counts 4 patients had azoospermia, 4 had severe oligozoospermia, 12 had oligozoospermia, and 1 had asteno-tetrazoospermia. The karyotype was 46XY in each case, the FSH hormone value normal or elevated. The biopsies were performed by atraumatic microsurgery. Two samples were taken from both testes, one from the inner-upper and one the lower-outer surface. Bouin fixed, paraffin embedded blocks were cut and HE slides examined. Sertoli cells and germ cells were counted within 10 round shaped seminiferous tubules.

**Results:** In azoospermic group Sertoli Cell Only Syndrome or inhibited maturation was found. In one of the cases in spite of high FSH level satisfying spermatogenesis was observed in one sample. In cases of severe oligozoospermia the spermatogenesis was affected, but in one quadrant the spermatogenesis seemed to be satisfactory again. In the largest group presented with oligozoospermia, 8/12 patients had normal spermatogenesis in both quadrant of both testes.

**Conclusion:** The sperm count alone is not satisfactory in assessing spermatogenesis. The four biopsy samples give more realistic information because the spermatogenesis is not homogenous in the testis. High level of FSH (ICSH) does not exclude the necessity of testicular biopsy.

## O-094

**INHIBIN-α, CD99, HEA125, PLAP, AND CHROMOGRANIN IMMUNOREACTIVITY IN TESTICULAR NEOPLASMS**

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**Aims:** Inhibin-α and CD99 have recently been identified as being useful diagnostic markers in ovarian sex cord-stromal tumors. Only limited information is available on the in situ distribution of these markers in testicular neoplasms.

**Methods:** In the present immunohistochemical study, we investigated 116 testicular and 3 epididymal tumors using monoclonal antibodies against inhibin-α, CD99, HEA125, PLAP, and chromogranin.

**Results:** Inhibin-α was detected in neoplastic tumor cells in 4/5 juvenile granulosa cell tumors (JGCTs), 6/20 Sertoli cell tumors (SCSTs), 27/27 primary Leydig cell tumors (LCTs), 1/1 metastatic LCT, and in 2/6 unclassified sex cord tumors (USCTs). CD99 was detected in 3/5 JGCTs, 1/7 SCTs, 10/15 primary and 0/1 metastatic LCTs, and in 1/6 USCTs. While strong inhibin-α immunoreactivity in the syncytiotrophoblastic cells but less intense staining of the cytotrophoblastic component was found in 2/2 choriocarcinomas, and in the choriocarcinomatous component of 1 mixed germ cell neoplasm, CD99 was not detected in any tumor outside the sex cord-stromal category. HEA125 expression was not observed in sex cord-stromal tumors except for 1/6 USCT. However, it was detected in some germ cell tumors including 3/12 seminomas, 2/12 embryonal carcinomas, 6/8 yolk sac tumors, and 1/2 teratomas. While PLAP was detected in 4/15 primary Leydig cell tumors and in most germ cell tumors, chromogranin immunostaining of tumor cells was rarely observed in the present study.

**Conclusions:** Inhibin-α, CD99, HEA125, and PLAP immunostaining may be helpful in the differential diagnosis of testicular neoplasms.

## O-095

LOW MICROSATELLITE INSTABILITY AND FREQUENT P53 PROTEIN ACCUMULATION IN SPORADIC AS COMPARED TO FAMILIAL COLORECTAL CANCERS

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**Aims**: Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant cancer-susceptibility condition characterized by early onset colorectal cancer (CRC). The underlying genetic abnormalities are germline mutations in one of five DNA mismatch repair genes (MMR) or in the *TGFbRII* gene. Gene-carriers are at high risk of developing multiple tumors, therefore distinction between inherited and sporadic CRC cases is of medical importance.

**Methods**: We studied three groups of patients, HNPCC kindred fulfilling the International Collaborative Group criteria (n=10), families in which at least one of the criteria was not satisfied (n=7) and sporadic CRC diagnosed before the age of 50 (n=17). In these three groups we searched for microsatellite instability (MSI), presence of *hMSH2* and *hMLH1* germline mutations, and expression of hMSH2, hMLH1 and p53 proteins in tumoral tissue samples by immunohistochemistry.

**Results**: Fifteen out of 17 (88%) of HNPCC and incomplete HNPCC cases were MSI, whereas all of the 17 early-onset sporadic cases were microsatellite stable (MSS). Nine germline mutations in *hMSH2* or *hMLH1* were detected out of 15 MSI cases (60%) but none in the 9 MSS investigated cases. Eleven out of 13 (85%) familial cases were MSI and p53 protein negative whereas 13/14 (93%) sporadic cases were MSS and p53 protein strongly positive. Such observations demonstrate the existence of an inverse correlation between high microsatellite instability and p53 protein accumulation in tumors.

**Conclusion**: Our results suggest that there are two different genetic pathways in colorectal carcinogenesis, one for familial cases, involving MMR genes inactivation without p53 protein accumulation, the other for sporadic cases involving p53 protein accumulation and without MMR genes inactivation.

## O-096

**RAPID SCREENING FOR PROGNOSTIC MARKERS IN RENAL CELL CARCINOMAS BY COMBINING CDNA-ARRAY AND TUMOR-ARRAY TECHNOLOGIES**

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**Aims**: Many genes and signalling pathways are involved in renal cell carcinoma (RCC) development. It was the aim of this study to identify genes with relevance in RCC.

**Methods**: A cDNA array (Release I of the human GeneFilters, Research Genetics) containing 5184 cDNA/EST clones was used to screen for genes with differential expression between the renal cancer cell line CRL-1933 and normal kidney tissue.

**Results**: There were 89 differentially expressed genes including vimentin which was highly overexpressed in the cell line. To test prevalence and prognostic significance of vimentin expression a renal cancer tumor array containing 532 RCC specimen was then constructed and vimentin expression was determined by immunohistochemistry. Vimentin expression was frequently seen in clear-cell (51%) and papillary RCC (61%), but rarely in chromophobe RCC and oncocytomas. This prevalence exactly matched with previous findings indicating that valid results can be obtaind examining minute arrayed tumor samples. Most interestingely, vimentin expression was significantly associated with poor patient prognosis ($p<0.007$) which was independent of grade and stage.

**Conclusions**: These results suggest that combining cDNA and tumor arrays is a powerful approach for identification and further evaluation of genes playing a role in RCC and other tumors. The prognostic significance of Vimentin expression should be further evaluated in prospective studies.

## O-097

**DIFFERENTIAL EXPRESSION OF TSH RECEPTOR IN ARCHIVAL THYROID CARCINOMAS USING 5' NUCLEASE ASSAY (TAQMAN).**

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**Aim:** Prognosis in thyroid carcinoma is generally dependent on the patient's age and stage of tumour at the time of diagnosis. However tumours with several adverse features such as necrosis or high mitotic index may follow an indolent course while other seemingly inert ones may rapidly progress and have a fatal outcome. Proliferation indices using Ki-67 or mitotic counts and apoptotic counts are useful at either end of the spectrum of differentiation of tumours, but more accurate prognostication is required to predict the outcome of tumours whose histological appearance belies their sinister intent.
Proliferation in thyroid carcinoma is variably TSH driven and TSH receptor (TSHr) status significantly relates to therapeutic response. The aim of this study was to quantify the level of TSH receptor expression in a series of 125 thyroid neoplasms.
**Methods:** TSHr expression was semi-quantitatively assessed in a series of archival thyroid carcinomas comprising follicular adenomas, follicular, papillary, medullary and anaplastic carcinomas. Total RNA was extracted from formalin fixed paraffin embedded tissues and reverse transcribed. To overcome the effect of different degrees of RNA degradation due to variations in storage conditions and duration of fixation, samples were analysed using GAPDH as a housekeeping gene.
The TaqMan detection system exploits the 5'- 3' endonuclease activity of Taq DNA polymerase, which digests a double labelled internal fluorogenic probe during the amplification reaction. Prior to PCR the intact probe fluorescence of the reporter is suppressed by the quencher due to its spatial proximity. Digestion of the probe by Taq DNA polymerase results in separation of reporter and quencher dyes and a concomitant increase in fluorescence.
**Results:** The fluorescent intensities obtained for TSHr and GAPDH were compared and a relative TSHr index was calculated for each sample. Results indicate the level of TSHr expression parallels the histologically graded degree of differentiation in the tumours assayed.
**Conclusion:** TSHr expression may prove to be an additional prognostic marker in thyroid carcinomas, and be of therapeutic value.

## O-098

USEFULNESS OF ANTIBODIES TO BRCA1 PROTEIN TO DETECT MUTATED BRCA1 GENE. AN IMMUNOHISTO-CHEMICAL STUDY.

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Aims: To assess the value of immunohistochemistry using commercially available BRCA1 antibodies to discriminate between breast tumors with and without BRCA1 germline mutations.
Methods: 22 paraffin-embedded tumoral samples from patients with (7/22) and without (15/22) BRCA1 germline mutations studied by Protein Truncation Test (PTT), Conformational Sensitive Gel Electrophoresis (CSGE) and direct sequencing of genomic DNA.
Immunohistochemistry was undertaken following the standard avidin-biotin immunoperoxidase method. Pretreatment with microwave oven and autoclaving were used for antigen retrieval. The antibody panel used comprised D-20 (1:500), I-20 (1:100) and K18 (1:100) primary antibodies was from Santa Cruz Biotechnologies (CA USA).
Results: No differences in BRCA1 expression were found between cases with and without BRCA1 germline mutations. All positive cases showed predominantly cytoplasmic staining, in tumoral and non-tumoral cells. After autoclaving pretreatment we found nuclear staining in tumoral and non-tumoral cells, with the I-20 antibody in 7 (5 without and 2 with BRCA1 germline mutations) of the 22 cases studied. A membrane pattern with the D-20 antibody and also cytoplasmic granules with the K18 antibody were found, but the significance of these findings, if any, is uncertain.
Conclusions: Commercially available BRCA1 antibodies lack the specificity required to unambiguously identify a protein as BRCA1, and so are not useful to establish differences between familial and sporadic breast tumors.

## O-099

**DETECTION OF TELOMERASE IN HEPATOCELLULAR CARCINOMAS (CHC) USING AN *IN SITU* TELOMERIC REPEAT AMPLIFICATION PROTOCOL (TRAP) ASSAY ON TISSUE SECTIONS**

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**Aims:** Telomerase activity has been widely detected by TRAP assay in germ cells and many tumor extracts. We performed a topographic assessment of telomerase activity (TA) in CHC, and adjacent liver tissue in order to precise the role of telomerase during hepatocarcinogenesis.
**Material and methods:** Frozen sections of 26 CHC, and 29 non tumoral liver tissues were studied. Sections from normal testis were used as positive control. In a first step, the elongation step and the PCR amplification of the standard TRAP Assay was performed *in situ* on frozen tissue sections using unlabelled primers. Telomeres amplified repeats were then detected using a complementary digoxigenin-labeled probe that was subsequently revealed with an anti-digoxigenin-FITC antibody.
**Results:** Testis showed a bright punctuate nuclear signal in immature germinal cells. Signal disappeared when slides were preheated, or RNAse pretreated, or when the PCR step was omitted. Twenty-two of the 26 (84,6 %) CHC exhibited a bright punctuate nuclear signal in areas of tumoral cells, sometimes associated with a diffuse cytoplasmic staining. Non tumoral liver tissues exhibited a nuclear staining in groups of periportal hepatocytes (7/29), but also in periveinular hepatocytes (5/29), and scattered intralobular hepatocytes (14/29).
**Conclusions:** These results confirm actual knowledge about TA in hepatocellular carcinogenesis. *In situ* TRAP assay on tissue sections is a new sensible and reliable method which allows the study of topographic distribution of TA in cancerous and precancerous lesions.

## O-100

**GENETIC ALTERATIONS IN GLIOSARCOMAS**

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**Aims:** Glioblastoma is the most frequent and malignant brain tumor in humans. Recent studies have shown that there are distinct genetic pathways leading to glioblastomas: Primary *(de novo)* gliobalstomas are characterized by high frequency of *EGFR* amplification/overexpression, *p16* deletion and *PTEN* mutations, whereas secondary glioblastomas which progressed from low-grade or anaplastic astrocytoma are characterized by frequent *p53* mutations. The objective of this study was to assess the genetic profile of gliosarcomas (GS), a rare glioblastoma variant, in which a mesenchymal component is present in addition to regions of gliomatous differentiation.
**Methods:** We assessed in 19 gliosarcomas, *p53* mutations (exons 5-8) and *PTEN* mutations (exons 1-9) using PCR-SSCP followed by direct sequencing, homozygous *p16/CDKN2* deletion, amplification of *MDM2*, *CDK4* and *EGFR* by differential PCR, and altered expression of MDM2, EGFR, p53 and Rb by immunohistochemistry.
**Results:** Six (32%) GS contained a *p53* mutation. In one case, the same mutation was detected in both mesenchymal and glial components. Mutations of the *PTEN* gene were found in 7 (37%) cases. In two cases the same mutations was found in both mesenchymal and glial components. *p16* homozygous deletion was found in 7 (37%) GS. In one case, the homozygous deletion was found in both mesenchymal and glial regions. Amplification of *CDK4* and *MDM2* was detected in one gliosarcoma. None of the GS showed *EGFR* amplification/overexpression. One case with *MDM2* overexpression in more than 50% of neoplastic cells showed gene amplification. One GS (5%) showed loss of Rb expression. Overall incidence of aberrant expression of the Rb growth control pathway (*p16*, *CDK4* and pRb) was 47%.
**Conclusions**: Gliosarcomas show the unique genetic profile, i.e., frequent *PTEN* mutation and homozygous *p16* deletions. The identification of same genetic alterations in mesenchymal and glial components suggest the monoclonal origin of both tumor components.

## O-101

**Thyrotropin receptor mutations in single and multiple autonomously functioning thyroid nodules are frequently associated with hyperplastic morphology**

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**Aims:** Autonomously functioning thyroid nodules (AFTN) frequently harbor constitutively activating mutations of the thyrotropin (TSH) receptor gene. To verify whether the clinical designation "autonomous adenoma" is justified for AFTN we correlated the presence of TSH receptor mutations in single and multiple AFTN with the histopathological features.

**Methods:** Surgically removed AFTN from 25 patients (19 with a single, 6 with multiple AFTN) were analyzed. DNA was extracted from formalin fixed paraffin embedded tissue after microdissection. A fragment of the TSH receptor gene (base pairs 1762-1976) was amplified by PCR and directly sequenced using fluorochrome-labeled dideoxynucleotides. All AFTN were histopathologically classified into adenomas and nodular hyperplasia.

**Results:** Sequence analysis was successful in 17 cases of single AFTN and 3 cases of multiple AFTN. TSH receptor mutations were found in 5 of 17 cases (29%) of single and in 1 of 3 cases of multiple AFTN. These mutations were detected in codons 629, 631 (2x), 632 and 633 (2x) which all have been previously identified as functionally active. All 5 single AFTN with mutation and all multiple AFTN were histologically classified as nodular hyperplasia whereas 5 of 12 single AFTN without mutation were classified as adenomas.

**Conclusions:** Most AFTN, regardless the presence of TSH receptor mutations are histologically nodular hyperplasia. However, only about 30% of single and multiple AFTN are associated with TSH receptor mutations in exon 10.

## O-102

PAPILLARY CARCINOMA OF THE THYROID: OVEREXPRESSION OF MET PROTEIN IN TUMOUR CELLS IS ASSOCIATED WITH INCREASED IN VITRO INVASIVENESS IN RESPONSE TO HGF

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**Aims:** We have previously shown that Met protein, the high affinity receptor for HGF, is overexpressed in >95% of cases of papillary carcinoma of the thyroid. In the present study, we have investigated the functional role of HGF/Met interaction in primary cultures of 20 papillary carcinomas and in normal thyroid cells obtained from the same patients.

**Methods:** Normal and tumour cells were cultured in DMEM + 10% FBS. The ability of HGF to stimulate tumour cell proliferation was investigated as $^3$H-thymidine incorporation. Tumour cell invasiveness was investigated in Boyden chambers, using filters coated with Matrigel. Tumour cell adhesion to extracellular matrix (ECM) components was investigated in 96 wells tissue culture plates coated with fibronectin, laminin, or vitronectin.

**Results:** HGF did not affect tumour cell proliferation. HGF-stimulated tumor cells were more invasive than the corresponding normal thyroid cells in 5 of 7 cases. Unstimulated tumour cells were 3-10 fold more adherent than normal cells to fibronectin, laminin, and vitronectin; moreover, HGF induced a marked increase of tumor cell adhesion to fibronectin. In 27 cases investigated in tissue sections, basement membranes of papillary carcinoma cells were rich of EDA and EDB oncofetal fibronectins, whereas normal thyroid follicles were negative. Normal cells and tumour cells did not differ in the level of expression of the high affinity receptors for fibronectin $\alpha5/\beta1$ and $\alpha v/\beta3$.

**Conclusions:** Our findings are consistent with the possibility that HGF/Met protein interaction stimulates adhesion of tumor cells to ECM components, especially fibronectin, and facilitates tumour cell invasiveness.

## O-103

CD15 (Leu-M1) AND CA19.9 IMMUNOREACTIVITY IN SOLID CELL NESTS OF THE THYROID GLAND

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**Aims:** We think that the solid cell nests (SCN) of the thyroid gland represent remnants of the ultimobranchial body and could contribute both C-cells and follicular cells to the thyroid gland itself as well as to some thyroid tumours. CD15 (Leu-M1) and CA19.9 are antigens detected inmunohistochemically in thyroid carcinomas, more frequently in the papillary type. We have investigated the expression of CD15 and CA19.9 in SCN.

**Methods:** We examined a series of seven cases of SCN. Immunohistochemical studies were performed on paraffin sections using the monoclonal antibodies to high molecular weight cytokeratins (34βE12, Enzo, Farmingdale, NY, USA, 1:10), CD15 (Leu-M1, Becton Dickinson, San Jose, CA, USA, 1:100) and CA19.9 (Sialyl Lewis$^a$, Novocastra, Newcastle upon Tyne, UK, 1:200), with the reaction detected by the streptavidin-biotin complex technique (StreptABComplex/HRP, Dako, Glostrup, Denmark).

**Results:** Solid cell nests were positive for high molecular weight cytokeratins, CD15 and CA19.9 antibodies. The background normal thyroid follicles were completely negative.

**Conclusions:** These results are in keeping with the endodermal origin of SCN and give further support to the link of these structures to (some) thyroid carcinomas.

## O-104

CORRELATION OF BCL-2 AND BAX EXPRESSION WITH APOPTOSIS IN HUMAN PITUITARY ADENOMAS

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**Aims:** Bcl-2 oncogene and Bax gene play an important regulatory role in apoptosis. Bcl-2 inhibits the programmed cell death without increasing cell proliferation and negatively regulates the apoptotic activity of Bax by the formation of Bcl-2/Bax heterodimers. In this study, the expression of bcl-2 and bax was investigated and correlated with apoptosis in human pituitary adenomas.

**Methods:** A series of 81 human pituitary adenomas were studied, including all the main representative morphologic types. Bcl-2 and bax proteins were detected by immunohistochemistry and the histoscore (HSC) was assessed by multiplying the extent of immunoreactivity (grade 1 to 4) by the staining intensity (grade 1 to 3). The bcl-2/bax protein ratio (BBPR) was separated in group A when ≥1 and group B when <1. Apoptosis was detected by the in situ end-labeling (ISEL) technique and the apoptotic index (ALI) was determined by estimating the percentage of positive nuclei.

**Results:** Bcl-2 protein HSC was significantly higher in nonfunctioning adenomas (P=0.02), whereas that of bax protein was significantly higher in functioning tumors (P=0.0008). In all adenoma types we observed variable mumber of scattered ISEL positive apoptotic nuclei. The mean ALI was significantly higher in functioning adenomas (P=0.03), and it was inversely correlated with bcl-2 HSC (r=-0.19, P=0.08) and directly with bax HSC (r=0.36, P=0.002). In addition, the mean ALI was significantly higher in the BBPR group B than in group A (P=0.006), and significantly predominated in functioning tumors (P=0.05).

**Conclusions:** Our findings indicate that bcl-2 and bax proteins significantly correlate with ALI and suggest that bcl-2 and bax molecules play an important role in the regulation of apoptotic mechanisms in human pituitary adenomas.

## O-105

### DISTINCT PATTERN OF *ret* REARRANGEMENTS IN MORPHOLOGICAL VARIANTS OF THYROID PAPILLARY CARCINOMAS

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Papillary thyroid carcinoma (PTC) is the most common thyroid cancer, accounting for 50-70% of all thyroid malignancies and generally has a more favorable prognosis than other carcinoma types. However, recently, within the group of PTC several morphologic variants have been reported, some of which seem to have a less favorable prognosis than the classical PTC. Among these, tall-cell variant (TCV) has been reported as a variant of PTC with a more aggressive prognosis than usual PTC. In attempt to identify a genetic marker correlated with the histologic appearance of the tumor we evaluated ret/PTCs rearrangements on a group of thyroid neoplasms operated at S. Chiara Hospital, University of Pisa between 1997 and 1998. In this study we evaluated 45 thyroid tumors including: 26 usual variants of PTCs, 5 tall-cell variants, 8 follicular variants, 1 solid variant, 2 microcarcinomas, one poorly differentiated carcinoma (PDC), one undifferentiated carcinoma, one oncocytic carcinoma. In addition we analyzed 7 non-malignant lesions. The analysis was performed by RT-PCR amplification of ret/PTC1,3 mRNA, followed by an hybridization with a specific probe. These are the results obtained: rearrangements of PTC1 type were found in 14% (6 out of 42) of the PTCs while PTC3 was observed in 31% (13 out of 42) of the cases; in particular the percentage of RET rearrangements according the subtype was the following: a) 50% (13 out of 26) of usual variant shows ret alteration, with a prevalence of ret/PTC3 31% (8 out of 26); b) 60% (3 out of 5) of the tall cell variants were rearranged as ret/PTC3; c) 12.5% (1 out of 8) of the follicular variant is rearranged as ret/PTC3.

Thus we have observed a high prevalence of ret/PTC3 rearrangements in usual variant; furthermore a high percentage of ret/PTC3 were found in tall cell variant tumors, while follicular variant shows a low incidence of ret rearrangements. This data could suggests that the different types of ret rearrangement confer neoplastic thyroid cells with distinct phenotypic properties and aggressiveness.

## O-106

### EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF α-,β- AND γ-CATENINS IN DIFFERENTIATED THYROID CARCINOMA

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**Aims:** The purpose of the study was to evaluate the role of catenins (α, β and γ) as prognostic factors in differentiated thyroid carcinoma (DTC).

**Methods:** The localization and signal intensities of α-, β- and γ-catenins were immunohistochemically analyzed in paraffin embedded specimens of 209 DTC patients treated in Eastern Finland between 1976 and 1995. All relevant patient records were reviewed for tumour characteristics, metastases, primary treatment, follow-up and possible tumour recurrence.

**Results:** The expression of α-catenin was related to tumour type ($p < 0.001$) and size of the primary tumour ($p < 0.05$). No associations between β-catenin expression and different clinicopathological factors could be established. The expression of γ-catenin was related to tumour size, distant metastases and recurrence of the primary tumour ($p < 0.05$, $p < 0.05$, and $p < 0.01$ respectively). In the univariate survival analysis γ-catenin was a significant prognostic factor for tumour recurrence ($p < 0.01$). Also in the multivariate survival analysis (including age over 60, tumour type, gender, pTNM stage and γ-catenin) γ-catenin was the only significant prognostic factor for disease-free survival.

**Conclusions:** Our results suggest that the loss of γ-catenin expression is associated with unfavourable outcome of DTC patients, and thus may be a useful marker in deciding more aggressive treatment procedures.

## P-001

DUCTAL CARCINOMA IN SITU (DCIS) AND ATYPICAL DUCTAL HYPERPLASIA (ADH) OF THE BREAST DIAGNOSED AT STEREOTAXIC CORE NEDDLE BIOPSY (SNCB). A MULTIINSTITUTIONAL STUDY.

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**Aims:** SNCB allows specific histopathologic diagnosis to be made without surgery. However, recent studies have concluded that diagnosis of ADH by means of SNCB misses about a 20% of cases that finally are carcinoma. Furthermore, SNCB diagnosed as DCIS can not reliably indicate the absence of invasion in nearly half of the patients. This is a review of the experience at three institutions, with analysis of diagnostic accuracy, when a diagnosis of ADH or DCIS has been made at SNCB.

**Methods:** SNCB of 1,221 consecutive, non-palpable breast lesions was performed between 1993 and 1998. Nineteen cases (1.6%) of ADH and 89 cases (7.3%) of DCIS were diagnosed at SNCB. Surgery was performed in 89 patients, which form the basis of the study.

**Results:** In 12 cases of ADH diagnosed by SNCB, surgery showed ADH in 7 (58%) and DCIS in the 5 (42%). No cases with benign results or invasiveness at surgery were recorded. In 77 cases of DCIS diagnosed by SNCB, surgery yielded DCIS in 56 (73%), 6 cases (8%) showed DCIS with microinvasion, and 15 (19%) showed invasive ductal carcinoma. Six (40%) of the latter corresponded histologically to invasive carcinoma with extensive intraductal component.

**Conclusions:** The diagnosis of ADH at SNCB indicates high probability of DCIS or residual ADH in the surgical biopsy. The diagnosis of DCIS at SNCB is confirmed in the majority of surgical biopsies, however, a significant number of cases may show microinvasion or invasive carcinoma.

## P-002

COULD THE STUDY OF HORMONAL RECEPTORS AND OTHER PROGNOSTIC FACTORS MODIFY THE STAGE OF T2N0M0 BREAST CARCINOMA?

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**Introduction:** Some authors suggest that T2N0M0 Invasive Carcinoma of the Breast, at present classified as Stage IIA, should be reclassified as Stage I. We pretend to know if the status of Hormonal Receptors, as well as the study of the new biological markers can contribute to this aim.

**Methods:** We have studied by immunohistochemical determination the status of Estrogen and Progesterone Receptors (ER and PR respectively), as well as the Prognostic Factors HER2/Neu, p53, and Ki67, in 103 consecutive cases of Ductal Invasive Carcinoma of the Breast. Thirty-seven of them were T1N0M0 (Stage I), nineteen were T1N1M0 (Stage IIA), and forty-seven were T2N0M0 (Stage IIA). The Statistics Computer Program Epi Info6 was used to calcule Chi square ($\chi 2$) and p of Mantel-Haenszel.

**Results:** Although the ER status of T2N0M0 tumours (Stage IIA) is more similar to T1N0M0 tumours (Stage I), than to T1N1M0 tumours (Stage IIA), we have not found significant statistical differences among them regarding Hormonal Receptors and the other Prognostic Factors.

**Conclusions:** Our study does not recommend to reclassify the T2N0M0 Invasive Carcinomas of the Breast into Stage I.

## P-003

**IMMUNOHISTOCHEMISTRY IN NEGATIVE AXILLARY LYMPH NODES IN BREAST CARCINOMA: PRELIMINARY RESULTS**

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**Aims:** To evaluate the sensibility of hematoxilin-eosin (HE) and immunohistochemistry (IH) in the detection of tumoral cells in axillary lymph nodes in the surgical treatment of breast cancer.

**Methods:** Fifty-six consecutive axilar lymphadenectomies carried out between the 1st October and 31st December 1998 have been studied for this work. Thirty-four out of the fifty-six cases studied had not metastases in the initial study with HE. Ordinary, the inclusion of the lymph nodes was total in to two o three macroscopic sections. Several microscopic sections were included in each slide.

For the present study, sections with HE have been repeated in a slide and another extra section for immunohistochemistry, using prediluted monoclonal antibody cytokeratin AE1-AE3 (Biomeda).

**Results:** In the thirty-four lymphadenectomies six hundred and four lymph nodes have been studied (average:18, range: 6 to 31). Six lymph nodes with positive cells of four different lymphadenectomies have been detected with AE1-AE3. In the new sections with HE, tumoral cells have been found in only two lymph nodes. In the four remaining lymph nodes, positive cytokeratin cells have not been recognised. The proportion of false negative is of 11.8% in the initial study with HE. The negative predictive value of HE in the routine was of 88%.

**Conclusions:** 1.- The IH improves the sensibility of the lymph nodes morphological study, increasing the number of discovered metastasic lymph nodes. This will determine the adyuvant oncologic therapeutics.

2.- It serves as the quality control of our observation with HE detecting truthful false negatives.

3.- Certain questions arise in relation to the usual methodology in the pathological study and with regard to the identification of the cytological malignity of certain keratin positive cells.

## P-004

**PROGNOSTIC VALUE OF NOTTINGHAM HISTOLOGIC GRADE IN EARLY (T1N0M0) BREAST CARCINOMA**

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**Aims**: To determine the prognostic utility of Nottingham histologic grade (NHG) and its components in a series of 278 stage T1N0M0 breast cancer patients with a median follow-up of 12 years.

**Methods:** Microscopic slides were re-examined and the degree of tubule formation, nuclear pleomorphism and mitotic rate were semiquantitatively assessed and scored according to the suggested guidelines. The association with cancer-specific survival (CSS) was evaluated by univariate and multivariate analysis.

**Results:** The proportion of grade 1, 2 and 3 was 36%, 39% and 25%, respectively. Whereas tumor size and patient age were not related to prognosis, NHG was strongly associated with CSS (P=0.0002). When evaluating the components of NHG separately, survival was significantly better in tumors with score 1 or 2 for tubule formation (10y-survival: 96% vs. 83%, P=0.0012) and in those with score 1 for mitotic rate (10y-survival: 92% vs. 80%, P=0.0013). An even stronger association with survival was observed when the proposed cutpoints for mitotic rate (fewer than 7/10HPF and more than 13/10HPF) were replaced by lower values (fewer than 3/10HPF and more than 8/10HPF; 10y-survival: 97% vs. 87% vs. 77%, P<0.0001). Regardless of the cutpoints used, mitotic score and tubule formation score retained their independent prognostic significance in multivariate analysis.

**Conclusions:** Our findings confirm the prognostic value of NHG in T1N0M0 breast carcinoma, show that evaluation of tubule formation and mitotic rate provides independent prognostic information and suggest that the proposed cutpoints for mitotic rate may be too high for this particular group of tumors.

## P-005

COMPARISON OF THE INCIDENCE OF BREAST CANCER IN THE REGION OF AACHEN IN 1996 AND 1997 WITH 16 EUROPEAN REGIONS

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**Aims:** The present study compares the rates of incidence of breast cancer in the region of Aachen in 1996 and 1997 with 16 European regions. This comparison is based on more than 50.000 breast cancer cases and a yearly population at risk of about 55 millions women.

**Methods:** These newest data on breast cancer incidence of varios regions in Europe are based on data from the period of years 1994-1996. Furthermore, the criteria of various cancer registries for the calculation of the incidence were considered. Data files of the Cancer Registry Aachen were used in the field studie of breast cancer witch is supported by the Federal Ministery of Health (Germany).
The comparison is made under the aspect that some European countries started nationwide mammographic cancer screening programs, others not. In Aachen and Germany in general there does not exist a nationwide mammographic screening program.

**Results:** The incidence of breast cancer values according to the European Standard Population amounted 94 cases in 1996 and 90 cases in 1997of invasive breast cancer per 100,000 women.This is just in the middle of the range of breast cancer incidence of other European regions without a nationwide screening programm. The increase of the breast cancer incidence in the regions without a national mammographic screening is about 15 %. The range of this increase differs between Norway with 7 % to 18 % in Saarland and Ireland. A higher increase was found for countries with a national mammographic screening programm. Interestingly a steep short time increase of the incidence is directly correlated with the beginning of the screening.

**Conclusion:** The incidence of breast cancer in Central,- and North Europe is high. According to the European Standart Population 95 invasive breast cancer cases per 100,000 women are registrated. In general, the concept of comparing regions with and without a screening programm seems to be relevant.

## P-006

MAMMOGRAPHIC FINDINGS AND HISTOPATHOLOGICAL CORRELATION OF ADENOID CYSTIC CARCINOMA OF THE BREAST

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AIMS: To describe the mammographic features of adenoid cystic carcinoma of the breast (ACC) as well as their possible correlation with histopathological findings.

METHODS: Clinical information, mammographies and histopathology of 8 ACC were reviewed. Histopathological evaluation included immunohistochemical analysis for diagnosis confirmation.

RESULTS: All patients had palpable nodules, and mammographies showed ill or partially-ill defined lesions in 5 cases. One case was a well defined nodule and another one displayed a focal distortion pattern with microcalcifications. One case was not visible on mammography due to the high density of breast parenchyma. Histologically, 6 tumors showed predominance of glandular component and 2 cases were solid.

CONCLUSIONS: In spite of its low incidence ACC is a type of breast tumor which must be recognized due to its favorable prognosis. Most ACC of the breast in our series had ill-defined margins on mammography.

## P-007

PRELIMINAR RESULTS OF A HISTOLOGICAL, FLOW CYTOMETRY AND CLINICAL FOLLOW UP STUDY. A 10 YEARS ANALISYS OF pT1 N0 M0 STAGE BREAST CARCINOMA.

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**Aims:** DNA-ploidy and S-phase fraction (SPF) determined by flow cytometry are considered to have prognostic value despite negative axillary nodes (N0) in breast carcinoma. We have performed a retrospective analysis of those flow cytometric criteria in relation to 5 years follow-up in a group of pT1NoMo breast carcinomas treated in our hospital since 1989.

**Methods.** 53 cases corresponding to 52 patients aged 32-82 years with a mean of 60.5 years were studied. Tumor size was always less than 2 cm in maximum diameter with 8 or more nodes studied. The tissue was fixed and included on paraffin . Flow cytometry was performed following Hedley's method using Propidium Iodide as fluorochrome. Uni- and multivariate statistical analysis was performed using the statistical package SPSS with the ji-square and Anova test for qualitative and quantitative variables respectively.

**Results**: The univariate analysis showed statistical significancy between histological types and SPF. In the multivariate analysis only DNA ploidy and global SPF showed statistical correlation with the development of high grade neoplasms. The survival ratio at 5 years was 79% (media of 58,8 months). Two patients had local recurrence and 2 had metastases. One patient died of unrelated cause.

**Conclusions:** Flow cytometry could provide information of prognostic value for treatment in low size breast tumor (DNA-ploidy, global SPF,diploid SPF, aneuploid SPF and DNA index). All our patients had at least one bad prognosis marker.

## P-008

**IS NOTHINGHAM PROGNOSTIC INDEX CORRELATED WITH APOPTOSIS AND P53 EXPRESSION IN INVASIVE DUCTAL CARCINOMA OF THE BREAST?**

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**Aims:** The role of the p53 as a prognostic factor is not clear in literature. P53 named as "guardian of the genome" plays an important role in many intracellular regulatory systems one of which is apoptosis having an impact on tumor kinetics. A retrospective study was undertaken to assess the relationship of the Nothingham Prognostic Index (NPI) to p53 expression and apoptotic cell counts.

**Method:** To conduct the study successively administered 160 cases of invasive ductal carcinoma of the breast were included. P53 was assessed on AP-AAP stained sections. Apoptotic cell counting was done on the HE stained routine sections on 10 HPF. Clinical data were driven from the hospital files.

**Results**: Apoptotic cell counts were higher in p53 positive group but this was not significant (p=0.079). P53 positivity was found to be related to the disease free survival (p=0.008). NPI was significantly higher in apoptotic cell containing group (p=0.006). There was a positive lineer correlation between apoptotic cell counts and NPI scores (p=0.004). This correlation was not present between apoptosis and disease free survival.

**Conclusion**: P53 expression was found to be related with disease free survival but not with the NPI which is a score composed of best prognostic indicators known today. In contrast to this apoptotic cell count was found to be closely and linearly associated to the known prognostic factors. This may suggest that the apoptotic cell counts done on routine sections may be used as a part of prognosis assessment in invasive ductal carcinomas.

# P-009

## BREAST INFILTRATING CARCINOMA WITHIN BREAST HAMARTOMA

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INTRODUCTION: Breast hamartoma is an infrequent entity with difficult clinical diagnosis and with no relationship with risk for breast carcinoma. Hamartomas, with different locations, are a well-known component of Cowden's disease, the latter conferring a mild risk increase of malignant tumour development, among them breast cancer.
CASE HISTORY: 73-year-old woman without any relevant antecedents. She had a one-year history of painless increase in the right breast size. After a radiological suspicious image, a well-delimited round mass was resected, measuring 9x 4,5 cm in its longest dimension, also having an irregular, hardened, and star-shaped area in one of its edges. Histologically the presence of a infiltrating breast carcinoma within a breast hamartoma was confirmed. Total mastectomy plus axillar lymphadenectomy were performed. Neither residual lesion nor lymph node involvement were observed. The patient did not have other lesions suggestive of Cowden's disease. The patient is currently free of lesion after the protocolised treatment.
CONCLUSIONS: In spite of the absence literature on this subject, breast hamartoma, which is not specially related to Cowden's disease, can harbour breast carcinoma.

# P-010

## CYSTATIN A IN BREAST CANCER

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**Aims**: Testing of the prognostic value of cystatin A (ACPI, acid cystein proteinase inhibitor) immunohistochemistry in breast cancer.
**Method**: 440 formalin-fixed paraffin embedded samples of breast cancer from years 1988-1991 were collected from the files of our department. After exclusion of patients with disseminated disease at diagnosis, previous contralateral breast cancer, or absence of follow-up, samples were left from 384 patients. Monoclonal cystatin A antibody WR-23/2/3/3 was used and the binding detected with the avidin-biotin peroxidase method.
**Results**: Positive staining for cystatin A was found in 52 cases. The staining was irregular showing islands of positive cells among negative background. Most positive tumors were of ductal infiltrative type, two were mucinous carcinomas, one medullary, and one squamous cell carcinoma. No positive lobular carcinomas were found. Focal cystatin A positivity was found in the myoepithelial cells of the benign ducts. Occasional apoptotic cells showed positive staining. Tumors with cystatin A positivity were large in size and had higher mitotic activity than cystatin A negative tumors. Cystatin A was negatively correlated with bcl-2 staining. The risk for breast cancer death was higher among cystatin A positive tumors than cystatin A negative tumors. The risk increase was significant also in lymph node negative patients. Adjustment for tumor size, histological grade, and lymph node status did not change the conclusions: cystatin A positive tumors were associated with higher risk of death than cystatin A negative tumors.
**Conclusion**: The study reveals a new variant of aggressive breast cancer. The development can be expected to be associated with neoplastic progression through genetic instability, which allows cystatin A expression and gives growth advantage to cell clones with cystatin A positivity.

# P-011

## PATHOLOGICAL RESULTS FROM A BREAST CANCER SCREENING PROGRAM IN TWO DISTRICTS OF BARCELONA

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**Aims:**We report the pathological findings from a breast cancer screening program (November 1995 to March 1998), in a population of 27,726 women from two districts in Barcelona.
**Methods:** Women between 50 and 64 years, not subjected to mammography in the last 12 months, without past history of breast cancer, were included in the study. A double - projection bilateral mammography was performed, with double -. blind readings, and a third reading in cases with disagreement. When the radiologic findings were consistent with or diagnostic of malignancy, fine needle aspiration cytology, stereotactic core biopsy, and/or excisional biopsy with radiologic localization were performed.
**Results:** From the screenable population, 15,173 women (76.1%) were enrolled in the study. A total of 98 breast cancer cases (6.4%) were identified, 56.1% of them in women between 60 and 64 years.
Histological classification of these cases was as follows: infiltrating ductal ca, 68; ductal carcinoma in situ, 9; infiltrating lobular ca, 7; tubular ca, 5; mucinous ca, 2; medular ca, 2; and not otherwise specified, 5 cases. From the 597 FNAC, there were 63 positive, 473 negative cases; 61 cases were not adequate. From 187 core biopsies, there were 22 positive, 134 negative and 31 non-adequate cases. From 36 excisional biopsies, 13 cases were positive and 23 negative. Tumor size was < 10 mm in 22, >10 mm in 69, and Tx in 8 cases. Lymph nodes were N0 in 69, N1 in 16, N2 in 5, and Nx in 8 cases. Distant metastatic stage was M0 in 89, M1 in 1 and Mx in 8 cases.
**Conclusions:** The proportion of lesions obtained, and the results of the enrolment are in agreement with similar studies reported in the literature.

# P-012

## CUTANEOUS ANGIOSARCOMA OF THE BREAST 2 YEARS AFTER CONSERVATIVE TREATMENT AND RADIATION THERAPHY.

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Angiosarcoma (AS) is an uncommon breast tumor that rarely develops after external beam radiation therapy. In view of the increasing use of breast conservative therapy (BCT) with radiation therapy (RT) in the last 15 years, the number of patients with BCT-associated AS is likely to increase. Cutaneous AS following radiotherapy for breast carcinoma has been rarely documented. We report an additional case of AS of the skin of the breast which develops after conventional postoperative irradiation.

In 1992, a 57 year-old woman had a segmental excision with axillary dissection for a microinvasive ductal carcinoma. Histologically the excision margins were free of tumor and there weren't nodal metastases. Postoperative RT was given (50.4 gy). Twenty-seven months after RT, the patient presented an area with an infiltrative lesion with oedema of the skin, erithema and annular ecchymosis at the site of radiation exposure. A punch biopsy of the skin revealed an increase of vascular structures with some diffuse clefts, focal perivascular inflammation and a small solid nest intravascular of atypical cells in dermis. The cells were negative for immunoreactive factor VIII and keratin- associated antigen and the diagnosis was carcinoma with vascular invasion. Five months later the patient underwent a mastectomy and the microscopic exam revealed three areas with moderate-differentiated (grade II) AS. Nine months later she presented a nodule in the mastectomy scar which histologically showed unremarkable infiltration of the full thickness of the dermis by typical AS with abundant mitosic figures. Positive findings at immunohistochemical staining (CD34 , Ulex , FactorVIII - related antigen) for endothelial tissue confirmed the diagnosis of high grade cutaneous AS. Since then five recurrences have ocurred around the mastectomy scar. After 11 months the patient is alive without evidence of other distant metastases.

The median patient age of 68 years at RT before developing an AS is higher than the media age of 49 years in a population undergoing BCT without subsequent AS. There is a posible higher susceptibility for radiation induced AS with advancing age.
The pathogenesis of AS of the breast is unclear. Radiation may play a role in the development of sarcomas, with unusual latency times of 10-20 years. The latency time for AS following BCT is generally shorter 74 months (range 29-106 months) and this might indicate a different mechanism of pathogenesis. In our case the latency time was 27 months.

The diagnosis of cutaneous AS should be considered in patients who have new lesions within a region previous RT. This diagnostic consideration should be given more importance as RT for the treatment of breast carcinoma becomes more popular. As this present case illustrates cutaneous AS may arise shortly after RT.

## P-013

**The prognostic significance Mib-1, p53, bcl-2 and cerbß-2 expression in screen detected breast carcinomas and correlation to histological parameters**. Susanne Duun, MD. Department of Pathology, University of Copenhagen, Bispebjerg Hospital, Copenhagen, Denmark.

**Aim**: To determine the pathological and biological characteristics of invasive breast carcinomas diagnosed by screening and examined in the Copenhagen screening programme trial.

**Material and methods**: Immunohisthochemistry with antibodies recognising Mib-1, c-erbß-2, p53 and bcl-2 encoded proteins was performed on 232 invasive carcinomas from a prevalence screening in Copenhagen 1991-1993. Immunohisthochemical findigns were compared with conventional pathological parameters.

**Results**: Bcl-2 protein was expressed in 87.5% of the carcinomas and was significantly associated with low grade, positive ER, PGR receptors, low Mib-1 expression and inversely associated with p53. Tumour type, size, lymph node involvement and c-erbß-2 expression did not show a statistical significant association with bcl-2. P53 was expressed in 25% of the carcinomas and was associated with high grade, size, lymph node involvement and receptor negative tumours and positive correlated to MIB-1 and cerbß-2 . Cerbß was expressed in 12.5% and was significantly correlated with high tumour grade, type, lymph node involvement and receptor negative tumours. High expression of Mib-1 was significantly associated with high grade and over expression of cerbß-2, while there was negative relation to ER and PGR.

**Conclusions**: The results of this study indicate that statistical analyses conducted on biological and clinico-pathological parameters might constitute an integrated approach to data analyses useful for distinguishing different biological behaviour and therapeutic groups in breast carcinomas.

## P-014

IMMUNOHISTOCHEMICAL EXPRESSION OF p21, p27 AND p16 PROTEINS IN $N_0$ INVASIVE BREAST CARCINOMA: PROGNOSTIC IMPLICATIONS AND RELATIONSHIP TO HISTOPATHOLOGICAL PARAMETERS.

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**Aims**: To investigate the expression of CDKIs (cyclin-dependent kinase inhibitors) in $N_0$ invasive breast tumours and compare their expression with the major biopathological prognostic indicators to identify more aggressive subgroups.

**Material and Methods**: Archival paraffin embedded tissues from 120 $N_0$ invasive breast carcinomas. CDKIs ($p21^{Cip1/Waf1}$, $p27^{Kip1}$ and $p16^{CDKN2/MTS1}$), oestrogen and progesterone receptors (ER/PR), MIB-1, pRb and p53 proteins were identified by immunohistochemistry and score values were recorded by image cytometric analysis. The follow-up time was 10 years.

**Results**: Low expression of p27 protein (< 50%) was seen in 57 cases and was associated with a poor prognosis ($p = 0.03$) and high-grade tumours and inverse correlation with oestrogen/progesterone receptors. p21 was expressed (> 10%) in 62 tumours and had inverse association with p53 protein, histological grade and ER/PR receptors. There was no association between p16 staining (43 cases) and any histopathological parameters.

**Conclusions**: The p27 protein could be useful to identify patients who might benefit from adjuvant therapy. In a Cox multivariate analysis neither p21 nor p16 were an independent predictor of patient outcome.

## P-015

CYTOLOGIC GRADING OF FINE NEEDLE ASPIRATES OF BREAST CARCINOMA – CORRELATION WITH HISTOLOGIC GRADE AND Ki-67 / PCNA IMMUNOSTAINING

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**Aims**: The reliability of breast cancer grading system in fine needle aspirates and correlation with immunohistologic application of Ki-67 and proliferating cell nuclear antigen (PCNA).

**Methods**: Thirty consecutive breast cancer fine needle aspirates and their surgical specimens were reviewed. The aspirates and histologic sections were graded according to simplified Black (SB) and Bloom- Richardson systems, respectively. Ki-67 and PCNA monoclonal antibodies were applied to the histologic sections by using immunoperoxidase methods.

**Results**: The cytologic and histologic grades were well correlated. A significant correlation between two proliferation marker labelings and histologic grade and mitotic activity was found.

**Conclusion**: The cytologic grade can be used to predict the histologic grade of the breast carcinoma. Ki-67 and PCNA immunostainings provide valuable information and are correlated with histologic grade. This information could be very important especially in chemotherapy planning of inoperable breast cancer patients.

## P-016

**STEREOTAXIC NEEDLE-CORE BIOPSY IN BREAST MICROCALCIFICATIONS.**

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**Aims:** Evaluation accuracy of stereotaxic needle-core biopsy (SNCB) in mammary nonpalpable microcalcifications and other ultrasonographically undetectable lesions.

**Methods:** We have retrospectively analyzed 100 lesions (1996-1998), from 85 women (37-68 years). Seventy-seven lesions, presented calcifications as major imaging feature and 23 were calcifications with nodule, asimetry or increased density. Fifty of them were classified as low-suspicion, and other 50 moderate or high-suspicion. In every case, three biopsies were performed with a 14 gauge tru-cut biopsy needle. Lesions classified as low-suspicion mammography with benign microscopic features were followed by mammography (mean 12 months) and the remaining ones were surgically removed.

**Results:** Five histological kinds were made: Normal or not valuable (2); benign (60); benign with atypia (2); suspicious (3) and malignant (33). Sensitivity: 90%; Specificity: 94%; PPV: 100%; PNV: 90%; Accuracy: 96%. Valid samples for diagnosis: 98%. False-positive: 0%. False-negative: 8%. Avoided surgical biopsies: 46. Malignant tumors presurgically diagnosed: 33.

**Conclusions:** SNCB-14G is an accurate method to diagnose breast calcifications and other nonpalpable lesions unrecognizable at sonography. A minimum number of three biopsies with SNCB-14G obtain better results than a single biopsy with a larger gauge needle (18 y 16 G), both in sensitivity and specificity. Moreover, the number of insufficient samples decreases significatively (from 16% to 2%).

In our series SNCB has false-positive rate 0%, so after malignancy results definitive surgical treatment must be made. Diagnostic surgical biopsy must be recommended when results of suspicious or atypia are obtained. After benignancy results in moderate or high suspicious mammographic lesions, confirmation biopsy must be made.

## P-017

ENDOCRINE CELLS IN THE BREAST CARCINOMAS AND THEIR PROGNOSTIC VALUE

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**Aims:** identification of endocrine cells (EC) in breast carcinomas (BC) and studying their influence on course of disease.

**Methods:** histochemical (Grimelius's reaction) and immunohistochemical (antibody to chromogranin A) methods were used for identification of EC in 175 BC. In statistical analysis criteria $\chi^2$ were engaged for determination of clinical differences between BC with EC and "common" carcinomas of the breast. The survival data of patients which had BC with EC were estimated by Kaplan-Meyer's method.

**Results:** the breast tumors with EC (60 cases) were divided into three groups depending on quantity of EC: 1- BC with single EC (30 cases); 2- cancers which contained 20-50% of EC (18 cases) and 3 - BC with more than 50% of EC from whole tumor cells (12 cases). The histological characteristics of all tumor groups were revealed. It was shown that forming of solid and alveolar structure is usual for BC with EC. Correlation analysis of the clinical characteristics like ages of patients, tumors size, lymph nodes conditions as well as differences between the groups of BC and following up BC with EC was made. We didn't find any significant differences between course of BC with and without EC. We also revealed visible tendency of BC with EC of 2-th group to aggressive behavior and this tendency influence on the criteria "survival" for patients of this group ($p<0,05$).

**Conclusions:** There are no significant differences between BC with EC and "common" carcinomas of the breast. But we found worth prognosis data for patients who had 2-d group of BC with EC.

## P-018

CYSTIC HIPERSECRETORY BREAST LESION

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**Aims :** Cystic hipersecretory breast lesions have a morphologic feature, marked secretory activity with formation of dilated ducts and cysts containing homogeneous, eosinophilic material which resembles thyroid colloid. These lesions are inusually and go from cystic hypersecretory hyperplasia (" CHH ") to cystic hypersecretory carcinoma ("CHC "). CHC is a variant of duct carcinoma with a histologic or cytologic features can be deceptively bland ; the majority of cases described thus far have been intraductal carcinomas ( with an epithelium of some cysts and ducts grows as micropapillary pattern). A biopsy that consists entirely of cystic elements, lacking the papilllary component, should be termed CHH.

**Methods :** We report a case of 63-year-old woman, with a 2-cm-diameter, firm mass in the left breast. Mammography and clinical exploratory showed a marked asymmetry breast. A FNA was performed without success. A biopsy was recommended. Preoperatory study included a frozen section and cytologic extension. Postoperatory study included H-E , histochemical stain ( orcein, mucicarmin and PAS ) and inmunohistochemical techniques ( ER, PRG, p53, c-erbB-2, bcl-2, MIB-1, CEA and Thyroglobulin ).

**Results :** The case reported shows after including of all lesion for microscopic examination, cysts lined by a bening-appearing flattened or single-layered cuboidal epithelium, other cysts, however, show multilayered lining of ductal epithelial cells without atypia, like in CHH. The intracystic material was stained intensely with PAS and was negative with mucicarmin stain.

**Conclusions :**This rare lesion has difficulty diagnostic in FNA or frozen section. We recommend to include all lesion for microscopic examination and follow up the patient even those cases of single CHH. Studies with p53, c-erbB-2, bcl-2 and MIB-1 could be useful in the diferencial diagnostic between CHH and CHC.

## P-019

ANGIOGENESIS IN DUCTAL CARCINOMA IN SITU OF THE BREAST. STUDY BY IMAGE ANALYSIS SYSTEM.

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**Aims**: Angiogenesis is a critical factor in both local growth and metastasis of solid tumors and can provide an important independent factor, which predicts prognosis in breast cancer. Studies in experimental animals and humans have indicated that ductal carcinoma in situ of the breast (DCIS) is capable of inducing neovascularization. The purpose of this study is to analyze angiogenesis and its relationship with nuclear grade, tumor size and histological subtype of DCIS.

**Methods**: Neo-vascularization was determinate labelling microvessels with factor VIII immunostain (Dako®, Denmark) and analyzed by image analysis system (Visilog5, Microptic®, France) Fifty-three cases of DCIS were included, studying 6 images at 200 x scanning power of each case. Endothelial area, relative and absolute, was counted in each image. Mean and standard deviation of endothelial areas of each case was correlated with histological grade (Van Nuys classification), tumoral size and histological type, using ANOVA test.

**Results:** The mean endothelial area was variable between 1.1% and 5.1% of the total field analyzed (absolute values, between 1428.5 and 6523.4 $\mu^2$). Mean endothelial area was higher in DCIS grade 3 (2.9% Vs 2.4% and 2.3% in grades 1 and 2), although without statistical significance. Mean endothelial area was higher in tumors greater than 4 cm (4.1% Vs 2.4% in tumors smaller than 15mm, p=,02). Relation between angiogenesis and histological subtype of DCIS was not found.

**Conclusion:** In conclusion, this study indicates a higher grade of angiogenesis in cases of DCIS of greater size and a tendency of increased number of vessels in cases of high histological grade DCIS.

## P-020

**APOPTOSIS DETERMINATION WITH TUNEL STAINING IN LOCALLY ADVANCED BREAST CANCER**

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**Aims:** Chemotherapeutic drugs can effect neoplastic cells by means of apoptosis which is a cellular response to fatal stimuli. Apoptosis together with mitosis determine the growth rate of a tumor tissue. In this study we aimed to understand the effects of chemotherapy both on mitotic and apoptotic cell counts by using paired specimens taken before and after.

**Methods:** The study group was composed of 25 cases of locally advanced breast cancer which were treated with neoadjuvant chemotherapy. Both biopsy and mastectomy specimens were examined for the mitotic and apoptotic cell counts. Counting was performed both at the center and the periphery of the tumors in 10 HPF, on H&E stained sections. TUNEL staining for apoptosis was also done both to the biopsies and the mastectomy specimens and counting was performed through out the tumor since staining cells were few in number.

**Results:** Both mitotic and apoptotic cell counts were found to be elevated following chemotherapy. This increment was only statistically significant for the apoptotic cell counting done at the tumor periphery ($p<0.005$). With TUNEL staining there was a significant difference in between the apoptotic cell counts of the groups when divided into two; as one with decreased size and the other composed of tumors with same or increased size ($p<0.005$). Poorly differentiated tumors with striking pleomorphism showed higher apoptotic cell count ($p<0.005$).

**Conclusion:** Following the chemotherapy apoptosis was found to be more prominent at the tumor periphery where as a rule tumor growth was expected to be more pronounced. Apoptotic cell counts were higher in the group with decreasing tumor sizes. Depending on these findings apoptotic cell count could be used as a part of chemotherapy response determination.

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## P-021

**GRANULOMATOUS STROMAL REACTION IN BREAST CARCINOMA: Report of a case and review of the literature**

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**Aim:** To present a case of granulomatous stromal reaction in invasive breast carcinoma and to review the literature of this comparatively rare condition.

**Material and Method:** A 66 year old woman presented with a mobile, 2 cm lump in the border of the inner quadrants of her left breast. Wide local excision and axillary dissection was performed. In her history, healed tuberculosis of the lung, partial thyroidectomy and appendectomy are to be mentioned.

**Results:** Histologically, the tumour was an invasive ductal carcinoma of no special type, modified Bloom-Richardson grade 2. No axillary lymph node metastasis was found. The stroma of the tumour showed widespread granulomatous reaction. The granulomas were of "sarcoid"-type: small granulomas composed of epitheloid cells and multinucleated Langhans giant cells were present. There was no associated necrosis. Acid fast bacilli could not be detected within the granulomas. Granulomas were not seen in the surrounding breast parenchyma, nor in the axillary lymph nodes.

Only sporadic cases of granulomatous stromal reaction associated with breast carcinoma have been reported in the literature. Bässler, in 1988, published five cases and gave a detailed discussion of the condition, which differs from the entity usually described as "breast carcinoma with osteoclast-like stromal giant cells".

**Conclusion:** Granulomatous stromal reaction in breast carcinoma is a rare condition, which is not associated with systemic granulomatous disease of the patients. In our case, the healed lung tuberculosis does not seem to be related to the unusual reaction, but should be regarded as a coincidence.

## P-022

**SOLID PAPILLARY CARCINOMA OF THE BREAST, DIMORPHIC VARIANT. STUDY OF FOUR CASES WITH CYTOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS**

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**Background:** Solid papillary carcinoma (SPC) of the breast is recognized as a rare type of intraductal carcinoma usually occurring in older women. A variant of this tumor is the dimorphic carcinoma (DC).

**Aims:** To evaluate the cytological, histological and immunohistochemical features of SPCDC.

**Methods:** We studied four cases of SPCDC. Cytological smears were stained with Diff-Quick and Papanicolaou Stains, Streptavidin complex using CAM 5.2, HHF35, Alfa-actin, S-100 protein, Ki-67, P53, Bcl-2, c-erbB-2, estrogen and progesterone receptors were applied.

**Results:** The ages ranged from 47 to 84 years-old (mean 67). Cytologically, the smears were hypercellular with low grade pleomorphism. Histologically the tumors consisted of solid masses of neoplastic cells with central tubular and fibrovascular structures. Immunohistochemical study showed strong positivity for cytokeratins (CAM 5.2) in the tumor cells whereas muscle-specific antigen (HHF35), alfa-actin and S-100 was uniformly negative. The tumors stained positively for estrogen and progesterone receptors.Ki-67 expression was low and P53, Bcl-2, c-erbB-2 were negative. Axillary lymph nodes were free of metastasis.

**Conclusions:** Cytologically it is not possible to recognize this variant. The nuclear features may allow the diagnosis of malignancy although because of the low cytologic atypia, these lesions may be misdiagnosed as benign. Immunohistochemical stains indicates a low grade tumor.

## P-023

C-erbB-2 ONCOGENE EXPRESSION DETECTED BY FISH IN CORRELATION WITH MEMBRANE EXPRESSION OF c-erbB-2 ONCOPROTEIN IN PATIENTS WITH BREAST CARCINOMAS

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**Aims:** c-erbB-2 oncogene plays an important role in the development and progression of breast carcinoma. Contemporary anti-cancer therapy is designed to use an antibody directed against the extracellular epitope of the c-erbB-2 oncoprotein. In decision whether such a therapy should be administered it is necessary to establish the status of the c-erbB-2 expression. In this study we compared the expression of the oncoprotein on cell membranes and the status of the c-erbB-2 gene using FISH in patients with breast carcinomas.

**Methods:** FISH with digoxigenin labeled probe and a signal amplification system were used. We utilized tissue imprints, nuclear isolates and paraffin sections to determine the most suitable method for detection of c-erbB-2 gene amplification. For assessing the expression of c-erbB-2 oncoprotein we used immunohistochemical (IHC) staining of membrane positivity on the tumor cells. Expression of the estrogen (ER) and progesteron (PR) receptors, and tumor cell positivity with proliferating cell nuclear antigen (PCNA) were compared with the c-erbB-2 status.

**Results:** Comparing the methods of tissue processing for FISH we have established that imprints and tissue sections yielded the most appropriate results. Imprints were easy to prepare and were readily assessed. The tissue sections offered the advantage of assessing the structural morphology and to evaluate the expression of the gene in defined cell populations. The membrane positivity of c-erbB-2 oncoprotein was detected in cases of the gene amplification (6 – 15 signals per cell). Cases negative for c-erbB-2 oncoprotein shoved two signals per a cell revealing a diploid status of the c-erbB-2 gene. A majority of cases positive for c-erbB-2 oncoprotein disclosed a high percentage of tumor cells positive with PCNA whereas a simultaneous expression of both c-erbB-2 and hormone receptors was variable.

**Conclusions:** FISH on imprints and paraffin sections is a suitable method for establishing the c-erbB-2 gene status and we recommend using these two approaches in combination. The status of the c-erbB-2 gene showed a good correlation with the c-erbB-2 oncoprotein expression on tumor cell membranes as demonstrated by IHC. The utilized methods are appropriate before therapy with antibodies directed against c-erbB-2 oncoprotein is considered.

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## P-024

**RELATIONSHIP BETWEEN HORMONE RECEPTOR EXPRESSION AND DNA FLOW CYTOMETRIC PARAMETERS IN BREAST CARCINOMA**

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**Aim:** To establish the possible relationship between hormone receptor expression and flow cytometric parameters (ploidy, S-phase) in infiltrating breast carcinoma.

**Methods:** 290 cases of infiltrating carcinoma of the breast were immunohistochemically analyzed for estrogen and progesterone receptors expression. Flow cytometric DNA analysis of ploidy and S-phase was performed on paraffin block sections.

**Results:** Results are summarized in the following table:

| | | HORMONE RECEPTOR STATUS | | | | |
|---|---|---|---|---|---|---|
| PLOIDY | | ER⁻/PR⁻ | ER⁻/PR⁺ | ER⁺/PR⁻ | ER⁺/PR⁺ | TOTAL |
| DIPLOID | S<7% | 20 | 3 | 26 | 70 | 119 |
| | S≥7% | 2 | 0 | 3 | 3 | 8 |
| ANEUPLOID | S<7% | 26 | 6 | 28 | 62 | 122 |
| | S≥7% | 18 | 2 | 5 | 16 | 41 |
| TOTAL | S<7% | 46 | 9 | 54 | 132 | 241 |
| | S≥7% | 20 | 2 | 8 | 19 | 49 |

**Conclusions:**

ER⁻/PR⁻ tumors were more frequently aneuploid than those with any positivity. (p=0.03)

High S-phase (≥7%) was more frequent in ER⁻/PR⁻ tumors. (p=0.0018)

Tumors with high S-phase were more frequently aneuploid (p=0.00004) irrespective of its hormone receptor status.

## P-025

**BENIGN AND BORDERLINE PHYLLODES TUMOR OF THE BREAST. CORRELATION WITH LOCAL RECURRENCES IN A SERIES OF 41 CASES.**

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**Aims:** To investigate the possible correlation of some clinicopathological features (age, tumor size, benign and borderline type) in order to predict local recurrences in Phyllodes tumor (Pht).
**Methods:** We studied a series of 41 female patients, the ages ranged from 17 to 74 yr.- old (mean 44), sizes ranged from 2 to 36 cm (mean 6,7), Histologically 31 were benign and 10 borderline, 11/41 cases exhibited local recurrences. Follow-up ranged between 2 to 10 years.
**Results:** Local recurrences were observed in 5/16 patients <40 yr.-old (31%) and in 6/25 patients >40 yr.-old (24%) (n.s.). Cases with recurrences showed a mean size of 5,6 cm vs. 7,1 cm without recurrences (n.s.). The mean size of tumors from patients with recurrences in <40 yr.-old was 4,9 cm vs. 7,8 cm in patients >40 yr.-old (n.s.). Recurrences were observed in 7/31 (23%) benign Pht vs. 4/10 (40%) borderline Pht (n.s.).
**Conclusions:** Our results showed that none of the clinical variables studied was useful in predicting local recurrences. Besides, because of the higher tendency to local recurrence in 40% of borderline Pht, with independence of age and tumor size, we justify the designation of this category.

## P-026

**EXPRESSION OF $p27^{kip1}$ PROTEIN IN DUCTAL CARCINOMA IN SITU OF THE BREAST**

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**Introduction:** $p27^{kip1}$ protein, a cyclin-dependent kinase inhibitor, has been reported to be a powerful prognostic marker in patients with breast carcinoma. However, to this day, studies of $p27^{kip1}$ protein expression in ductal carcinoma in situ (DCIS) have been extremely limited. The detection of DCIS has increased with the advent of mammography but the controversy in the appropriate treatment of this lesion still remains. Therefore, a comprehensive understanding of the biologic features of DCIS is needed.
**Methods:** We studied the immunohistochemical expression of $p27^{kip1}$ protein in 49 cases of DCIS and compared the findings to the clinicopathologic parameters (age, gross type, histologic type and nuclear grade), and the expression of cyclin D1, p53 and estrogen receptor (ER).
**Results:** Strong nuclear staining of $p27^{kip1}$ protein was seen in 23 (46.9%) cases. The $p27^{kip1}$ protein expression correlated with the cyclin D1 immunopositivity ($p<0.005$) and ER expression ($p<0.05$). No significant associations were seen in the $p27^{kip1}$ protein expression and clinicopathologic parameters. The overexpression of cyclin D1 (59.2% of the cases) correlated positively with ER expression. The p53 protein expression was identified in 30.6% and the poorly differentiated DCISs were more likely to be p53-positive ($p<0.05$).
**Conclusions:** Our data suggest that the expression of $p27^{kip1}$ protein as well as cyclin D1 and p53 protein may be influenced by the ER status in DCIS. The significantly positive correlation of $p27^{kip1}$ protein and cyclin D1 expression supports the theory that the balance of the two opposing signals is important in determining the cell proliferation in breast cancers. Therefore, a loop reaction of ER-$p27^{kip1}$-cyclin D1 may be the most useful prognostic marker in DCIS.

## P-027

**BREAST CANCER PROGNOSIS MARKERS AND THE EXPRESSION OF HSP-70 AND HSP-27.**

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The expression of two major stress proteins, hsp 70, hsp 27 was analysed in this study to find a correlation with other known tumoral markers for a better prognosis/diagnosis in the clinical management of breast cancer. Thirty four samples of human breast tumor, were analysed by immunohistochemistry using a broad range of current tumor markers: cell proliferation markers (PCNA, Ki67), oncogenes (p53,c/erbB2), and hormonal receptors (estrogen and progesterone). In addition heat shock protein, hsp 70 and hsp 27, were studied by immunohistochemistry and Western blot, as novel prognostic factors.
The main results may be summarized as follows. There is a positive relationship between hsp70 and PCNA expression, whereas hsp27 is inversely correlated. There is also positive correlation between hsp70 and hormonal receptors (estrogen and progesterone), but these correlations do not exist for hsp27 expression. However, there is a positive correlation between hsp27 and c/erbB2.
In summary, it seems that both hsp70 and hsp27 expressions are rather high in human breast cancers. The expression of hsp70 is much higher than hsp27. They might be proposed as markers for tumoral diagnosis, and it seems that hsp70 is rather correlated with proliferation, whereas, hsp27 would be in relation with differentiation as shown by its correlation with c-cerbB2.

## P-028

**EXPRESION OF ESTROGEN RECEPTORS AND RELATIONS WITH PROTEIN P-53 IN BREAST CANCER**

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**AIMS**
A group of 85 breast cancer patients were analyzed inmunohistochemically for the expresion of estrogen receptors (ER) and p-53, protein to determinate the relationship between them.
**METHOD**
Tissue blocks fixed in 10% formaldehyde solution and embedded in paraffin were available for all the patients. The estrogen receptors inmunohistochemistry was carried out using and a commercially avalilable kit ( H-222, ER-ICA-Abbot Laboratories) and was valorated using the HISTOCORE method (threshold of positivity >100).
The immunolocalization of p-53 protein in formalin-fixed and paraffin-embedded tissues was obtained by the monoclonal antibody DO-7 ( 1:100 dilution )
**RESULTS**
Fifty nine percent (50 cases)of the 85 patients with breast cancer examined were ER positive. The p-53 protein was positive in 22 cases (25%), 11 had ER positive and 11 ER negative; but only 7 had a staining percentage >10%. Of this cases 4 had infiltrating ductal carcinoma (27% ) and 3 medullary carcinoma (75% of all the medullary carcinomas in the serie).
**CONCLUSIONS**
In our study no association was demostrable between estrogen receptors and the p-53 protein ($p=0,07$). We confirm the positive correlation p-53 protein with the medullary carcinoma ($p<0'001$).

## P-029

**PROGNOSTIC SIGNIFICANCE OF HORMONAL RECEPTORS AND Ki-67 EXPRESSION IN BREAST CANCER AND ITS RELATIONSHIP TO OTHER CLINICAL AND HISTOLOGICAL PROGNOSTIC FACTORS.**

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**Aims :** To evaluate the relationship between clinical, histological and immunohistochemical prognostic factors in breast carcinoma.

**Methods :** Primary breast carcinoma tumours from 257 patients were examined for clinical and histological variables, hormonal receptors and cell proliferation index (Ki-67). The estrogen and progesterone receptors were assessed according to the intensity of staining (I), with values from 0 to 3, and the percentage of positive cells (P). A histoscore was calculated by the formula, (I + 1) x P (range 0-400). Cases with a histocore value of over 100 were considered positive. The cell proliferation fraction (Ki-67) was assessed by counting 500 cells in the infiltrative areas of maximum positivity, expressing the number of positive cells as a percentage.

**Results :** Positive estrogen receptors stains were found in 61.5 % of the tumours, whereas 47.3 % were progesterone receptors positive. The percentage of tumour cells stained for Ki-67 ranged from 0 to 69% (X=9). The statistical analysis showed a relationship between age, histologic type, nuclear grade, tumour necrosis, inflammatory cell reaction and immunohistochemical markers. Moreover, estrogen receptor absence and high positivity of Ki-67 were indicative of poor prognosis.

**Conclusions :** Patients without estrogen receptors and a high cell proliferation index (Ki-67) have a high rate of recurrence or metastasis and they usually die from this illness.

## P-030

**PATHOLOGY OF CHEMOTHERAPY-RELATED CHANGES IN BREAST CANCER. Preliminary evaluation of 150 cases treated with primary (neoadjuvant) chemotherapy.**

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**Aims:** To study morphologic changes related to chemotherapy in breast cancer.

**Methods:** A series of 150 patients with operable breast tumors (≥ 30 mm.), submitted to primary chemotherapy (Protocol 3490). Malignancy was confirmed by a small open biopsy (30 cases) or by fine needle aspiration cytology. Primary chemotherapy started with 3 cycles of CMF (600/40/600,days 1 and 8). Assessment of response was made by mammography. The response was classified as Complete (CR); Partial (PR) ≥ 50%; Partial (PR) < 50%; and Negative. The percent of microscopic infiltrating tumor reduction (replacement by fibrosis) was calculated with two independent observer agreement and added to the mammographic reduction. After surgery, patients with response (complete or > 50%) received three new CMF cycles. Patients without response (< 50%) received doxorrubicine.

**Results:** Changes secondary to chemotherapy, representing at least 10% of tumor volume were identified in 59 tumors (39.6%) and in at least one metastatic node from 8 tumors (11.6%). They include distinct loose fibrosis with capillary neoformation, that trend to confluence, periductal inflammatory infiltrate (ductulitis), stromal microcalcifications and accumules of foamy macrophages outside the ductal lumen. In situ ductal component and intravascular tumor cells seems to be little affected by chemotherapeutic regression. In all cases with RC the mammographic and pathologic evaluation were concordant (only isolated remaining tumor cells in occasional lesions). However pathologic evaluation represented an increase ≥ 20% in response in 13% of tumors with RP≥50%, 20.6% of tumors with PR<50% and interestingly in 5% of tumor lacking any clinical evidence of response.

**Conclusion:** Pathology can add valuable information to the assessment of response to primary chemotherapy.

## P-031

**DIABETIC MASTOPATHY: REPORT OF 3 CASES**

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**Aims:** Diabetic Mastopathy is a clinico-pathologic entity. It represents less than 1% of benign breast diseases and it is mainly related to type I diabetes. Pathogenesis is unknown: extracellular deposit of collagen and B-cell predominant inflammation with autoimmune response are the the most convincing hypothesis. The clinical, radiological and pathological -including cytological and immunohistochemical- features of three patients with diabetic mastopathy are presented.

**Methods:** Three female patients with "breast masses" of uncertain nature were studied. Two of them were diagnosed and treated of their diabetes. An excision biopsy was performed in all cases.One of them had a previous FNA citology.Material was routinely processed. Sections were obtained for H-E and immunostaining studies. Clinical and mammographical data were also registered.

**Results:** The three cases were long standing right breast nodules. One of them was highly suspicious of inflammatory carcinoma. Mammographic features ranged from high density irregular mass to stellate and microcalcified nodule. Macroscopically, all of them were firm, white, fibrous and well defined masses. Microscopically, wide areas of keloid fibrosis were seen. One of the cases showed mononuclear vasculitis. Two cases presented ductitis. All cases showed lobulitis with a lymphocitic infiltrate. In one case sparsed epithelioid fibroblasts could be seen between the bands of dense hyalinized collagen. The cytological study of one case showed fibroblasts with isolated epithelial cells. In two cases immunohistochemical study showed immunoreactivity for CD20 in the mononuclear cells. Epithelioid fibroblasts were positive for vimentin. The three patients remain well after surgical excision. The woman who was suspected of having an inflammatory carcinoma was not treated for her diabetes previously. She has improved after antidiabetical treatment.

**Conclusions:** The importance of this entity is that it can be usually confused clinically and radiologically with a breast carcinoma.The biopsy is essential in these cases for excluding carcinoma. The histopathological data were those expected for a diabetic mastopathy, although we have not found in all cases the "epithelioid fibroblasts" that some authors consider as a basic feature of this condition. We believe that keloid fibrosis and ductal and/or lobular lymphoid B-cell infiltrate, in an appropiate clinical context, are enough to diagnose diabetic mastopathy.

## P-032

DIAGNOSTIC ACCURACY OF LARGE-CORE NEEDLE BIOPSY (LCNB) AND FINE-NEEDLE ASPIRATION BIOPSY (FNAB) IN PATIENTS WITH PALPABLE BREAST LESIONS.

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**Aims:** LNCB has been considered to have higher diagnostic accuracy than FNAB in the study of non-palpable breast lesions. Comparative utility of these techniques in palpable breast lesions has received scant attention in literature. The purpose of this study is to compare diagnostic accuracy of both procedures in this kind of lesions.

**Methods:** Simultaneous LNCB and FNAB were performed in 69 consecutive patients with palpable breast lesions, between May 1997 and February 1999 (22 months). Two pathologists examined each proof blindly and independently. Surgical excision, or follow-up of at least one year in benign cases, was used as gold standard.

**Results:** Results of LNCB: 4 cases (5.8%) insufficient samples; 5 (7.2%) benign; 1 (1.5%) atypical ductal hyperplasia; 4 (5.8%) ductal carcinoma in situ (DCIS); 53 (76.8%) invasive carcinoma and 2 cases (2.9%) malignant phyllodes tumour. False-positive rate of 0% and false-negative rate of 1.45% (1 case diagnosed as DCIS at surgical specimen). Sensitivity: 98.3% (ci 95%: 91.2-99.9). Specificity: 100%.

Results of FNAB: 8 cases (11.6%) insufficient samples; 10 (14.5%) benign (negative and atypical); 51 (73.9%) malignant (suspicious and positive). False-positive rate of 1.45% (1 case) and false-negative rate of 11.6% (8 cases, 6 of them were correctly diagnosed at LNCB). Sensitivity: 86.2% (ci 95%: 74.6-93.8). Specificity: 75%.

No relevant complications were detected in the series.

**Conclusions:** LNCB revealed higher values for sensitivity and specificity and lower rate of insufficient samples than FNAB. Specificity of 100% for LNCB allows a better planning of definitive surgical procedure without intraoperative study.

## P-033

**PROGNOSTIC VALUE OF EXPRESSION OF p53 PROTEIN AND CLINICOPATHOLOGIC FACTORS OF INVASIVE BREAST CANCER. A STUDY OF 257 PATIENTS.**

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**Aims :** To know the factors related with the breast cancer prognosis.

**Methods :** In our study, we controlled 257 patients with breast cancer. The following factors were valued : Age, menstrual status, tumour size, lymph nodes status, histologic type, nuclear grade, tumour necrosis, inflammatory cell reaction, blood or lymphatic vessel invasion and expression of p53 protein. Also, the achieved treatment, complementary treatment, presence of metastasis (local and to distance) and death of the patient (distinguishing death caused by breast cancer or another) were assessed. A multivariate study was carried out to detect the usefulness that every factor could have to estimate the disease prognosis.

**Results :** The tumour size, lymph node status, number of mitosis, presence of necrosis, presence of blood or lymphatic vessel invasion and presence of p53 protein have been shown independent prognostic factors in our study.

**Conclusions :** A great deal of information about the factors related with the breast cancer prognosis has been acquired in the histological study. Also, the expression of p53 protein play a central role in the development and progression of breast cancer.

## P-034

ANALYSIS OF TUMOR ASSOCIATED ANTIGENS EXPRESSION IN SURVIVAL PROGNOSING IN PATIENTS WITH BREAST CANCER.

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**The aims of the study** were: 1)to compare the survival time, the appearance of metastases, degree of cancer staging (pTNM) and its histological differentation with tumor associated antigens (TAA's) expression (LeY,LeX,LeB,LeA,sialylLeA) and 2) to compare these features with the composition of lymphocytic infiltration in carcinoma microenvironment.

**Methods.** The examinations were performed on 43 female patients (mean age 53+/-16.6 years) operated due to invasive ductal carcinoma. Postoperative specimens were subjected to routine histological processing and were stained immunohistochemically using monoclonal antibodies against TAA's and lymphocytic antigens (CD3,CD20,CD56). The examination of antigen expression was performed quantitatively by two pathologists. Statistical significance was obtained using Mann-Whitney test

**Results.** The analysis of TAA's showed increased expression of LeX($p=0.03$) in patients with the survival time shorter than 2 years and increased expression of LeB and LeA($p=0.03$) in patients in stage N1 than stage N2. The expression of LeB and LeY was more increased in patients in stage T1 than in stage T4($p=0.02$). No differences were observed for histological differentation. Lymphocytic infiltration consisted mainly of B cells(CD20) and was more intensive in T1 and T2 tumors than in T4 tumors ($p=0.005$). No other differences were found.

**Conclusion.** The expression of LeA and LeB antigens may predict metastases to lymphatic nodes.

## P-035

BENIGN BREAST PHYLLODES TUMOR WITH LOCAL RECURRENCE 2 YEARS LATER AND DEATH CAUSE BY SYSTEMIC METASTASES 3 YEARS AFTER THE INITIAL DIAGNOSIS.

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Recurrent breast phyllodes tumor is the consequence of inadequate excision. Have been described more severe recurrences. Sometimes have been reported cases of death cause by systemic metastases after malignant recurrence of phyllodes tumor. Stromal hipercellularity overgrowth is the most closely associated with the malignant transformation of the original tumor.

We present a case in a 51-year-old woman with a breast mass of 21 cm in diameter. The local excision with adjacent normal tissue was the treatment of choice. The diagnosis was a benign phyllodes tumor. Fibroadenoma was detected in breast tissue axillary. Tumor showed stromal overgrowth with absent of ductal elements in 2 low-power fields and 1-2 mitoses per 10 high-power fields, moderate cellularity and lack cellular atypism. Two years later a core-biopsy was performed because a local recurrence was detected. The diagnosis was phyllodes tumor with absent of ductal elements and 2 mitoses per 10 high-power fields, then tumorectoy was performed. There was a tumor of 10 cm in diameter with a malignant phyllodes tumor with biphasic composition with more than 10 mitoses per 10 high-power fields, stromal hipercellularity with associated cellular atypism. Mastectomy showed a residual foci (0.3 cm in diameter) of low-grade phyllodes tumor with epithelial elements. Seven months later a new recurrence with invasion of pectoral muscle was diagnosed of fibrosarcomatous malignant phyllodes tumor without ductal elements. There were two recurrences with similar features at 5 and 7 months later. The patient died cause by systemic metastases 2 months after the last recurrence.

The prognostic prediction have to be maded on the basis of the size, cellular atypia, mitotic count, peripheral margins and stromal overgrowth. Some references in the literature described the importance of stromal overgrowth in higher degree recurrences and ocasional cases with systemic metastases. In our case, the first biopsy showed low mitotic activity, stromal overgrowth, large size and doubtful margin of normal tissue. Phyllodes tumor with benign appearance should be investigated thoroughly to rule out invasion of margin and foci of stromal overgrowth that could suggest malignant recurrences or systemic metastases.

## P-036

ASSESSMENT OF BREAST CANCER SIZE:
ULTRASONOGRAPHIC AND PATHOLOGIC CORRELATION

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**Aims**: Pre-surgical assessment of tumor size in breast cancer is important for therapeutical choice including prescription of primary systemic therapy.

**Methods**: In 188 cases, tumor size was measured by ultrasonography and correlated with values obtained by pathologic examination of the specimen. Ultrasonographic configuration of the tumor, number of lesions and the presence of extensive intraductal component were considered.

**Results**: Ultrasonographic measurements of tumor size showed a good correlation with histological size ($R^2 = 0.38$; 95 % confidence interval: 0.25 - 0.49). The correlation was higher for those lesions of 20 mm or less when compared with those measuring more than 20 mm. Tumors with extensive intraductal component revealed a lower correlation than those tumors without extensive intraductal component. The ultrasound and pathologic correlation of tumor size was less accurate when the lesion was multiple.

**Conclusion**: Ultrasonography is a useful method for pre-surgical assessment of tumor size in patients with breast cancer, specially in single lesions of 20 mm or less and without extensive intraductal component.

## P-037

**TENASCIN AND NM23 EXPRESSION IN INVASIVE CARCINOMA OF THE BREAST**

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**Aims**: The aim of this study was to predict the accurate biological behaviour and the metastatic potential of the invasive breast carcinomas.
**Methods**: Immunohistochemistry was performed with Biotin StreptAvidin Amplified System .nm23-H1 and tenascin monoclonal antibodies were used in 15 ductal, 2 medullary and 1 lobular carcinomas of the breast.
**Results**: Strong immunoexpression of tenascin was observed in 13 of the 18 cases surrounding the tumoral islands. Antimetastasis gene (nm23H-1) expression was found stronger (+++) in 14 of the cases compared to (++/+) 4 of the cases which had lymph node metastasis.
**Conclusion**: We observed positive correlation between the histopathological parameters (grade, stage ) and the immunohistochemical markers. The results suggest that tenascin and nm23-H1 could be useful to predict the prognosis of the breast carcinoma patients.

## P-038

**Reliability of intra-operative frozen section and imprint cytological investigation of sentinel lymph nodes in breast cancer**

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The sentinel node (SN) procedure enables selective targeting of the first draining lymph node, where the initial metastases will form. A negative SN predicts the absence of tumour metastases in the other regional lymph nodes with high accuracy. So, in case of a negative SN, regional lymph node dissection is no longer necessary. This will save costs and prevent the side effects of lymph node dissection. The aim of this study was to evaluate the reliability of intraoperative cytological and frozen section (FS) investigation of the SN to detect metastases. This would allow the axillary lymph node dissection to be performed in the same session as the SN procedure and the excision of the primary tumour in case of a positive SN.
74 SNs were detected by gamma probe detection of nanocolloid and visual localization of patent blue accumulations in 54 women with T1-2N0M0 invasive breast cancer. The SNs were investigated by FS and imprint cytology. Diagnoses were confirmed on the paraffin material, with skip H&E sections and immunohistochemistry.
31 SNs (42%) contained metastases, of which 27 were detected by FS (sensitivity 87%). There were no false positives (specificity 100%). The sensitivity and specificity of the imprints were 62% and 100%. When evaluating the data per patient, for the FS the sensitivity and specificity were 91% and 100%, and for the imprints, 63% and 100%. There were no SNs in which the imprints showed metastases and the FS did not.
In conclusion, intraoperative frozen section analysis is a reliable procedure by which a high percentage of sentinel lymph node metastases can be detected in breast cancer patients without false positive results. In up to 10% of cases, the final paraffin sections will reveal micrometastases that were not detected by the frozen section, and in these patients axillary lymph node dissection will have to be performed in a second session. Imprints are less sensitive than the FS but may be used as an alternative.

## P-039

THE EXTENT OF PROLIFERATIVE AND APOPTOTIC ACTIVITY IN INTRADUCTAL AND INVASIVE DUCTAL BREAST CARCINOMAS

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**Aims**: To improve our understanding of the pathogenesis of breast cancer, it is important to determine whether there is a relationship between cell proliferation, cell differentiation, and cell death (apoptosis) in invasive and in situ ductal breast carcinomas.
**Methods**: This study examined the occurrence of apoptosis and proliferative capacity in 153 breast carcinomas: 50 ductal carcinomas in situ (DCIS) and 103 infiltrative ductal carcinomas (IDC). Terminal deoxynucletidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) and immunostaining with the Ki-67 antibody (MIB-1) were used in the examination and they were correlated with p53 overexpression, histological grade and oestrogen receptor (ER) protein expression. A quantitative immunohistochemistry investigation with a Computerised Analyser System was used to score individual nuclei for the presence of p53 and oestrogen receptor protein, MIB-1 antibody and TUNEL label. The apoptosis and proliferation of each cancer were expressed as and apoptotic index (AI) and a proliferation index (PI).
**Results**: The extent of apoptosis was more frequently observed in DCIS than in IDC (3.9 ±3.1 vs 1.7± 3.2) and a high AI is more frequently found in DCIS than in IDC (AI>3=38% vs AI>3=14.2%) and the proliferation activity was higher in IDC than in DCIS (20.2±5.9 vs 8.3±1.0). In DCIS and IDC AI and PI were not correlated.
**Conclusions**: The results of this study show that a higher degree of apoptosis and lower proliferation activity in intraductal carcinoma leads to a steady-state and the disturbance of the balance between apoptosis-proliferation might be a fact that contributes to the development of invasive carcinoma and thus obtains the capacity to disseminate.

## P-040

**FROZEN SECTIONS AND CYTOKERATIN STAIN IN INTRAOPERATORY EVALUATION OF SENTINEL NODE IN BREAST CANCER.**

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**Aims:** To evaluate the utility of intraoperatory evaluation (frozen sections and cytokeratin stains on frozen sections) in the pathologic study of sentinel node biopsies in breast cancer.
**Methods:** A series of 50 patients with breast tumors were submitted to a preliminary evaluation of sentinel node biopsy. Technetium-99m sulfur colloid was injected in the breast tissue surrounding the tumor and a gamma counter was used to detect the radioactive sentinel node. Sentinel nodes were bisected; one half was immediately frozen, serial sections were obtained and stained with H&E and Cam5.2 (TechMate 500 immunostainer), residual tissue was formalin fixed for control. The other half was formalin fixed. Fixed tissue was routinely processed with serial sections stained with H&E and Cam5.2. In all the patients a complete axillary dissection (three levels) was performed
**Results:** Sentinel node could not be detected in 6% of the tumors. In an additional 6% the sentinel node was located in the internal mammary chain. In the remaining patients, the sentinel node (one to three nodes) predicted the status of the axilla in 93.2% of the cases (the sentinel was the only involved node in 5 of the 19 metastatic tumors). The intraoperatory examination yielded two false negative and one false positive result. Intraoperatory Cam 5.2 immunohistochemical stain helped in one of those false negative cases. Unfortunately, Cam5.2 stain over frozen sections failed in two cases. The duration of the process in gross room (median value) ranged between 19 (cases with one sentinel node detected) and 23 minutes (case s with 2 or 3 nodes detected).
**Conclusion:** Intraoperatory evaluation of sentinel node in breast cancer is a time consuming procedure without benefit for the patient. The use of immunohistochemical stains of cytokeratins (Cam 5.2) on frozen sections does not improve significantly the technique.

## P-041

IMMUNOHISTOCHEMICAL STUDY OF PHYLLODES TUMOR OF THE BREAST

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Phyllodes tumor (PT) is a rare, fibroepithelial breast neoplasm with unpredictable prognostic and controversial therapeutic aspects.

**Aims:** The aim of this retrospective study is to evaluate Ki-67 and CD34 antigen expression, as well as estrogen (ER) and progesterone receptor (PgR) status in stromal cells, as additional diagnostic criteria in assessing different types of PT.

**Methods:** Routinely processed, formalin-fixed and paraffin-embedded surgical specimens from 47 cases of PT were stained by immunoperoxidase technique using Ki-67, CD34, ER and PgR monoclonal antibodies. On the basis of histopathological criteria proposed by Azzopardi, 6 malignant, 12 borderline and 29 benign PT have been evaluated. The mean size of the tumour was 9.4 cm (range 2.8-20 cm). During the follow-up period (mean 50, range 2-127 months), recurrences were observed in 8 patients (17%).

**Results:** The proliferating index determined by Ki-67 antigen expression was significantly different between histologically benign PT (19%), borderline PT (25%) and malignant PT (50%). There was a difference of the human progenitor cell CD34 antigen expression in malignant PT (50%), borderline PT (50%) and benign PT (30%). As expected, the stromal cells in various types of PT were mostly ER negative and PgR positive.

**Conclusions:** Our preliminary results suggest that CD34 positivity and high proliferative index of Ki-67 antigen in stromal cells are more frequently associated with high grade PT. Therefore, the immunohistochemical features could provide better discrimination between different PT types. The prognostic implications of these observations should be evaluated in additional studies.

## P-042

**CYTOGENETICAL ANALYSIS OF 70 DUCTAL BREAST CARCINOMAS BY COMPARATIVE GENOMIC HYBRIDIZATION (CGH): PRELIMINARY RESULTS.**

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**Aims:** The purpose of this study is to identify cytogenetic abnormalities in a group of ductal carcinomas using CGH, in order to determine the correlation between these data and known prognostic and diagnostic parameters.

**Material and methods:** DNA samples were obtained from formalin-fixed, paraffin-embedded tissues. Tumor and reference DNA were labeled by nick translation with Fluorescein-dUTP and Texas Red-dUTP. Negative and positive controls were included in each experiment. Other known parameters of breast cancer were studied (histological type/grading, tumor size, lymph node status, estrogen/progesterone receptors, bcl-2, c-erb-B2, Ki-67, angiogenesis grade/intensity).

**Results:** DNA sequence copy number changes were present in the first ten cases analysed. The most frequent aberrations were: losses of 1p, 11q21-qter (37% samples) and Xp, and gains in 8q (50% samples), 11q11-21 and 6q16-22.

**Conclusions:** Some of our CGH results have been frequently reported: gain of 8q (where c-myc and a possible and still undescribed oncogene in region 8q12-22 are affected), gain of 11q21-qter (the third most frequently amplified region in breast cancer, after MYC and ERBB2) and loss of 11q23 (whose loss of heterozigosity is linked to 5 times increased risk of disease). We have also found a rarely described amplification in 6q16-22 (2/10), that will be the subject of further study by FISH. Clinical correlations will be established when more cases are analysed.

## P-043

MICROSCOPIC, ULTRASTRUCTURAL AND INMUNOHISTOCHEMICAL PROFELE OF CYSTIC ADENOID CARCINOMA OF THE BREAST.

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Aims: We present a case of cystic adenoid carcinoma of the breast on a 43 year old female patient.

**Result**: This woman complained of a three week history of a breast palpable mass; on mammograms there were signs of malignancy. FNA was diagnostic of carcinoma Cystic Adenoid. Gross: It was a solid, lobulated mass measuring 6 cm. Histology showed a cystic adenoid carcinoma, mainly cylindroid, with large solid areas where atypia was more pronounced (grade II of Ro. J. Y.). At the margin, ducts containing an in situ cystic adenoid carcinoma were also identified. Electron microscopy revealed the presence of epithelial and myoepithelial cells, among which there was membranous material. Immunohistochemisty studies: This tumour stained as follows: ER (-), PR (-), p53 (-), c-erbB-2 (+/-), Keratin (+) (epithelial cells), actin (+) (myoepithelial cells), S-100 (+) (epithelial-myoepithelial cells), type IV collagen (+) at the cystic adenoid areas, Ki-67 (+), more intense at the solid regions.

**Conclusions**: FNA is a suitable diagnostic tool for low grade cystic adenoid carcinomas, but not for the higher grade ones, where differential diagnosis between DCIS, variants of infiltrating ductal carcinoma and collagen microspherulosis must be made. Hormonal receptors are usually positive in low grade tumours, which is not our case. Although p53 was negative, we expect a poor evolution of thes particular tumour, due to Ki-67 overexpression, size the carcinoma and large solid areas..

## P-044

OPTICAL, ULTRASTRUCTURAL AND INMUNOHISTOCHEMICAL PROFILE OF ADENOMYOEPITHELIOMA OF THE BREAST..

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Aims: We present two cases of adenomyoepithelioma of the breast, studied with a combination of optical and electron microscopy, and immunohistochemistry.

Results: Two female patints aged 76 and 90 presented with a palpable breast tumour of 3 and 7 cm. respectively. FNA suggested carcinoma in one case. On section, both masses were solid with cystic areas. Histology showed tubular cystic adenomyoepithelioma with atypia, microcalcifications, and squamous and sebaceous differentiation. Electron microscopy confirmed the presence of two cellular lines (epithelialy-myoepithelial) in these neoplasms. Tubular epithelial cells showed ER(-), PR(-), p53(-),c-erbB-2(+/-), bcl-2(+), cytokeratin (+), S-100(+) patchily distributed, and EMA (+) in the apical pole. Myoepithelial cells were actin (+) and S-100(+) regardless of their volume, Ki-67 was low in both cases, specially in the one with more marked atypia.

Conclusions: The most suitable antisera for detection of the two cellular types in a case of adenomyoepithelioma are actin (myoepithelial), and cytokeratin (epithelial). S.100 identifies both types in an irregular fashion. We have found no use for determination of hormonal receptors, p-53, c-erbB-2 and EMA. There could be certain correlation between bcl-2, Ki-67 expression, nuclear atypia and tomour progression.

## P-045

PROGNOSTIC VALUE OF TUMOR NECROSIS IN PATIENTS WITH OSTEOSARCOMA OF BONE TREATED WITH PREOPERATIVE CHEMOTHERAPY.

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**Aims**: The prognostic value of tumor necrosis percentage as an evidence of response to preoperative chemotherapy (PCh) was studied in a series of 36 osteosarcomas.

**Methods**: A retrospective review of our Surgical Pathology files from 1983-1998 disclosed 109 osteosarcomas. We selected 36 patients with localized (stage $II_{A+B}$) conventional osteosarcoma of bone who had received PCh (cisplatinum and adriamycin) prior to radical surgery (amputation or en bloc resection) and in whom follow-up of at least 3 years was available. In these tumors, four to 28 (mean 12.3) H-E stained histologic sections were evaluated.

**Results**: Twenty-one patients were males and 15 females. Ages ranged from 8-45 (mean 19.3) years. Locations included: femur 19; tibia 13; skull 1; humerus 1; radius 1; fibula 1. Tumor necrosis was 80-100% in 9 patients; six (66%) of them had no evidence of disease (NED) at from 3 to 13 (mean 7) years. Three patients died of disease (DOD) at 3, 5 and 6 years postoperatively. Twenty-seven patients had less than 80% tumor necrosis. Sixteen (59%) were NED at from 3 to16 (mean 7) years and 9 DOD. Interestingly, eleven of the survivors (68%) had 40% or less tumor necrosis.

**Conclusions**: Tumor necrosis has been found to be an excellent prognostic factor in patients with osteosarcoma treated with PCh. Our results, however, do not support this as many patients with poor response to chemotherapy had a long-term survival. Microscopically recognizable tumor cells may be non viable even when the tumor does not appear necrotic. The role of PCh is mainly to prevent distant metastasis improving survival despite its effect on local tumor.

## P-046

FROZEN SECTION BIOPSY ASSESSMENT FOR THE PRESENCE OF POLYMORPHONUCLEAR LEUKOCYTES IN PATIENTS UNDERGOING REVISION OF ARTHROPLASTIES.

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**Aims**: To determinate the reliability of intraoperative frozen sections (FSs) during revision hip arthroplasties for the diagnosis of infection by the identification of polymorphonuclear leukocytes.

**Methods**: 62 patients with prosthetic hip replacement: 19 men, 43 women. Mean age 65 y. Mean interval between primary and revision arthroplasty 12.3 y. FSs were obtained from the host-implant interface membranes in the suspicious areas and were stained with H-E. A FSs was considerated positive for infection if there were more than 5 polymorphonuclear leukocytes (PMNs) per high power field (HPF) in at least five separate fields. A positive intraoperative culture was the criterion for a diagnosis of infection.

**Results**: Of 62 patients, 9 had more than 5 PMNs per HPF and were considerated infected. Of these nine, 2 had 6 to 10 PMNs per HPF and 7 had more than 10 PMNs per HPF. The remaining 53 had 5 or less PMNs per HPF and were considerated negative. Of these 53 patients, 11 had 1 to 5 PMNs per HPF and 42 had no evidence of PMNs. Intraoperative cultures were positive in 10 patients . Of the 53 patients who had negative FSs, 3 were considerated infected on the basis of final cultures. Of the 9 patients who had positive FSs, 7 were considerated infected on the basis of final cultures. The sensitivity and especificity of the analysis of FSs using an index of more than 5 PMNs per HPF were 70% and 96% respectively. If an index of more than 10 PMNs per HPF is used, sensitivity decreases to 60% and especificity increases to 98%.

**Conclusion**: The presence of more than 5 PMNs per HPF on analysis of intraoperative FSs during a revision hip arthroplasty is highly suggestive of active infection. Analysis of intraoperative FSs aids in the surgical decision of doing a primary exchange arthroplasty versus delayed reimplantation after antibiotic therapy.

## P-047

**SYSTEMIC SECONDARY (AA), AND ISOLATED AMYLOIDOSIS LOCALIZED TO ARTICULAR CARTILAGE OF HIP JOINT IN RHEUMATOID ARTHRITIS**

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**Objective**: *Systemic secondary (AA)* and *isolated amyloidosis (IA) localized to the articular cartilage* were studied in a randomized autopsy population of 37 in-patients with *rheumatoid arthritis (RA)*, to determine: the prevalence of systemic secondary (AA) amyloidosis in the synovial membrane, and bone marrow of femoral heads and acetabula, the prevalence of isolated amyloidosis (IA) localized to the articular cartilage of femoral heads and acetabula, the possible qualitative differences of (AA) and (IA) amyloid deposits, the possible correlation between (AA) and (IA) amyloidosis, the possible role of (AA) amyloidosis and (IA) in degenerative processes of hip joints.

**Material and Methods:** 74 samples of synovial membrane, 74 samples of the femoral head, and 68 samples of the acetabulum of 37 patients were examined. The formaldehyde fixed and in paraffin embedded tissue samples were stained with HE or Congo red according to Romhányi. The (AA) and (IA) amyloid was determined and characterized histochemically by congo red staining after performate pre-treatment, with $KMnO^4$ oxidation-induced proteolysis by 0.1% trypsin digestion, and by immunohistochemical reactions. The correlation between (AA) and (IA) amyloidosis was determined by $\chi^2$-test.

**Results**: (AA) amyloid deposits were observed in 10 (13.5%) of 74 synovial membranes, in 16 (21.6%) of 74 femoral heads, and 14 (20.6%) of 68 acetabula. (AA) deposits were usually detected in blood-vessels, collagen or reticulin fibres of connective tissue or bone marrow. (IA) amyloid deposits were observed in 52 (70%) of 74 femoral heads, and 46 (67.6%) of 68 acetabula localized to the superficial zone of articular cartilage. *(AA) amyloid* is *sensitive* to *$KMnO^4$ oxydation, followed by trypsin digestion* (for 1 min), green birefringence with polarized light disappear, while *isolated (dystrophic) amyloid* localised to the articular cartilage is *resistant* (for 1-10 min), and amyloid deposits remain birefringent. *Performate pre-treatment* is followed by pronounced congophilia. *(AA) amyloid* is *sensitive* to performate pre-treatment (1 sec), while *isolated (dystrophic) amyloid* deposits (1-25 sec) are *resistant*. There was no significant correlation between (AA) and (IA) amyloidosis ($\chi^2$=0.3136, c = -0.1429, p<0.5755). (AA) and (IA) amyloid deposits were found in almost intact as well as in markedly damaged and distroyed joints.

**Conclusions**: Different types of amyloidosis may exist simultaneously. Systemic secondary (AA) amyloidosis is one of the main complications in RA. (AA) amyloid deposition in blood vessels of synovial membrane and bone is not directly associated with cartilage destuction, but an indirect role in the pathogenesis of damage to cartilage by diminished nutrition can not be excluded. Local (isolated) amyloid deposits seem to be independent of articular destruction and of RA.

## P-048

GENETIC INSTABILITY IN OSTEOBLASTIC BONE TUMORS

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**Aims**: At the histological level, the differential diagnosis of osteoblastic bone tumors is characterized by several problems that cannot be solved by conventional histological methods including immunohistology. Differentiating aneurysmal bone cyst from telangiectatic osteosarcoma or giant cell tumor from giant cell-containing highly malignant osteosarcoma are only two examples reflecting the complexity of this field. To develop a new approach to these diagnostic problems, we analyzed the genetic instability in a large number of bone-forming tumor-like lesions as well as in benign and malignant osteoblastic tumors.

**Methods**: Our research concentrated on genetic alterations in cell cycle regulator genes: mutations in the p53 and ras genes, loss of heterozygosity at the p53, 16 and Rb-locus, and amplification of the mdm2 gene and the c-myc gene. In addition to cell cycle regulators, telomerase activity was also analyzed.

**Results**: The data show that the number of genetic alterations increases with the malignancy of the tumors. The highest number of genetic alterations could thus be found in conventional intraosseous osteosarcoma. In tumor-like lesions, genetic alterations have rarely been observed. Rb-LOH could be found in more than 50% of the highly malignant osteosarcomas, but in no case of low malignant osteosarcoma.

**Conclusions**: The results of this study show that analyzing the genetic instability probably contributes to improving the differential diagnosis of osteoblastic bone tumors. There seem to be considerable genetic differences between low and highly malignant osteosarcomas.

## P-049

SMALL ROUND CELL TUMORS OF BONE IN CHILDHOOD. A CLINICOPATOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF 44 CASES. J Vila Torres*; A. Llombart-Bosch**; S. Navarro**; V. Cusi*; R. Huguet*; J. Illa *; (*) Servei d'Anatomia Patológica, Hospital Universitari Sant Joan de Déu, Barcelona. (**) Departamento de Patología. Hospital Clínico Universitario. Valencia.

**BACKGROUND** SRCT of bone include Ewing/PNET, lymphoma, neuroblastoma, alveolar Rahbdomyosarcoma, mesenchymal chondrosarcoma and small cell osteosarcoma. EW/PNET are characterized by their incidence in young people and a highly agressive behaviour. Tumors with neuroectodermal differentiation (NED) are classified as PNET whereas those without, are labeled as EW. NED indicates a poor prognosis: higher incidence of metastases, relapses and poor response to treatment. **MATERIAL AND METHOD** Imprints and frozen sections were taken to check the amount and quality of the tissue. The following markers were applied: vimentin desmin, HBA-71, LCA, NSE, osteonectin, desmin, HHF35 and S-100. Up to 44 SRCT of bone with clinicopathological characteristics of EW/PNET and HBA-71(+) were collected. The following markers were used to search for NED: NSE, NF, Leu-7, Trk and PGP 9.5. **RESULTS** EW/PNET showed a monotonous pattern of round cells with- out estroma, few vessels and scarce reticulin. Cells were round or elongated, with scarce cytoplasm, poorly defined borders, and fine nuclear chromatin with nucleoli. Apoptotic dark cells with condensed chromatin were intermin gled with clear cells. HBA-71 was (+) in all cases.NED was characterized by lobular pattern, Homer-Wright pseudorosettes, and variable positivity for NSE, NF, Leu-7, and PGP 9.5. In some cases, NSE was (-) but other more sensitive neuroectodermal markers were focally (+). Trk A was a feature of PNET whereas Trk B/C were expressed in EW. Leu-7 and PGP 9.5 were limited to cells clusters in PNET. **CONCLUSIONS** HBA-71 is mandatory in the differential diagnosis of SRCT of bone. More sensitive markers of NED should be used in addition to NSE. The cases with NED (PNET), showed a more agressive outcome when matched with those without. (Supported with grant number 98/0600 from FISS Madrid, Spain).

## P-050

INFECTIVE ENDOCARDITIS. STUDY OF 37 PATIENTS FROM 5888 AUTOPSIES.

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**Aims:** The present study was performed to determine the frequency of infection as a cause of death in bacterial endocarditis, as well as the pathogens and the predisposing factors.

**Methods**: We reviewed 37 patients with bacterial endocarditis obtained from 5888 autopsies performed in our hospital from 1970 to 1997.

**Results**: The incidence was 0.63%. The mean age was 59.8 years (30-85 years), There were 19 men and 18 women. The pathogens associated with endocarditis were: S. viridians (12), S. aureus (5), S.coagulase negative (1), E. Coli (3), Serratia marcences (1), Erysipelothrix rhusiopathial (1), Candida sp (2) and Brucella (1). The locations were: mitral valve (25), aortic (23) and tricuspid (4). One valve was affected in 25 patients, two valves in 9 patients and 3 valves in three patients. Predisposing factors were: rheumatic disease (11), immunodeficiency (8), calcification (5), valve prosthesis (4), bicuspid aortic valve (1) and Marfan disease (1). Undeaching factors were surgery (4) and catheterism (2). Septic embolism was located in kidney (14), spleen (13), brain (13), heart (5), skin (2), lung (2), liver (2) and gastrointestinal tract (2). Cardiac complications were valve rupture (5), myocarditis (5) and myocardic perforation (4).

**Conclusions**: The most frequent agent was S. viridians. Among predisposing factors, the rheumatic disease was the most frequently seen followed, in recent years, by immunodeficiency, calcification and valve prosthesis. The valves more frequently affected were mitral and aortic. Most septic thrombus were located in kidney, spleen and brain.

## P-051

PREMATURE DEATH AND CARDIAC INSUFFICIENCY IN MALE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-α DEFICIENT MICE OVEREXPRESSING LIPOPROTEIN LIPASE

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**Aims:** We established a model for myopathy associated with increased import of fatty acids (FA) by generating mice that overexpress human lipoprotein lipase (LPL) specifically in skeletal and cardiac muscle. By crossbreeding these mice with mice having both alleles of the peroxisome proliferator-activated receptor alpha (PPARα) disrupted, we aimed to elucidate the role of PPARα in FA mediated cell damage.

**Methods:** Life span and weight gain were recorded over a 12 months period. Metabolic parameters in fed and fasted state, functional tests on isolated hearts and morphological changes were evaluated at 3 months.

**Results:** Male animals lacking PPARα and overexpressing LPL in cardiac and skeletal muscle (PPARα-/- LPL high) did not survive more than 3 months whereas PPARα+/- LPL high male animals had an average life expectancy of 6 months and all females were still alive after 12 months. Morphologic investigation of deceased animals revealed signs of acute cardiopulmonary congestion but no lipid storage in heart and liver as reported after etomoxir treatment of male PPARα-/- mice. Glucose levels were reduced by 50% in both PPARα-/- LPL high and normal animals of both sexes after overnight fasting. Plasma free FA, triglyceride and cholesterol levels did also not discriminate between male and female PPARα-/- LPL high animals or between PPARα-/- high and normal animals. Left ventricular developed pressure, however, was significantly lower in PPARα-/- LPL high mice.

**Conclusions:** Since the reduction of myocardial function is the only factor discriminating between mLPL high and normal animals, we believe that it is causally linked to the increase of FA in cardiomyocytes and to the premature death of the animals. It is also suggests that FA could indeed be a pathogenic factor in diabetic cardiomyopathy.

## P-052

ENDOTHELIAL INJURY AND TRAPPING OF BLOOD CELLS IN HUMAN MYOCARDIUM FOLLOWING CORONARY BY-PASS SURGERY

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**Aims:** During the first hour of reperfusion after hypothermic cardioplegic cardiac arrest, focal myocytic and microvascular injury and edema develops.

**Methods:** Repeated atrial biopsies were obtained from fourteen patients undergoing uneventful coronary by-pass-surgery: before the cardioplegia, at the beginning of reperfusion, and following 20 and 60 min of reperfusion.

**Results:** Transmission electron micrographs of biopsies examined by stereological techniques, revealed endothelial injury. Following 20 min of reperfusion accumulation of both red blood cells (P=0.03) and polymorphonuclear leukocytes (P= 0.0004) were found. There was also an intravascular accumulation of platelets (p= 0.008) and an extravasation of red blood cells (p= 0.02), which increased throughout the observation period.

**Conclusions:** If reperfusion was started with a gradual rise in temperature and pressure the number of platelets in the microvessels were lower than following an ordinary, abrupt reperfusion (P = 0.06). It is concluded that reperfusion injury is associated with microcirculatory damage and trapping of blood cells which may be favorably modified by a gentle reperfusion technique.

# P-053

**BLUE NITROTETRAZOLIUM USE IN SUDDEN DEATH CASES**

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**Aims:** To study the set of enzymhistochemical methods for determination of most important intramitochondrial enzymes parameters on early stages of myocardial ischemia, and to use the discovered pathognomic enzymes markers characteristics in determination of cause of death from AMI.

**Methods:** Pieces from top area, both ventricles and interventricular septum of heart had been taken for analysis in 62 autopsy cases of sudden death, each case. All taken material has frozen for preparation of cryostat sections. Later these sections have dried in cryostat camera and incubated into incubation solutions for enzymes activities' determination. To study intracellular localization and activity of mitochondrial enzymes we used tetrazolium reactions. Histochemical results assessment carried out visually by diformazan content' gradient. Sizes of the standard diformazan or its aggregate forms have been measured.

**Results:** Enzymhistochemical analysis has shown itself as quite effective diagnostic method in the ischemic zone identification in heart muscle of 42 cases. Aggregate condition of enzymes' markers was the basic histochemical parameter in such diagnostics. Big aggregated marker complexes in homogenous colored blue or pink sarcoplasma have been seen in these 42 cases. Dehydrogenases of ischemic zone were marked by standard granules in none of cases. Only in the boarder zone diformazan granules have appeared with nonsignificant disturbances in sarcoplasma. Certainly, in intact myocardium we could also see absolutely standard granules. They were small, oval, dark-blue and not bigger than mitochondria themselves.

**Conclusions:** This study has allowed to carry out the precise assessment of cardiomyocytes mitochondria physical condition changes under acute insufficiency of the oxygen and energetic material supply to the heart muscle. All histochemical changes in studied cases have been connected with stable and obvious parameters of nitrotetrazolium salts. Mitochondrial enzymes in all cases of ischemia have marked by rough-aggregated, non-standard diformazan or monoformazan that easily may be seen by the light microscopy.

# P-054

**ORAL ADMINISTRATION OF A TURMERIC EXTRACT IN RABBITS WITH EXPERIMENTAL ATHEROSCLEROSIS. ROLE OF LDL OXIDATION AND EXPRESSION OF ENDOTHELIN-1.**

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**1.-Aims:** The oxidation of low-density lipoproteins (LDL) plays an important role in the development of atherosclerosis. Curcumin and turmeric extracts are known to exhibit a variety of pharmacological properties. We seek to evaluate the effect of one ethanol-aqueous extract obtained from rhizomes of *Curcuma longa* on LDL oxidation susceptibility in rabbits with experimental atherosclerosis, on atherosclerotic lesion formation and on expression of endotehlin-1 (Et1) in arterial wall.

**2.- Methods:** Eighteen male New-Zealand rabbits were fed for 7 weeks on a diet to provoke an atherosclerotic process. Parallel, two of the experimental groups were orally treated with a turmeric hydroalcoholic extract at doses 1.66 **(Group A)** and 3.2 **(Group B)** mg/kg body weight, respectively. The third group **(Group C)** acted as a control and received a curcumin-free hydroalcoholic solution. Plasma and LDL lipid composition, plasma a-tocopherol, plasma retinol, LDL TBARS, LDL lipid hydroperoxides, histological analysis of aortic atherosclerosis lesions and IHQ with MoAB to Et1 were measured in all experimental groups.

**3.-Results:** The first dosage decreased the susceptibility of LDL to lipid peroxidation (low production of TBARS), whereas the 3.2 mg dosage did not show any antioxidant effect. The plaques in most rabbits were relatively uniform in appearance but the most remarkable changes were observed in the aortic arch. Et1 was expressed as on plaque's foam cells as on smooth muscle cells. There no were significant differences in the type and degree of lesions and in the expression of Et1 among the groups.

**4.-Conclusions:** a) The oral dosage of 1.6mg/kg of curcuma longa extract decreased the susceptibility of LDL to lipid peroxidation but in our experimental condition the extract of curcuma had not effect in the development of atheroma plaque and in Et-1 expression.

# P-055

THE PATHOLOGY OF THE TRANSMYOCARDIAL LASER REVASCULARIZATION (TMLR).

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**Aims:** TMLR is an investigational procedure designed to reperfuse the heart with blood from the left ventricular cavity through the TMLR tracts in patients who are not candidates for angioplasty or coronary bypass surgery. There is a paucity of histologic data regarding the effects of the laser on myocardium. The aim of this study is to evaluate the acute histologic effects of laser over the myocardium.

**Methods:** Six minipigs (body weight range 20-30 kg) were entered in the study. Myocardial ischemia was created by proximal left anterior descending coronary ameroid constrictors. TMLR was performed in the anterior left ventricular free wall (ischemic area) using an Excimer laser. Hearts were harvested acutely (G1; n=1), at 48 h (G2; n=2), at one (G3; n=1), two (G4; n=1), and three weeks (G5; n=1). Channels in each heart were examined macroscopica and histologically by H&E, and Masson's trichrome.

**Results:** Epicardial portions of the channels were easy to localize grossly in G1 and G2 after TMLR because of prominent pointed fibrin, but they were more difficult to identify in G3 to G5 after TMLR because they were smaller and scarred. Epicardial lesions varied from 2 mm on G1 and G2, to <1 mm on G3 to G5. Grossly, myocardial channels in G1 and G2 were easily identifiable as red linear lesions surrounded by a pale myocardium 1-mm thickness. Microscopically, G1 and G2 laser-induced channels were filled with abundant fibrinous network, platelets, and leukocytes, surrounded by a ring of necrotic (coagulated) myocyes. An intense acute inflammatory reaction was induced within the channels, which spills into the surrounding epicardium. On G3 to G5 (one to three weeks after TMLR) laser-induced channels were filled with macrophages, foreign-body giant cells, and exuberant granulation tissue with thin small walled vessels within a fibroblastic tissue. None of the treatment sites studied showed a patent channel.

**Conclusions:** 1) We found different stages of wound healing in channels after TMLR, without evidence of patent and endothelialized laser-created channels. The results suggest that transmyocardial blood flow may not be the mechanism of clinical benefit of this procedure. 2) The clinical benefit of TMLR may result simply from a nonspecific histologic response to injury, through the release of angiogenic growth factors and new blood vessels formation (granulation tissue).

# P-056

INTRACARDIAC FIBROHISTIOCYTARY PSEUDOTUMOR.

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**Aims:** There are only scant references of intracardiac inflammatory psedotumors. They are described as plasmatic cells granulomas or fibroblastic proliferations with inflammatoy component.

**Methods:** A 69-year-old man was referred to the Hospital because of congestive cardiac insufficiency, dyspnoea, ascitis, and maleolar edemas. He also showed a diffuse infiltrating mass that caused colapse of the right cardiac cavities and severe pericardiac effusion. Lipid analytical values were normal. Surgery was performed by pericardiectomy and a biospy of the auricular walls was taken.

The microscopic study showed auricular tissue with a diffuse lesion charactized by groups of foam and eosinophilic hystiocityc cells with single nucleous or multinucleated, embebbed in a fibrous stromal with areas of storiform pattern. Inespecific mononuclear inflammatory cells together with neutrophils were also seen.

**Conclusions:** We have observed a pseudotumoral lesion of fibrohistiocytary origen affecting the cardiac cavities. Although it has been previously reported in other organs, we have not found references in the literature describing this fact.

## P-057

INFECTIOUS PROCESSES DIAGNOSED POST MORTEM IN HEART-TRANSPLANTED PATIENTS.

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**Aims:** To establish the frequency of infection as the cause of death after heart transplantation (HT) and to compare the last clinical diagnostic with autopsy findings.
**Methods**: Review of the autopsy findings in 60 HT patients seen in this hospital from 1984 to 1997.
**Results**: Infections caused death of 23 among 60 HT patients. Transplant rejection participated in 8 of these. An acute rejection took place in another group of 11 patients and a non-infectious cause of death was found in the remaining 26 Among the 23 infectious cases, a bacterial etiology was determined in 11, fungal in 5, viral in 3, toxoplasma in one. Three patients suffered polymicrobian infections; (anaerobic and Cytomegalia virus, Candida sp. and S. Faecalis and P. Carinii, CMV and Candida). The infections affected mainly lungs (16) and heart (5). It acquired a septic syndrome in 11. Three patients presented with dissemination of fungi (Candida sp, CMV, and Candida with aspergillus). The microbiologic etiology was established postmortally in 8/23 cases (4 fungal, 1 tbc and 3 CMV).
**Conclusions**: Infectious processes of clinically unrecognized etiology constituted a relatively frequent finding in autopsies of patients submitted to HT. Among these, fungal and viral agents were the most frequently detected in cases of dissemination or affectation of unexpected organs.

## P-058

**NICK END LABELING METHOD VERSUS DNA AGAROSE GEL ELECTROPHORESIS FOR DETECTION OF APOPTOSIS IN MYOCARDIAL INFARCTION**

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**Aims:** The purpose of this study was to compare two widely used apoptosis detection methods: TUNEL assay and agarose gel electrophoresis, using formalin-fixed, paraffin-embedded tissue sections of infarted miocardium.
**Methods:** Myocardial tissue from a total of 14 autopsy cases were examined: 9 corresponded to myocardial infarction cases and 5 to healthy hearts. As positives controls for apoptosis sections from 2 tonsils were used.
The TUNEL assay was performed with the ApopTag® Kit, according to the manufacturer protocol. After DNA isolation, electrophoresis was carried out in 1.7% agarose gels for 1h at 115 V and bands were visualized by ethidium bromide.
**Results:** The nine miocardial infarction cases gave interpretable results with the ApopTag® Kit. The percentage of stained nuclei ranged from 0 to 74.7% (average 47.3%). Cytoplasmatic staining was noted in some obviously infarcted areas. Nuclear positivity in controls ranged from 0 to 18.9% (average 8.2%).
DNA fragmentation detected by agarose gel electrophoresis was confirmed in only two out of the nine infarctions tested.
**Conclusions:** The sensitivity of the ApopTag® Kit to detect apoptotic cells in myocardial infarction seems to be higher than the agarose electrophoresis method. This may be due, at least partially, to the use of paraffin-embedded tissue and the coexistence of necrosis, which may result in a continuous smear making difficult the recognition of DNA bands in the gel.

## P-059

THE ADVENTITIAL MAST CELLS CONTENT OF CORONARY ARTERIES WITH PATIENTS WITH ACUTE HEART INFARCTION

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**Aims:** To investigate the mast cells (MC) content of coronary arteries with patient with acute heart infarction.
**Methods:** Three groups were investigated: 36 patients with coronary atherosclerosis who died of heart infarction, and two groups for comparison. One is the group of 25 young persons who died of trauma suddenly and second one is the group of 21 old persons with coronary atherosclerosis who died without clinical manifestation of ischemic heart disease. Samples of the proximal part of the three major coronary arteries were obtained by multiple transverse sections with following formalin fixation. Histologic sections were stained with toluidine blue. In each section an average number of MC per section were calculated.
**Results:** Statistically significant distinctions of the content of MC were found among three groups. Maximum quantity of MC were found in the group of young people – $64.37\pm2.51$, and, in contrast ($p<0.001$), minimal number of MC were found in the group of aged people with atherosclerosis – $27.44\pm1.77$. Number of MC in the group of patients with acute infarction was increasing in comparison with the group of aged atherosclerosis ($p<0.001$) – $50.23\pm2.23$, but less than in the group of young people ($p<0.001$).
**Conclusions:** Minimal content of MC in the wall of coronary arteries in the group of old people without ischemic heart disease possibly reflects inactive phase of atherosclerosis. Increasing of the number of MC in the group with acute heart infarction is important prerequisite for coronaro-spasm because MC consist of the number of vasoactive substances. Increasing of MC in this group could be valued as a sign of acute condition of atherosclerosis. The maximum quantity of adventitial MC in the group of young people is a sign of participating of these cells in physiological processes of development and functioning arteries with possible balance of vasospastic and vasoprotective substances.

## P-060

**DNA ASSESSMENT AND MORPHOMETRICAL MEASUREMENT OF URINE SAMPLES FROM CASES OF TRANSITIONAL CELL CARCINOMS ASSOCIATED WITH SCHISTOSOMIASIS VER SUS TRANSITIONAL CELL PAPILLOMA IN EGYPTIANT PATIENTS**

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**Aims:** To find out the value of DNA content of urothelial cells in diagnosis of early neoplastic changes in Egyptian schistosomiasis.
**Methods :** This work was performed in Theodor Bilharz Research Institute on 29 urine samples obtained from patients suffering from urinany diseases including transitional cell papilloma (3 cases), and Transitional cell carcinome(26cases), 16 associated with schistosomiasis and 10 cases without as well as 8 normal control urine samples. Routinly stained sections were cytopathologically examined followed by morphological measurement of nuclear area and nucleocytoplosmic ratio and assessment of the DNA content of urothelial nuclei using the computerized image analysis (I.A) system on paraffin section stained with felgen stain.
**Results:** It was found that there was a highly significant increase in MNA(mean nuclear area) in TCC (Transitional cell carcionoma) compared to control cases($p<0.01$). It was also found that the N/C ratio showed a highly significant increase in cases of T.C papilloma and TCC ($P<0.01$). A significant difference between T.C.C with schistosomiasis and T.C.C without as regard both nuclear area and N/C ratio. Aneuploid histogram were elaborated by 76.9% of urine samples of TCC and 66.67% of samples of T.C papilloma, Tetraploid histograms represented 3.85% of TCC cases. Cases of T.C.C with schistosomiasis elaborated a significance increase in aneuploid histogram compared to T.C.C without $p>0.05$ and a significant increase in the mean percentage of the proliferating cells of S phase in cases of T.C.C with schistosomiasis in comparison to cases of T.C.C without schistomiasis. TCC cases exhibited a significant increase in the mean percentage of aneuploid cells, 5(exceeding rate% and mean unclear ploidy (MNP) compared to much lower values in cases of T.C. papilloma. T.C.C cases associated with schistosomiasis showed a significant increase in the mean percentage of the proliferating cells at S phase, MNP in comparison to cases of T.C.C without.
**Conclusion:** Image analysis of the cellular morphometric objectiVe parameters (nuclear area and N/C ratio), and assessmient of nuclear DNA content of urothelial cells can be applied on routinely processed voided urine smears. This help in detecting early urinary bladder percancerous lesions and confirming the diagnosis of urinay bladder cancer. It also refllect the cell proliferation and nuclear activity, and it suggest to be of value in distinguishing between the different lesions. The use of more than one parameter to differentiate between the different lesions, give nearly accurate results.

## P-061

MAMMARY CARCINOMA WITH OSTEOCLASTIC CELLS: A CYTOLOGYCALLY AND MACROSCOPICALLY STRIKING TYPE PREDOMINATINGLY DISPLAYING AN ADENOCYSTIC PATTERN Review of the literature and additional four cases

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**Aims:** Mammary carcinoma with osteoclastic cells (MCOCC) are extremely rare tumors with puzzling features. In this study we reviewed the unique cytologic, histologic and clinicopathologic traits of this entity.

**Methods:**We studied air-dried and May-Grünwald-Giemsa stained cytologic preparations of four patients initially. Then we examined histologic sections of the biopsy and mastectomy materials some supported by immunohistochemistry. Finally we evaluated the medical records and compared all these findings.

**Results:**All patients were premenopausal women with ages 46 to 52 years. All tumors were solitary and one bilateral varying from 1.8 to 2.4 cm. in size. All were nodular tumors with well-demarcated margins both mammographycally and grossly having a soft consistency with a red to brown color. Both cytologycally and histologycally there were intimacy of mono and multinuclear OCCs with small monotonous tumor cells. All had adenocystic except one of the patient with bilateral masses in which it was colloid carcinoma. Two cases had axillary lymph node metastasis, 3/13 and 2/16 each. Non of them had visceral metastasis and all were free of the disease.

**Conclusions:** OCCs seen easily and plentiful in cytologic aspirates makes diagnosis very easy, and expectation of a better prognosis in most of these well differentiated tumors makes it more essential to diagnose these earlier by cytology, while they give a benign impression clinically and radiologically. On the other hand one must keep in mind that monotonous and small bland tumor cells may cause a false negative cytologic diagnosis.

## P-062

MERKEL-CELL CARCINOMA OF THE SKIN WITH PLEURAL EFFUSION: CYTOLOGYCAL FINDINGS OF ONE CASE.

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Aim: Pleural effusion by Merkel-cell carcinoma of the skin rarely occur. A 78 year-old female with two year history of a Merkel-cell carcinoma of the skin of the right thigh and associated renal cell carcinoma of right the kidney, developed a right pleural effusion.The original tumor of the skin is compared with cytologycal material. Differential diagnoses are discussed.

Methods:Air dried smears were stained according to May-Grumwald-Giemsa. The avidin-biotin complex method was employed for Cytokeratin, Leucocytes Common Antigen, Chromogranin and Synaptophysin.

Results: Cytologycal smears were composed of a monotonous, hight cellular population of small and round cells, with eccentrically placed nucleus and indistinct nucleoli: only small rim of cytoplasm was present. The cells formed loosely cohesive clusters, Indian files, curved rows and rosettelike structures and revealed positivity for Cytokeratin, Chromogranin and Synaptophisyn. Light microscopy of the skin tumor showed a small cell tumor arranged in solid arcas.The tumor cells recapitulated the morphology of neoplastic cells seen in the pleural effusion, two years later.

Conclusions: Main differential diagnoses were metastasis from small cell carcinoma of a neuroendocrine or undifferentiated type, malignant non Hodgkin Lymphomas and malignant melanoma. Small cell carcinomas and carcinoid tumors are the most difficult to distinguish cytologycally from primary small-cell carcinoma of the skin because no significant difference exist in the chromatin pattern between these neoplasms. Lymphomas are noted for a noncohesive monomorphic population of tumor cells, in wich irregularities, indentation and cleaved appearance of the nuclear membrane exist and lymphoglandular bodies are noted. The malignant melanoma cells exibit abundant cytoplasm, bi or multinucleation, eosinophilic macronucleoli and growth in isolated or rare loose clusters. Clinical correlation is essential and immunochemistry may be helpful to avoid misdiagnosis.

## P-063

**CANCER CELLS IN EFFUSIONS. DIAGNOSTIC SIGNIFICANCE OF TELOMERASE ACTIVITY.**

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**Aims :** Most somatic cells in adult tissues have no telomerase activity but this activity is present in a great majority of cancer cells. We have compared the presence of telomerase activity using the telomeric repeat amplification protocol (TRAP) assay, with cytological examination for the detection of cancer cells in pathological effusions.

**Methods :**TRAP assays and cytologic examinations were performed in 91 effusions (pleural, ascitic and pericardic) from 86 unselected patients. TRAP assays and cytologic examinations were performed in a double-blinded fashion. In order to reduce the chance of false-negative TRAP assay results, the integrity of rRNA in tissues was verified in each case.

**Results :** Medium concordance (63/91 cases, 69%) between cytological examination and telomerase assay was observed. According to the cytological diagnosis achieved, the effusions were classified into malignant (27 cases) and non-malignant (64 cases). In the 27 cytologic positive cases concordance between the telomerase assay and cytologic examination was observed in 19 cases (70%). Among the 8 cytologic positive cases without telomerase activity, 6 had highly RNA degraded materiel. Telomerase activity was detected in 27% (17/64) of non-malignant cytologic effusions, and in 11 of them there was a strong clinical suspicion of malignancy.

**Conclusions :** These results suggest that, provided that the cell sample is sufficiently well preserved to allow for undegraded rRNA, telomerase activity could be a more sensitive parameter for the presence of malignant cells than cytology. This, however, needs to be verified in large scale prospective studies before the telomerase assay can become a useful adjunct in routine cytologic examination.

## P-064

GERM CELL TUMOURS OF EXTRAGONADAL LOCATION. Cytological and immunocytochemical study.

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**Aims:** FNA is an effective method well suited to the diagnosis of GCT and the determination of the correct therapy. This is a report of a series of 10 primary and 22 metastatic GCTs, corresponding to 27 patients.

**Methods:** Cytological and immunocytochemical study of 28 FNA from: mediastimun (7), retroperitoneum (3), lymph nodes (13), abdominal mass (1), liver (1), kidney (1), adrenal (1) and lung (1). Also 1 sputum and 3 serous effusions were studied. Beta-hCG was used in 14 cases, alpha-fetoprotein (AFP) in 6 , cytokeratin in 7, CEA in 5 and placental alkaline phosphatase (PLAP) in 4. In a few cases vimentin, EMA and enolase were used.

**Results:** Cytological diagnosis was stablished in 29 cases: 6 embryonal carcinoma(EC), 9 choriocarcinoma, 4 yolk-sac tumour(YST), 2 seminoma, 1 teratoma and 7 GCT witthout specification. In 3 cases the diagnosis was erroneous. In 16 the diagnosis was confirmed by biopsy and autopsy was performed in 2 cases. In cases where GCT without specification was diagnosed and cases with erroneous diagnosis, histological analysis showed a mixed tumour made up predominantly of EC and YST, one case showed areas of choriocarcinoma.

**Conclusions:** The main diagnostic difficulty is presented by EC and YST as they can be identified as metastasis of epithelial tumours without differentiation or adenocarcinoma. Pleomorphic cells, light empty-looking nuclei, atypical, eosiniphilic and multiple nuleoli, mitosis and haemorrhage are points to bear in mind in the cytological diagnosis of these neoplasms. Expression of beta-hCG, PLAP and AFP help and may indicate a GCT.

## P-065

**FINE NEEDLE ASPIRATION OF PAROTID GLAND LESIONS. CYTOHISTOLOGICAL CORRELATION**

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**Aims**: To evaluate the utility of fine needle aspiration (FNA) in the study of parotid gland tumours and to assess its capacity to provide and accurate diagnosis and a correct histological identification.

**Methods**: 148 FNAs were carried out on parotid gland lesions at our Hospital between 1991 and 1998. In 67 patients that underwent surgery, comparison between cytological and histological diagnosis was made.

**Results**: In histological study 57 cases (85,07%) were benign lesions and 10 (14,92%) malignant tumours. In 5 benign lesions, the material obtained by FNA was inadequate for diagnosis and was excluded from the series (benign n=52; malignant n=10).

FNA yielded a correct diagnosis in 45 of 52 benign lesions: 32/34 (94,1%) pleomorphic adenoma; 11/15 (73,3%) Warthin's tumour; and 2/2 (100%) benign cyst. In the other 7 cases a correct diagnosis was not achieved but no false positive was made.

FNA yielded a correct diagnosis in 9 of 10 malignant tumours: 3/3 (100%) acinic cell carcinoma, 2/3 (66,6%) mucoepidermoid carcinoma, 2/2 (100%) malignant lymphoma, 1/1 (100%) adenoid cystic carcinoma and 1/1 (100%) poorly differentiated carcinoma. The false negative diagnosis was a mucoepidermoid carcinoma arising in a pleomorphic adenoma in which only the benign component was evaluated in FNA.

**Conclusion**: FNA of parotid gland tumours allows a correct diagnosis in 86,5% of benign lesions and 90% of malignant tumours. The sensitivity and specificity are 90% and 100%, respectively.

## P-066

**FINE NEEDLE ASPIRATION CYTOLOGY OF 119 ADRENAL MASSES**

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**Aims:** the use of radiological procedures has led to the identification of an ever increasing number of adrenal masses. However, only few series discussing FNA cytology of adrenal lesions have been published.

**Methods:** one hundred nineteen consecutive adrenal FNAs were examined in our department between 1992 and 1998. FNAs were performed via a posterior or anterior approach under TAC guidance using a 21-gauge needle. Histological examination of the aspirated lesions was available in 55 cases.

**Results:** the aspirates were divided in diagnostic (108) or nondiagnostic samples (11). The diagnostic aspirates were classified in three groups: benign cortical nodules (BCNs), malignant tumours and pheocromocytomas. Malignant smears were divided in primary, secondary neoplasms and malignant cells of undetermined origin. BCNs were 54 (28 confirmed by pathological follow-up). Five adrenal cortical carcinomas were diagnosed by cytology, four with histological assessment. Metastatic adrenal lesions were 26 (four had histology); the most common primary was lung carcinoma with 15 cases. Malignant cells of undetermined origin were diagnosed in 10 cases: at histology, four were primary adrenal carcinomas, one was a paraganglioma, one was nondiagnostic. In ten cases cytology was consistent with pheochromocytoma. Moreover, at cytology we diagnosed also a paraganglioma, a ganglioneuroma and a lymphoma.

**Conclusions:** the high degree of adequacy can be explained by the immediate assessment of the samples. The cases with a pathological follow-up have shown that cytology of adrenal masses has a high degree of accuracy in classifying lesions as benign or malignant, therefore resolving the most important clinical question. A limit of the procedure may be in distinguishing between malignant tumours of different origin.

## P-067

**VALUE OF EXFOLIATIVE BILE CYTOLOGY FOR DIAGNOSIS OF MALIGNANT OBSTRUCTIVE JAUNDICE**

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**Aims:** To assess the value of exfoliative bile cytology for diagnosis of obstructing cancer of the biliary tract.

**Methods:** 168 specimens were obtained by percutaneous transhepatic biliary drainage from 128 consecutive patients with obstructive jaundice due to biliary tract stricture (73 males, 55 females). 104 patients had malignant obstruction and 14 had benign disease, confirmed by fine-needle aspiration cytology in 12, percutaneous endobiliary biopsy with forceps in 40, endoscopical biopsy in 6, surgical specimen in 12, necropsy in 1, or a combination of percutaneous cholangiography findings and clinical follow-up in 47 patients. 13 samples of ten patients were excluded from analysis because of inadequate follow-up information.

**Results:** From 155 bile samples studied, 86 were benign (55%) (epithelial cells were not present in 27 of them), 29 were atypical (19%), and 40 were suspicious or positive for malignant cells (26%). All the patients who had samples reported as suspicious or positive for malignancy were confirmed as having malignant disease. None of the 14 patients with benign disease had positive or suspicious cytology. From 29 atypical reports, two patients had benign disease (bile duct stones and chronic pancreatitis). In 12 patients, cytology alone established the presence of cancer. Overall specificity and sensitivity of bile cytology were 100% and 30%. Overall positive predictive value was 100% and negative predictive value was 18%.

**Conclusions:** Samples from exfoliative bile cytology are simple to obtain, the positive results are highly specific and could be a routine part of the obstructive jaundice diagnosis.

## P-068

**FINE NEEDLE ASPIRATION CYTOLOGY OF GYNECO-MASTIA. A STUDY OF 245 CASES.**

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**Aims :** To define the distinctive cytologic features of gynecomastia and their relation with the numerous clinic factors.

**Methods :** We examined the FNA cytology of 245 cases of gynecomastia and we studied 64 clinic and cytomorphologic parameters.

**Results :** The FNA cytologic features of gynecomastia included a variable number of cohesive monolayered sheets of epithelial duct cells with surrounding and superimposed myoepithelial cells. The ductal cells had scanty, basophilic cytoplasma and single, round, vesicular nuclei with finely granular chromatin and inconspicuous nucleoli or chromocenters. A variable number of bipolar naked nuclei, single tall columnar cells and stromal fragments were seen in the clean background. Histiocytes and apocrine metaplastic cells were occasionally noted. We observed significant differences between cytologic and clinic parameters, in terms of cellularity and aging, drug-induced, non-physiologic and systemic illnesses-associated gyneco-mastias and short duration of symptoms. Also, statistically nodular, unilateral, left-side and intermediate size masses showed more cellular smears.

**Conclusions :** In conclusion, this study support that the different cytologic appearance of gynecomastia is related to the duration of the breast enlargement and the etiologic factors. Previous reports do not mention any clinical and cytological association.

## P-069

USEFULNESS OF THE BRONCHOALVEOLAR LAVAGE (BAL) CYTOLOGY IN THE CRITICALLY ILL PATIENTS.

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**Aims**: The causes of respiratory insufficiency in mechanically ventilated patients are various and may overlap infectious and non-infectious etiologies. The cytologic examination of BAL fluid (type of cells and presence of intracellular bacteria in PMN) is analyzed in these patients.

**Methods**: Thirty intubated patients who developed chest radiographic infiltrates and clinical deterioration or fever during their evolution in the Intensive Care Unit were included. Respiratory samples were obtained by bronchoalveolar lavage in the first 48 hours of the new infiltrates. The sampling method was selected according to the clinical state of the patient (bronchoscopy or mini-protected-BAL). The cytologic study of BAL fluid informed about: type of inflammatory cells, presence and percentage of intracellular bacteria in PMN (threshold level ≥2%) and presence of other opportunistic infections or atypical cells. The smears were stained with Diff-Quik, H-E and Papanicolaou. The results were delivered within two hours. An aliquot of the BAL fluid was sended for culture.

**Results**: Initial diagnosis were: pneumonia (19), ARDS (6), COPD (3), bronchoaspiration of gastric content (1) and pulmonary embolism (1). Cytology changed diagnosis and treatment in 10 patients (33%). When results of cultures arrived, changes in diagnosis rose to 50% and treatment to 83%. The final diagnosis were: bacterial pneumonia (17), viral pneumonia (2), ARDS (4), lung carcinoma (3), pulmonary tuberculosis (1), aspergillosis (1), pulmonary edema (1) and lung contusion (1). The total mortality was 73% (22/30).

**Conclusions**: Cytology of BAL fluid is a useful tool in critically ill patients, giving rapid and valuable information in order to establish a correct diagnosis and, therefore, a more accurate treatment.

## P-070

**CYTOLOGICAL EXAMINATION OF LIVER FNA. INTRA AND INTEROBSERVER VARIABILITY.**

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**Aims**: To determine the diagnostic value and reproducibility of cytological parameters in liver FNA from benign and malignant lesions and to evaluate the intra and interobserver variability in such samples.

**Methods**: A total of 140 FNA samples from benign and malignant liver lesions were analyzed by five independent observers, utilizing a model with 28 different cytological parameters. There were 29 benign lesions, 49 hepatocellular carcinomas (HCCA), and 62 metastatic tumors (MT). Step-wise logistic regression was used to analyze the multivariate contingency tables, and the kappa values were used for evaluation of intra and interobserver agreement.

**Results**: Irregular nuclear contour, three-dimensional groups, and atypical naked nuclei were strongly associated with malignancy. The presence of granular cytoplasm and capillaries were most commonly associated to HCCA, while eccentrically placed nuclei and necrosis were strongly associated to MT. With consensus diagnosis, the sensitivity of cytological analysis was 100%, and the specificity 97%. There was excellent interobserver and excellent to perfect intraobserver agreement for the final cytological diagnosis. There was a fair to very good interobserver agreement for 22 of the 28 cytological parameters studied.

**Conclusions**: There are several cytological parameters that are highly diagnostic in distinguishing benign from malignant and primary from metastatic liver disease by FNA analysis. Most of these parameters are reproducible by observers with different experience in the field of cytology. The sensitivity of FNA cytological evaluation of liver masses varies from 95% to 100%, and the specificity from 83% to 100% depending on the different observers.

## P-071

PITFALLS IN THE CYTODIAGNOSIS OF HODGKIN'S DISEASE

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**Aims:** To describe the cytodiagnostic accuracy and pitfalls in a large series of Hodgkin's disease (HD).

**Methods:** All the cytodiagnosis of HD during a 19-year period (1980-1998) were reviewed. Cyto-histologic correlation was present in 142 while 15 were discordant. Thirty-one cases of HD with a previous cytodiagnosis different than HD were also selected. Patients with known HD or without biopsy were not included in the study.

**Results:** The sensibility was 82.1% and the positive predictive value 90.4%. Pitfalls could be divided as follows:

1. Non-Hodgkin lymphomas (15): 10 large cell lymphomas and 5 cytodiagnosis of lymphoproliferative disorder without further specifications. They represent the most common pitfall during the last years.
2. Reactive lymphadenopathies (6): they concern cases with suppurative and granulomatous changes and one case of infectious mononucleosis.
3. Metastatic carcinoma (5): none of these errors were performed during the last 10 years. Related to tumors with suppurative changes and lymphoid stroma.
4. Related to scarce material (20): negative (8), non-representative (8) and suspicious of malignancy (4).

**Conclusion:** FNAC constitutes an excellent diagnostic method for the initial evaluation of HD. Cytologic pitfalls have changed from one decade to another reflecting the increase experience of cytopathologists. At the present, Ki-1 positive- and T cell lymphomas constitute the most important diagnostic challenges.

## P-072

**FINE-NEEDLE ASPIRATION CYTOLOGY IN DERMATOLOGY**

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Cytology is a well-established diagnostic method. However, pathologists have been reluctant to use FNAC in dermatology, mainly for two reasons, namely their scarce experience in cytological diagnosis of skin diseases, which is dealt with in very few papers or textbooks, and the accessibility of skin for biopsy purposes. Nevertheless, in some circumstances FNAC may be more adequate than biopsy: (1) to avoid unnecessary scars, (2) to avoid unnecessary surgical procedures of lesions which prove to be benign after the clinico-cytological diagnosis is made, (3) to rule out metastasis in oncologic patients, (4) for reassurance of patients who deny permission for biopsy, (5) to determine patient priority without compromising the eventual pathologic diagnosis in surgical facilities with long waiting lists, (6) in children and (7) on special anatomical locations, or (8) even because of economical concerns, as in underdeveloped countries. In situations like those, unfamiliarity with the cytologic features of cutaneous diseases precludes the use of FNAC.

Even though skin FNAC may prove to be necessary in only a few occasions, we must be prepared to cope with the subtleties of this reproducible diagnostic method. We have used a simple post-exeresis self-teaching technique that simulates a real working situation without damaging the biopsy or bothering the patient, and has allowed us to acquire the necessary expertise to face cutaneous cytologic diagnosis with confidence.

In this poster you will be able to experiment yourself with our method of cutaneous FNAC.

## P-073

HPV DETECTION USING HYBRID CAPTURE II IN PATIENTS REFERRED TO A CERVICAL PATHOLOGY UNIT

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Aims

Detection of HPV in cytology samples can be helpful in the management of lesions of the uterine cervix. Hybrid Capture II (Digene/Abbot) is an alternative to PCR methods in clinical cytology laboratories that has to be evaluated.

Material and methods

A series of 108 patients referred to our hospital for having an abnormal cytology was included in the study. Colposcopy, cytology and sampling for HPV detection were performed in each patient at the same time. Biopsies were taken in 84 cases. Mean age was 39 years (19-69). Cytology results were grouped according to the Bethesda system. Hybrid Capture II , using low and high-risk HPV types probes, was applied.

Results

HPV was detected in 57/113 cases (50%): high risk types in 39 ( 34.5 %), low risk in 8 (7%) and both high and low risk HPV types in 9 (8 %). HPV was found in 12/48 (25%) normal cytology cases, 3/11 (27%) ASCUS, 24/35 (68.5%) LSIL and in 17/18 (94%) HSIL. HPV testing was positive in 14/36 (39%) patients with normal biopsies, 20/32 (62.5%) with LSIL and in 16/16 (100%) with HSIL.

Conclusions

- HPV detection by Hybrid Capture II is a sensitive test that can be easily implemented in clinical cytology laboratories.
- HPV detection in HSIL was very high (94-100%). However, many LSIL cases (31.5-37.5%) were negative for HPV.
- Infection by low risk HPV types was infrequent
- HPV was detected in a high proportion of cytology (25%) and biopsy negative cases (39%).

## P-074

IMMUNOCYTOCHEMISTRY IN THE DIFFERENTIAL DIAGNOSIS OF SEROUS EFFUSIONS: A COMPARATIVE EVALUATION OF EIGHT MONOCLONAL ANTIBODIES IN PAPANICOLAOU STAINED SMEARS.

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**Aims:** The distinction between pleural mesothelioma (MS), reactive mesothelium (RM), and adenocarcinoma (AC) in serous effusions continues to be a diagnostic problem in pathology. The use of various immunohistochemical markers to facilitate this differential diagnosis has become common, specially in surgical samples. But, as yet, no antigen is expressed specifically in mesothelial proliferations nor in adenocarcinomas. Thus, the optimum panel of antibodies has to be reported. Most of these antibodies have also been applied to serous effusions also with variable results. The aim of this study is to evaluated the usefulness of eight antibodies in the diagnosis of these type of effusions.

**Methods:** A total of 44 cytological specimens of serous effusions (26 pleural, 15 peritoneal, and 3 pericardial), from 30 AC, 3 MS and 11 RM previously stained with Papanicolaou were selected and stained with HMBE-1, Trombomodulin, Calretinin, MOC-31, ESA, E-Cadherin, CEA and CD-15. The immunoreactions were independently evaluated by two observers and considered 0 if negative, and 1 if clearly positive (cytoplasmic and cell membrane reactivity). A stepwise logistic regression analysis was applied to our data to select an appropriate panel of antibodies.

**Results**: Statistical significance was found with HMBE-1, trombomodulin, ESA, MOC-31 and CD-15, when compared both AC versus MS, and AC versus any type of mesothelial proliferation (MS plus RM). Using HMBE-1, 80% of ACs were negative while all 3 MS react strongly with a p value of 0,003. A value of p = 0,02 was reached with trombomodulin antibody with 76,5% of AC showing no immunoreactivity. ESA and MOC-31 emerged as great discriminators, with p value <0,01 and 0,001 respectively. CD-15 was also useful with a p = 0,034. No differences were found using the other antibodies. Ten ACs, all 3 MS and 10 RM were double immunostained with HMBE-1 and/or MOC-31 and ESA with good results.

**Conclusions**. Immunohistochemical studies performed on Papanicolaou stained cytological smears are useful in the differentiation between metastatic AC and mesothelial proliferation. HMBE-1, trombomodulin, ESA, MOC-31, and CD-15 are the most useful. If the sample is scarce, double immunostaining with two different antibodies could be of help.

## P-075

**CYTOLOGICAL GRADING OF DUCTAL CARCINOMA IN SITU (DCIS)**

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**Aims:** To identify features that distinguish between the subtypes of ductal carcinoma in-situ and possibly differentiate in-situ disease.

**Methods:** 66 cases of histologically proven pure DCIS (39 high grade, 12 cribriform and 15 low/intermediate grade) with a preoperative cytology report of carcinoma were retrieved for review. 7 cytological features were assessed and scored from 1 to 3: cellularity, cell dissociation, nuclear size cell uniformity, nucleoli, nuclear margins and chromatin pattern. We also recorded the presence of calcium, necrosis and foamy macrophages, a cribriform pattern in cell clusters, and evidence of infiltration by tumor cells into fat and stroma. Clinical features were correlated with the cytological features.

**Results:** High grade DCIS has predominantly large, pleomorphic cells, low grade including cribriform DCIS shows small, well-differentiated cells. Most high grade lesions demonstrated calcium and necrosis, this was less frequent in the low grade and cribriform cases. Foamy macrophages were more common in high grade and cribriform types of DCIS as opposed to the non-cribriform, low/intermediate grade DCIS None of the cases showed infiltration of fat and stroma by tumor cells.

**Conclusion:** In this study the absence of tumor infiltration into fat and stroma was a constant feature but cannot be taken as definite evidence of pure in-situ disease . However, together with pleomorphic carcinoma cells, calcium, necrosis, macrophages and 'casting calcification' on mammograms with no soft tissue mass, this is virtually diagnostic of comedo-type high grade DCIS. Cribriform DCIS was always low grade with a typical cribriform appearance. Low/intermediate grade DCIS has no particular diagnostic features.

## P-076

**FINE NEEDLE ASPIRATION OF ADNEXAL TUMOURS: CYTOHISTOLOGICAL CORRELATION**

Méndez I, Combalia N, Ferreres JC, Orellana R, Ferran A, Gallardo J, Antoni J*, Corona M*, Mellado F*, Rey M. Dept of Pathology and *Gynecology Corporació Sanitària Parc Taulí. Sabadell. Barcelona. Spain.

**Aims**: To evaluate the utility of transvaginal fine needle aspiration (TFNA) for diagnosis study ovarian tumours.

**Methods**: 1330 US-guided of adnexal masses have been performed in 1.149 patients during the last ten years. Tumours were surgical removed in 505 cases and cytohistological correlation was made. Cytologic diagnosis has been assembled in positive for carcinoma, negative for malignant cells and inadequate. Diagnosis as suspicious or atypia have been included in positive group because it implies a similar surgical attitude.

**Results**:

| Histologic diagnosis | Cytologic diagnosis | | | |
|---|---|---|---|---|
| | Positive | Negative | Inadequate | **Total** |
| Benign | 8 | 360 | 20 | **388** |
| Malignant | 87 | 5 | 5 | **97** |
| Borderline | 8 | 11 | 1 | **20** |
| **Total** | **103** | **376** | **26** | **505** |

With these results, we have obtained a sensitivity of 85,5 % and specificity of 97,8 %, a positive predictive value of 92,2 % and negative predictive value of 95,7 %.

Serous cystadenomas, endometriosis and reactive mesothelial cells are the most important causes of false positive diagnosis and mucinous tumours of borderline malignancy and adenocarcinomas arising in endometriosis cysts are causes of false negative diagnosis.

30 of the ovarian carcinomas diagnosed by TFNA were histologically on stage Ia (FIGO). None of them has recidivated after a follow-up between 3 months and 9 years with a average of 51 months.

**Conclusions**: TFNA of adnexal tumours is an effective and reliable method to avoid unnecessary surgery in cases of benign pathology, and allows a preoperative diagnosis of malignancy.

## P-077

FINE NEEDLE ASPIRATION CYTOLOGY OF AN ADULT MULTIFOCAL RHABDOMYOMA OF THE NECK.

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Aim: The fine needle aspiration cytology (FNAC) of an adult multifocal rhabdomyoma (AMR) of the neck region in a 61-year-old male is documented. The patient presented with five nontender masses (0,5-4 cm) in the left side of the neck and parapharyngeal space.

Methods: A FNAC of the main mass with a 25x0,5 mm gauge needle was performed. Smears were stained by Papanicolaou and Romanowsky techniques. Histological and immunohistochemical studies were also made on the formalin-fixed tissue.

Results: The aspiration smears were cellular, showing polygonal-to-round or elongated individual and cluster cells, with abundant well-defined granular amphophilic to eosinophilic cytoplasm, and measured 15 to 80 μm. Ocassional cross striations were also seen. The nuclei were uniform, round and peripherally located. The background was clean without any necrotic debris or inflammatory cells. The cytologic picture was interpreted as rhabdomyoma, and the patient was operated on. Histologically proved to be an adult rhabdomyoma. The tumor cells showed strong positivity for desmin, myoglobin, actin and weak positivity for S-100 protein and vimentin.

Conclusions: AMR are rare benign tumors of striated muscle origin. Only very few cases have been reported. They have an unexplained predilection for head and neck region and affect more males patients than females. Most cases occur in individuals over 40 years of age. The characteristic cytology, histology and immunophenotype, distinguish AMR from other lesions with which it is frequently confused, including rhabdomyosarcoma, granular cell tumor, oncocytoma, Hürthle cell tumor of thyroid gland, acinic-cell carcinoma of salivary gland, hibernoma, etc.

## P-078

**LUNG CANCER: BRONCHIAL ASPIRATES AND BIOPSY SPECIMENS IN ITS DIAGNOSIS. STUDY OF 112 PATIENTS WITH BRONCHIAL ASPIRATES.**

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Introduction: Lung cancer (LC) is one of the most frequent tumors and its pathologic typification decisively affect in prognosis and antineoplastic treatment. The cytology of sputum has been used as diagnosis method in these patients, but this technique is being displaced by others as aspirates with flexible fibrobronchoscopy (FBC) or transbronchial fine-needle aspirates.

Methods: We reviewed correlation of cytologic study of bronchoaspirates (BAS) and clinics, radiologics and FBC findings in 112 patients who came to our hospital during the years 1997-1998. In 36 of these patients bronchial biopsy was also made.

Results: In 92,3% of patients with non-suspicious radiology of neoplasia and 91,6% of normal FBC the cytology was negative (p=0,0003). Of patients with LC corroborated by lobctomy or pneumectomy, 91,3% had positive cytology before and in 65,5% of these the simultaneous biopsy corroborated the diagnosis (p=0,0023). 20% of patients with negative cytology had positive biopsy (p=0,0023). No significative relation found between clinic symptoms and cyto-histological findings.

Conclusions: The correlation found between radiologic and FBC findings and the cytologic study of patients support these techniques for diagnosis of LC. The clinics sympton's valoration is more subjective and non-valuable. The biopsy specimens are available for confirmation of cytology or as/like diagnostic technique in cases of negative cytology.

## P-079

**FINE NEEDLE ASPIRATION CYTOLOGY OF CLEAR CELL HIDRADENOMA OF THE BREAST. A CASE REPORT.**

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**Aims :** To describe the cytological features of a benign tumour of the sweat gland, presented as a breast lump, that can be mistaken with a primary or metastatic tumor of the mamary gland.

**Case report:** A 88-year-old woman was seen in our Hospital with a lump in the breast. Phisical examination revealed a 2 x 2 cm, firm mass in the upper, outer quadrant of the right breast. The skin over was ulcerated. It was clinically diagnosed of carcinoma. Mammography revealed a well circumscribed lesion. Ultrasound investigation demonstrated a solid-cystic nodule. A fine-needle aspiration cytology of the tumour was performed. Cytologic examination revealed a moderately cellular specimen containing sheets of cohesive epithelial cells, numerous multinucleated giant cells, histiocytes and cellular detritus. Bipolar naked nuclei were absent. Some epithelial cells were cuboidal with scant cytoplasm and others had polyhedral, escamoid cytoplasm. Nuclei were round to oval, without atypia. A small number of cells had clear cytoplasm and vesicular nuclei. There were fragments of hialynized stroma. In view of the unusual clinical and cytological findings, excision biopsy was recommended. The histopathologic study showed an ill-defined, ulcerated and lobulated tumour with marked focal stromal hyalinization. The solid component was a mixture of small basaloid cells and polygonal cells with clear appearance. Numerous histiocytes with foamy cytoplasm, cholesterol clefts and multinucleated cells were seen at the periphery of the tumour. There was evident connection between the tumour and the overlying epidermis. The diagnosis was that of clear cell hidradenoma.

**Discussion** : Fine needle aspirates of clear cell hidradenoma have been rarely reported, and it is recognized that they can be a source of diagnostic pitfall. An accurate knowledge of their cytologic features could be extremely helpful in order to identify them correctly.

## P-080

MONITOR CONTROL OF COLPOSCOPY, CYTOLOGY, HISTOLOGY IN 146 PATIENTS DURING 4 YEARS WITH PRIMARY HPV TYIPING BY PCR.

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Aims: This study was devoted to the analysis of the relationship between colposcopy and cytology-histology in the presence of high and low risk HPV infections.

Methods: Colposcopy, cytology, histology, HPV tyiping by PCR.

Results: We examined 146 patients in the period 1994-1998. These patients were divided according to the colposcopy diagnosis into the following groups: ANTZG0* (I), G1* (II), G2* (III), NTZ* (*italian classification). The objects analyzed have been the cytological and histological diagnoses, as well as the HPV tyiping by PCR. The first cytological exam has demonstrated the presence of HSIL and HSIL with HPV's alterations in 9% (I), 16% (II) and 44% (III). The 30% of the I group, 33% of the II group and 67% of the III group have been shown to contain DNA sequences from high risk HPV types. The first histologic exam has pointed out the presence of moderate and high grade dysplasias as well as in situ carcinomas in 17% (I), 35% (II), 55% (III). These patients were controlled after the therapy four years later. The colposcopy was NTZ in 63% (I), 53% (II), 75% (III). The cytology in I group did not demonstrate the alterations of high grade, while they were present in 2% of the II group and in 33% of the III group. In the group of patients with NTZ the dysplasias of high grade was exhibited in 17%. The histological analysis has shown CIN 1 in 46% and CIN 2-CIN 3 in 18%. The presence of high risk HPV types was emphasized in 31%.

Conclusions: It was demonstrated that there is an evident correlation among cyto-histological alterations and the presence of high risk HPV types in the patient with ANTZ. The cyto-histological alterations in the absence of colposcopic alterations should also not be neglected.

## P-081

**FINE NEEDLE ASPIRATION (FNA) OF THYROID LESIONS. CYTOHISTOLOGICAL CORRELATION AND DIAGNOSIS VALUE OF FINDINGS.**

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**Aims**: To evaluate the usefulness of thyroid FNA to provide an accurate diagnosis and to identify cases tributary of surgical treatment; and to determine cytological findings with diagnostic value.

**Methods**: 92 thyroid FNAs with adequate material from patients with subsequent thyroidectomy were reviewed by 2 pathologists without clinical information, making an agreement diagnosis and evaluating many cytological parameters about cellularity, colloid, other findings and usefulness of cell block. Correlation with histological diagnosis and therapeutic orientation was done. Cytological parameters were studied statistically with chi-square and exact Fisher tests.

**Results**: Diagnosis accuracy was 77,1%. About theoretic therapeutic orientation (surgery/no surgery), results were: sensitivity: 86,6%, specificity: 96,7%, PPV: 92,8%, NPV: 93,7%. Cell block was useful for diagnosis in 20,8%. In the most prevalent histological diagnosis, cytological findings with significant value ($p \leq 0.001$) were the following: for nodular hyperplasia (NH) vs. neoplasm (N): quantity of epithelial cells, quantity of colloid, cell size, nuclear size, irregular nuclei and nucleoli; for NH vs. follicular neoplasm (FN): follicular pattern; for papillary carcinoma (PC) vs. follicular lesions (NH and FN): intranuclear inclusions, nuclear clefts and multinucleated giant cells. Macrophages and fibrous tissue were no statistically significant.

**Conclusions**: Thyroid FNA is useful for therapeutic orientation, and acceptable for diagnostic accuracy. Some cytological findings have significant value and can be useful in the cytological diagnosis, while others are not in our series.

## P-082

**NUCLEAR GRADE BY FNA CYTOLOGY IN BREAST CARCINOMA. CORRELATION WITH THE HISTOLOGICAL GRADE IN CORE BIOPSY.**

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**Aims.** To correlate the nuclear grade obtained in FNA cytology with the histological grade in the core biopsy made at the same time, and comparison with the definite grade on the surgical specimen when available.

**Methods.** 89 cases of breast cancer diagnosed in 1998. 87 invasive carcinomas and 2 CIS. 79 cases with definite surgical specimen. Fisher criteria (1-3) for cytology grading and Elston Ellis criteria for biopsy grading.

**Results.** 63% correlation between nuclear grade obtained by FNA and the histological grade in needle biopsy.
61% correlation between the nuclear grade and the definite histological grade on surgical specimen.
No case with a discrepancy superior to one grade. The histological grade tends to be higher than cytology (nuclear) grade.

**Conclusion.** Nuclear grade in FNA cytology have and acceptable correlation with surgical biopsy so as to allow the prediction of the definite histological grade in 61% of cases and having no more than one grade discrepancy in the rest of the cases.

## P-083

**THYROID ANAPLASTIC CARCINOMA: CYTOLOGIC FINDINGS ON 7 CASES DIAGNOSED BY FNA.**

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**Aims:** To review cytological aspects of anaplastic thyroid carcinoma and to correlate them with surgical specimens.

**Methods:** 7 cases of anaplastic thyroid carcinoma diagnosed by Fine Neddle Aspiration (F.N.A.), with histological correlation, accounted between 1992-1998.

**Results**: Patient age ranged from 51 to 72 years (mean 62 ), 3 were female and 4 male. In three cases, a well-differentiated component was identified in the biopsy (not present in F.N.A.), two papillary and one follicular carcinoma. Cytologically all cases showed numerous highly malignant cells, with spindle, giant and/or squamoid appearance.

**Conclusion:** FNA yields a correct diagnosis in all of the anaplastic thyroid carcinomas studied by this procedure registered in our hospital. The bizarre cells present in these cytologies are diagnostic and they were found in all the cases.

## P-084

**DIAGNOSTIC ACCURACY OF INTRAOPERATIVE CYTOLOGY IN LESIONS OF THE NERVOUS SYSTEM**

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**Aims** Intraoperative cytologic touch imprints (IOC) are frequently used in frozen section examinations. The objective of this study was to evaluate the accuracy of IOC in the diagnosis of lesions of the nervous system.

**Material and Methods** We evaluated IOC and frozen section preparations from forty-two central and peripheral nervous system lesions obtained from craniotomies and stereotactic biopsies. Cytologic diagnosis was compared to the final histologic diagnosis.

**Results** Patient ages ranged from 7-79 years. Accurate IOC diagnoses was obtained in cases of metastatic carcinomas (8), chordoma (1), malignant melanoma (2), high grade astrocytoma (6), meningioma (6) and pituitary adenoma (2). Both cases of oligodendroglioma were interpreted as benign neural tumors. 4 atypical meningiomas were classified as meningiomas on IOC 2 cases of anaplastic meningiomas were incorrectly diagnosed as carcinomas. The remaining 9 cases of astrocytosis/low grade astrocytoma were favored to be benign neural lesions on IOC.

**Conclusion**

1. IOC is diagnostically accurate in classifying metastatic carcinoma and high grade astrocytoma
2. Diagnostic problems are encountered with low grade gliomas and atypical and anaplastic meningiomas

## P-085

CHILDHOOD RHABDOMYOSARCOMA IN FINE NEEDLE ASPIRATION BIOPSY SMEARS SIMULATING BENIGN LESIONS

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**Aims:** to point out some of the lesions that could represent a pitfall when differentiating them from a less common pattern of rhabdomyosarcoma (RMS).

**Methods:** During a 23 year period, 37 cases of histologically and/or immunocytochemically confirmed FNAB aspirates of childhood RMS were collected at our institute. Among these there were 8 cases of embryonal RMS with a benign cytomorphologic appearance. We compared their morphology to the morphology of three benign tumors: a neurofibroma, a benign hemangioendothelioma and a juvenile hemangiopericytoma. Morphologic comparison was based on Giemsa stained smears. The Papanicolaou stained smears, when available, were used for immunocytochemical staining .

**Results:** The morphology of the selected 8 cases of embryonal RMS was indistinguishable from that of the three benign tumors. In all cases smears contained a moderate number of very cellular tissue fragments and few dissociated cells. Tightly packed cells looked immature. They were small, unimorphous, with scant cytoplasm and round or slightly oval nuclei. Tissue fragments also contained various amounts of stroma. The RMS cases contained no classical rhabdomyoblasts and no bi or multinucleated cells.

Desmin was positive in all performed cases of RMS and was negative in neurofibroma and hemangiopericitoma. S100 protein and CD31 did not prove helpful.

**Conclusion:** Neurofibroma, benign hemangioendothelioma and juvenile hemangiopericitoma can morphologically be indistinguishable from embryonal RMS in FNAB smears. Positive reaction to desmin is helpful in differential diagnosis.

## P-086

**HORMONAL RECEPTOR STATUS DETERMINATION IN CELL-BLOCKS FROM BREAST FNA. CORRELATION WITH BIOPSIES AND INFLUENCE OF INTERCURRENT THERAPY.**

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**Aims:** To stablish the usefulness of a simple method of hormonal receptor determination in cell-blocks from breast FNA by comparing results with those of subsequent surgical specimens.

**Methods**: 30 cases in which immunohistochemical determination of estrogen and progestorone receptors was performed in cell-blocks from FNA and in further biopsies. Cell-blocks were obtained after rinsing the needle and syringe with formalin. Evaluation was performed following the same criteria as for biopsy material. 14 patients had been treated with neoadjuvant chemotherapy between FNA and surgery. No treatment had been administered to the remaining 16.

**Results:**

| | CELL-BLOCK | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Untreated patients (n=16) | | | | Treated patients (n=14) | | | |
| | ER | | PR | | ER | | PR | |
| **BIOPSY** | Pos | Neg | Pos | Neg | Pos | Neg | Pos | Neg |
| Positive | 9 | 2 | 11 | 1 | 6 | 3 | 3 | 2 |
| Negative | 1 | 4 | 1 | 3 | 2 | 3 | 5 | 4 |

A high concordance was observed in untreated patiens for ER (p=0.03; Kappa value: 0,59) and PR (p=0,02; kappa value: 0,67). Correlation was poor in treated patiens (p>0,3, kappa value<0,3).

**Conclusions:** Intercurrent therapy effect is the most likely cause for discrepancies in treated patients. Inmunohistochemical determination of hormonal receptors in cell-blocks from FNA avoids unnecessary biopsies in patiens not suitable for surgical therapy, and allows to know the hormonal status prior to the application of neoadjuvant chemotherapeutic regimens.

## P-087

**CYTOLOGIC CHARACTERISTICS OF PERIPHERAL NEUROECTODERMAL TUMORS IN FINE NEEDLE ASPIRATION CYTOLOGY AND INTRAOPERATIVE SMEARS.**

**Retrospective study of three cases**

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*Abstract:*

AIMS: The aim of this report is to contribute to the cytodiagnosis and categorization of this interesting group of tumors, that represents the 6% of the malignant soft tissues tumors. They present in a broad range of age, its behavior is highly agressive with recurrence and metastases shortly after the diagnosis.

METHODS: We describe retrospectively three cases, two from fine needle aspiration cytology (FNAC) and one from intraoperative smear. The diagnosis was suggested, and subsequently confirmed by histological and ancillary studies. The cytologyc material was stained with Giemsa, Hematoxilin and eosin and Papanicolaou methods.

RESULTS: Cytologic features described were identified, including monotonous appearance of the cells, -which showed scanty clear cytoplasm-, nuclei with fine chromatin and one or two small nucleoli, and cellular arrangement in cohesive and organoid fashion or as single cells. We did not find, large nucleoli, cellular debris, nuclear pleomorfism and inflammatory component, and the mitosis were scanty.

CONCLUSION: Peripheral neuroectodermal tumors (**PNET**) are rare lesions of the family of the Ewing tumors, including osseous and extraosseous lesions, mainly localized in soft tissues. Their cytologic diagnosis by **FNAC** or scrimp smears is difficult. However their morphological characteristics strongly suggest the correct diagnosis, without the use of any other ancillary studies, being a good orientative procedure in order to head toward the correct management of the tumor.

## P-088

**HEPATOCELLULAR CARCINOMA AND APOPTOSIS: STUDY WITH ANCILLARY TECHNIQUES IN FNAC MATERIAL**

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AIMS: FAS/APO1/CD95 is a 48-kd transmembrane glycoprotein member of the tumor necrosis factor/nerve growth receptor superfamily, originally identified as the mediator of apoptosis in T lymphocytes. Now it is know to be expresed in a variety of normal human tissues and tumors. The bcl-2 gene product is a potent inhibitor of apoptosis induced by a variety of stimuli. We investigated the role of apoptosis and their implications in carcinogenesis in hepatocellular carcinomas (HCC) diagnosed by ultrasound guided FNAC, using 1) *in situ* DNA nick end labeling (INSEL) method studied by fluorescence microscopy, and 2) immunohistochemical study with a specific antibody for bcl-2 protein.

METHODS: 30 samples diagnosed as HCC are selected in patients with enough follow-up and material available for ancillary tecniques. The slides stained with Papanicolaou method are selected and bleached in order to perfom the ISEL method with TdT-mediated dUTP nicke end labelling and studied by fluorescence microscopy, and immunostaining for bcl-2 protein. We analyze the relationship between size of tumor (range 3 cm/ multifocality), grade of differentiation observed by cytologic methods and apoptotic index.

RESULTS: The apoptotic index based on the percentage of positive cells on ISEL, ranges from inapreciable to 15,6 per 1000 cells, and it is unrelated to differentiation or tumoral size. No nuclear staining is observed in nude nucleus or in multinucleated tumoral cells. Bcl-2 expression is correlated with the lowest apoptotic index. Refered to the survival of the patients, the most important criterion is the size of tumor, but the the apoptotic index could be important in the smallest ones.

CONCLUSION: It is possible to detect apoptosis using ancillary methods in material from FNAC of HCC, the only method of diagnosis in some cases. The apoptotic index is not related with the grade of the tumor, but it is interesting its determination in the tumors of little size in order to study it as a possible prognostic factor.

## P-089

**DEDIFFERENTIATED CHONDROSARCOMA: CYTOMORPHOLOGIC FINDINGS OF 7 CASES.**

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Aims: Dedifferentiated chondrosarcoma is a rare variant of chondrosarcoma associated with a very aggressive behavior. It is characterized by areas of low-grade chondrosarcoma juxtaposed to areas of high-grade sarcoma. This study was undertaken with the objective of studying the cytological findings of dedifferentiated chondrosarcoma in CT-guided fine needle aspiration biopsies.

Methods: The cytology files were reviewed for all bone and soft tissue lesions diagnosed by fine needle aspiration in the period from January 1990 to December 1998. Seven cases of dedifferentiated chondrosacoma diagnosed by FNA were identified. Retrospective review of the clinical, radiographic, and cytomorphologic findings of these cases was performed.

Results: All patients were men, 44 to 81 years old (mean age, 67). Five patients presented with tumors involving pelvic bones, one involving the proximal femur and the other involving the tibia. Pain was the most common symptom at presentation (5 patients). One patient presented with pathological fracture, and in the other patient the pelvic tumor was discovered during work-up for metastatic disease in the orbit. All patients had CT-guided FNAB as the first diagnostic procedure. The smears showed fragments of chondroid matrix or clusters of atypical chondrocytes reminiscence of well-differentiated chondrosarcoma in 5 specimens. Features of a high-grade anaplastic sarcoma were seen in all cases. A thin core biopsy was obtained in 5 cases. Surgical resection was performed in 3 patients. Four patients are dead of their disease (mean follow-up, 5 months); 2 patients are alive with disease (follow-up, 6 and 36 months), and a single patient is alive free of disease (8 months).

Conclusions: The diagnosis of dedifferentiated chondrosarcoma is reliable by FNA. Correlation between the radiographic image with the cytological findings of a biphasic neoplasm, containing features of a chondroid lesion associated with areas of an anaplastic sarcoma, allows to the correct diagnosis in most of the cases.

## P-090

CYTOLOGIC FINDINGS OF AN EXTRASKELETAL MYXOID CHONDROSARCOMA

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**Aim:** we present the cytologic findings of an extraskeletal myxoid chondrosarcoma and emphasize their similarity to myxoid liposarcoma.

**Case:** a 54-year-old woman presented with a tumour in the left popliteal fossa which appeared 1 year ago. NMR showed a large well-defined tumor that extended from the posterior part of the left thigh to the popliteal fossa and displaced adjacent structures. This mass was fine-needle aspirated.

**Results:** the cytologic smear showed an abundant myxoid matrix and a moderate amount of fusocellular cells with mildly pleomorphic and hyperchromatic nuclei. The cytoplasms were scant and eosinophilic. The tumour cells were arranged in an arborescent pattern. A delicate branching capillary network and some cells with vacuolated cytoplasm were also observed. It was diagnosed as a fusocellular tumour with abundant myxoid component, suggesting myxoid liposarcoma.

The lesion was surgically resected. Grossly, it was composed of two large lobulated masses that measured 10 cm each one. The cut surface was solid and bright, with an homogeneous gelatinous appearance. Microscopically, it was composed by oval or fusiform cells with little nuclear pleomorphism, low mitotic activity, and eosinophilic, and sometimes vacuolated, cytoplasms. The cells were arranged in strands embedded in an abundant myxoid matrix with some areas that showed a chondroid aspect. The tumour exhibited a proliferation of small vascular channels. The final diagnosis was extraskeletal myxoid chondrosarcoma.

The patient is currently alive and receive complementary radiotherapy.

**Conclusion:** the cytologic features of extraskeletal myxoid chondrosarcoma are similar to those of myxoid liposarcoma. This two entities may be confused not only due to location and gross appearance but also at the cytologic level because of the myxoid stroma, mildly pleomorphic nuclear appearance, presence of some vacuolated cytoplasms, and a prominent capillary network.

## P-091

**Occurrence of ciliated adenocarcinoma cells in pleural effusion from a patient with (an unknown) ovarian carcinoma.**

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**Aims**: To demonstrate an unusual finding in the pleural effusion from a patient with metastatic ovarian carcinoma.

**Methods and results**. A 74 years old woman, without any pulmonary symptoms or clinical evidence of abdominal disease, was referred to the hospital with a pleural effusion. The effusion contained malignant tumor cells, often arranged in clusters and papillary formations, the tumor cells being large with the presence of cytoplasmic vacuoles. No psammoma bodies were noted. By light microscopy, most of the tumor cells were ciliated having a tuft of hairlike processes, observed as unipolar, bipolar and pericellular structures (Fig.). As we suggested, that these cells were metastatic tumor cells from an ovarian carcinoma, the patient underwent an explorative laporotomy and malignant tumor tissue was localized to the right ovary with carcinosis. The histology of the tumor tissue revealed a serous type of ovarian carcinoma with plenty of psammoma bodies.

**Conclusion**. The presence of tufts of hairlike processes, observed by light microscopy, is extremely rare on malignant cells, but has once been described (1). This case confirms the diagnostic value of finding these ciliated adenocarcinoma cells in pleural effusions from a patient with ovarian carcinoma.

Reference: 1) Gupta PK.*et al.* Diagn Cytopathol 1985: 1(3), 228-31.

## P-092

**CYTOPATHOLOGIC ASPECTS OF LEIOMYOBLASTOMA. REPORT OF SEVEN CASES.**

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**Aims: Leiomyoblastomas (LMB) are relatively uncommon neoplasms of controversial histogenesis, they are most frequently located in the stomach, but are also encountered outside the digestive tract. Few reports exist on their cytomorphologic features.**

**Methods: Seven cases of LMB obtained by FNA (3) or imprint cytology (4) are reported ; four of them were diagnosed as malignant by histopathology. Our purpose is to contribute to the still inadequately known cytomorphlogy of this tumor.**

**Results: Cytologic smears contained numerous epithelioid and spindle-shaped cells. Bi and multinucleate cells and naked nuclei were also seen. Intranuclear pseudoinclusions were observed in three cases. No mitotic figures were found. Lymphocytes and hemosiderin-laden macrophages were also present.**

**Conclusions: The varied morphology of LMB points to the diagnosis but the potential aggressive behavior of the tumours cannot be established only on the basis of cytologic material. The use of immunohistological markers on smears or cell block indicates the mesenchymal origen due to positivity for vimentin and negativity for desmine , S 100 and epithelial markers . In our opinion the cytologic spectrum of LMB permits their diagnosis by using "epithelioid mesenchymal tumor" (EMT) as the best term to designate them. The nomenclature EMT indicates their epithelioid appearance and mesenchymal histogenesis.**

## P-093

**CYTOMORPHOLOGY OF PITUITARY ADENOMAS ON FNA. REPORT OF FOUR CASES.**

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**Aims: Pituitary Adenomas (PA) constitute about 10 to 15 % of all intracraneal tumors. The classification is based on size, histologic pattern and hormonal secretory activity; electron microscopy is also relevant. The cytologic features of PA have been described on smears, imprints or touch preparations from biopsy specimens for intraoperative diagnosis of brain tumors. Cystic changes are possible but unfrequent. We present four cases of non-functioning PA, three of them as cystics tumors of sellar region, FNA was realized intraoperatorely. One case was a clinically suspected meningioma extending from the base of skull and parasellar region into paranasal sinuses. FNA was performed througout nasal sinus.**

**Method: Cytologic features are revised. Hormonal immunohistochemical analysis on biopsy material was performed. Ki 67 was studied in case fourth on cytologic material.**

**Results: PA present with trabecular,acinar and round glandular groups. Dens , granular and clear cytoplasm . Monomorphic,round nuclei with prominent nucleoli ; frequently denuded (case four).**
**All cases were negative for: PRL, FSH, LTH,STH, TSH and ACTH. Ki 67 showed sparsely positivity.**

**Conclusions: Differential cytologic diagnosis must be established with oligodendroglioma, meningioma and craneopharyngiomas.No correlation was seen among atypia and more aggressive behavior. Proliferative nuclear antigen (Ki 67) has been used to determine prognosis. Our fourth case presented as a giant and invasive PA and no relationship could be verified with nuclear immunoreactivity and growth or invassiveness.**

## P-094

**UTILITY OF CYTOKERATINS AND CEA IN PRIMARY AND METASTASIC CARCINOMAS IN PA.A.F. OF THE LIVER.**

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**Aims:** The aim of this study is to examine differences in the expression of Citokeratins (CK) and CEA in hepatocellular carcinoma (HC) and metastasic carcinomas (MC) of the liver in PAAF specimens.

**Methods**: We examined 29 liver PAAF: 13 HC and 16 MC (9 colorectal, 2 pancreatic, 3 of lung, 1 of tongue and 1 of unknown origin). Immunohistochemical studies were performed in cell blocks using avidin-biotin-peroxidase complex technique. We used: CK7, CK20, AE1 (CK 10, 14, 15, 16, 19), and CEA. Statistical analysis was made by using $\chi 2$ analysis and Fisher exact test.

**Results:** The results of immmunostaining, specificity and sensibility in the diagnosis of MC, and statistical significance (p-value) for each antibody are shown in the next table .

| | CK7 | AE1 | CK20 | CEA |
|---|---|---|---|---|
| **HC** | 1/13 | 2/13 | 0/13 | 3/13 |
| **MC** | 7/16 | 15/16 | 8/16 | 13/16 |
| -Colorectal | 0/9 | 9/9 | 8/9 | 9/9 |
| - Pancreas . | 2/2 | 2/2 | 0/2 | 2/2 |
| - Lung | 3/3 | 2/3 | 0/3 | 1/3 |
| - Others | 1/2 | 1/2 | 0/2 | 1/2 |
| Sensibility | 43,8% | 93,8% | 50% | 81,3% |
| Specificity | 92,3% | 84,6% | 100% | 76,9% |
| P-value | 0,03 | 0,00002 | 0,002 | 0,002 |

**Conclusions**: This study confirms the usefulness of CK7, CK20, AE1, and CEA in the differential diagnosis between HC and MC in PAAF of the liver. CK20 is specially useful for diagnosis of colorectal MC.

## P-095

FINE-NEEDLE ASPIRATION CYTOLOGY OF PANCREAS: A REVIEW OF 237 CASES

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**Aims:** The use of fine-needle aspiration cytology (FNAC) of the pancreas has had a great impact on the diagnosis of pancreatic masses. Since the introduction of FNAC, some centers have adopted a policy of no obtaining confirmatory tissue samples from the pancreas. We undertook a retrospective study to evaluate the efficacy of FNAC in assessing pancreatic masses.

**Methods**: Two hundred and thirty-seven radiologically guided FNAC of the pancreas performed in a period of ten years were reviewed. All material was stained with Papanicolaou and when available a cellblock was obtained. Surgical material from pancreas and other metastatic organs when available were also evaluated and compared to the FNAC material.

**Results:** Patients were 142 men and 95 women. One hundred and twenty-five aspirates (52,7%) showed malignant tumors, two of them were metastatic neoplasms. One hundred and twenty-three (98,4%) were primary malignant tumors: 107 adenocarcinomas, 6 cystadenocarcinomas and 10 neuroendocrine tumors. Forty patients (32%) had available surgical specimens and thirty-eight (95%) were malignant: 27 adenocarcinomas, 10 endocrine tumors and one metastatic neoplasm. Fifty-nine patients (24,9%) had benign aspirates: 28 ductal epithelium, 17 inflammation, 7 pseudocysts and 7 cysts. Two benign aspirates turned to be malignant in the biopsy. Twenty-four (10,1%) aspirates were suspicious for malignancy. Eighteen had biopsy, corresponding all of them (100%) to malignant neoplasms. Twenty (8,4%) aspirates were unsatisfactory for diagnosis; four of them were malignant in the biopsy.

**Conclusions**: FNAC is a simple and highly accurate method in the diagnosis of pancreatic lesions. In our experience radiologically guided FNAC of pancreatic masses is highly sensitive (90%) and specific (96%). All (100%) suspicious lesions showed malignant neoplasm in the follow-up biopsy.

## P-096

**Morphofunctional paculiarities of the thyroid gland in children & in teenagers in endemic goitre in a district of Azerbaijan.**

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The main part of the research represents material in 34 inlarged thyroid glands taken from dead bodies of people living in endemic goitre district of Azerbaijan. Patients were basically admitted due to mechanical trauma incidents, with ages from 3- 15 years.

Together with developmental actions, our study of the thyroid gland was characterized by morphological paculiarities showing a local effect of goitre causing factors. Most important from them is the development of Ganglion centers,which are first seen in children of 7 yrs. In Children between 7- 12 yrs the ganglionic activity was abruptly activated & in ages 12- 15 yrs together with the development of small ganglionic centers growth isolation was observed. Formation of ganglionic centers coincided with the start of the rapid growth & physiological malfunctioning of the gland.Thus noted that in endemic goitre regions the thyroid gland in children & teenagers are characterized by ganglionic centers of the colloidal type.Structural differentiating of the thyroid gland lays in the $10^{th}$ year, but in ages 12-15 when the thyroid gland was activated the number of gland parenchymal follicular type may be connected with the action of the endemic goiter causing factors.In conditions of deficiency of iodine in external environment and other goitre causing factors disturb the normal activity of the gland 7 is accompanied by increase in the functional activity of the thyroid gland in puberty, given preconditions for external secretory cycle and reinforced proliferation of the thyroidal parenchyma.

Deficiency in teenagers may be considered as a result of the activation of extra follicular proliferation of the thyroidal epithelium 7 in relationship of the secretorial cycle phase the secretion destroyed its product.The study also showed such formations that together with its growth actions the tissue undergoes changes by influence of endemic goitre causing factors to gether with adaptation to growth activity.In ages 3-7 yrs intra-follicular proliferation of the thyroidal epithelium is evoked with the growth of bumpy nipple like protrusions. These are seen in connection with the secretion & production of the thyroidal hormone. After the age of 7 yrs intrafollicular proliferation noticeably slackens but increase the collection & accumulation of colloids which increases the development and size of the follicles of the central colloid and most important the increase in ganglions.

Development period of 12-15 yrs is characterized by constant progress and increases the activity of extra follicular proliferation.

## P-097

**E-CADHERIN MUTATIONS IN MUCOEPIDERMOID CARCINOMA OF THE THYROID**

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**Background** E-cadherin expression has been described as minimal or absent in poorly differentiated and undifferentiated carcinomas of the thyroid in contrast to a variable degree of immunoreactivity/mRNA expression in papillary and follicular carcinomas. An association between reduction or loss of E-cadherin immunoexpression and unfavourable prognosis was found in papillary carcinomas. In a previous study we found a missense mutation in a single case of diffuse sclerosing variant of papillary carcinoma (DSV) (Soares *et al* Int J Cancer 70:32-38,1997)

**Aim & Materials and Methods** - In order to find if E-cadherin mutations are associated to DSV, we studied six additional cases of this variant, as well as 10 cases of mucoepidermoid carcinoma which is a variant of thyroid carcinoma that shares some features of DSV, and 13 "classic" papillary carcinomas. Every case was studied by imunohistochemistry and PCR/SSCP.

**Results** – Mutations were found in one out of the seven DSV and in all the 10 mucoepidermoid carcinomas ; in the latter we observed a mutational hot spot in exon 8 with predominance of a splice site point mutation. There was an association between histotype, mutation and immunohistochemical staining pattern. In papillary carcinomas and DSV the absence of mutations was accompanied by a heterogeneous cytoplasmic expression of E-cadherin together with focal membrane expression. In mucoepidermoid carcinoma, there was a clearly abnormal or absent immunoexpression.

**Conclusion** – We conclude that E-cadherin gene mutations are associated in thyroid, as elsewhere, with particular tumour histotypes: mucoepidermoid carcinoma in the thyroid, diffuse carcinoma in the stomach and lobular carcinoma in the breast.

## P-098

**EXPRESSION OF LEUKEMIA INHIBITORY FACTOR (LIF) AND LIF RECEPTOR (LIF-R) IN THE HUMAN ADRENAL CORTEX: IMPLICATIONS FOR STEROIDOGENESIS**

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**Aims:** It is well established that steroidogenesis in the adrenal cortex is regulated by extraadrenal factors, such as ACTH and angiotensin II. However, over the last years, it has become increasingly clear that paracrine and autocrine mechanisms are also important for steroid synthesis in the adrenal gland. The current study was designed to analyze whether the pleiotropic cytokine leukemia inhibitory factor (LIF) and/or its receptor (LIF-R) are expressed in the normal human adrenal cortex, and whether they may play a role in regulating steroidogenesis.

**Methods:** Expression of LIF and LIF-R in the human adrenal gland was analyzed by RT-PCR and by immunohistochemistry, using LIF- and LIF-R-specific primers and antibodies, respectively. The effect of LIF was studied in steroid-producing NCIh295 adrenal carcinoma cells.

**Results:** Both LIF and LIF-R mRNA are expressed in the adrenal gland, as well as in the NCIh295 adrenal carcinoma cell line. The correct sequences of the PCR products were verified by restriction enzyme analysis and DNA sequencing. Immunocytochemistry reveals expression of both proteins in the normal human adrenal cortex. Finally, we show that LIF can significantly enhance basal and ACTH-induced production of cortisol and aldosterone in NCIh295 cells.

**Conclusions:** We show for the first time that LIF and its receptor are expressed in the normal human adrenal cortex. Our functional data indicate that the intraadrenal LIF/LIF-R system may participate in regulating adrenal steroidogenesis.

## P-099

**ANALYSIS BY FLOW CYTOMETRY OF THE THYROID NODULE CYTOLOGY. STUDY WITH CYTOKERATIN, VIMENTIN, KI-67, BCL-2 AND CD44.**

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**Aims:** There is considerable controversy in the diagnosis and interpretation of the thyroid cytology. The features used to distinguish the lesions of nodular goiter from follicular adenoma or follicular carcinoma may not be seen cytologically. We postulate to use the flow cytometry to increase the efficiency of the fine needle cytology of the thyroid nodule.

**Methods:** We have studied a total of 67 euthyroid patients with palpable thyroid nodule. In 53 patient the sample was obtained by fine needle aspiration and in 14 by surgical biopsy. All the samples were studied for cytokeratin, vimentin, Ki-67, bcl-2 and CD44. The statistic study was accomplished with the program SPSS 6.1.1 for Windows.

**Results:** The obtained material was satisfactory in 50 cases. The histologic diagnoses were: colloid nodule 32 cases, follicular adenoma 7 cases, Hürthle tumor 2 cases, follicular carcinoma 3 cases, papillary carcinoma 4 cases and Hashimoto thyroiditis 2 cases. The cytokeratin, vimentin and CD44 were positive in practically the whole amount of the different diagnostic groups. Ki-67 and bcl-2 showed meaningful differences ($p < 0.04$ and $p < 0.0001$ respectively) among follicular neoplasm and the rest of pathologies. CD44 presented higher mean values in follicular carcinoma ($m = 60\%$) that in follicular adenoma ($m = 40\%$), but there was no statistical significance.

**Conclusions:** The cytometric evaluation of the thyroid cytology with Ki-67 and bcl-2 could be of great help for the differential diagnosis between adenomatous nodule and follicular neoplasm and between follicular adenoma and follicular carcinoma. Cytokeratin, vimentin and CD44 are not useful markers, individually considered, to establish differences among the different diagnostic groups. CD44 values present differences between follicular carcinoma and follicular adenoma, though a greater casuistry would be necessary to accomplish the study.

## P-100

**VALUE OF FINE-NEEDLE ASPIRATION CYTOLOGY (FNAC) REPETITION IN FOLLOW-UP OF THYROID NODULES**

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**Aims**: To determine the value of FNAC in follow-up of patients with thyroid nodular disease.

**Methods**: We have revised 4938 cytologies performed on 4099 patients in our Hospital between 1984-1997. The aspiration was repeated in 473 patients with a previous cytological diagnosis.

**Results**: Second FNAC showed identical cytological results in 429(90.7%) cases and was different in 44(9.3%). 30 patients of the last group were suspicious or malignancy and 26 of them, underwent surgery. At surgery, 14 out of 26(53.8%) were malignant: 5 well differenciated follicular arcinomas, 7 papillary carcinomas, 1 lymphoma and 1 extrathyroid paraganglioma and 12 were benign lesions: 7 follicular adenomas, 4 nodular goiters and 1 Hashimoto thyroiditis. All the discordants non suspicious cases(14) were nodular goiters with lymphocitic (9) or granulomatous (5) thyroiditis in the second aspiration. None of them were surgically removed.

**Conclusions**: Repeatted FNAC performed in patients with prior benign cytological diagnosis resulted in reclasification in 44 of 473(9.3%) patients. Repeat aspirates increase the malignancy yielt. The malignancy was confirmated in 53.3%. The cause of all non suspicious discordances has been the persence in the second aspiration of a component of thyroiditis, probably consecuence of tisular lesion by reaspiration.

## P-101

**THYROID CANCER WITH DISTANT METASTASES: A REVIEW OF 122 CASES.**

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**Aims** : distant metastases (DM) are rare in differentiated thyroid carcinomas and usually associated with a poor long-term survival. In order to assess the characteristics that could predict a worse prognosis, we reviewed the initial thyroid cancer of patients with DM.
**Methods** : among 1161 thyroid cancers (medullary excluded) enrolled in our institution from 1965 to 1998, 122 patients (9.5%) developped DM.
**Results** : DM occured between a few months to 40 years after the initial diagnosis. In 12 cases, DM were precessive. This series included 90 female and 32 male patients whose age ranged from 15 to 83 years with a mean age of 53.6 years. Eighty-six patients (70%) died of their cancer. DM were located in bone (21 cases), lung (45), bone and lung (25) and 31 elsewhere (liver, brain, skin...). Histologic slides were available in 91 cases. The primary thyroid tumors were classified as papillary carcinomas (49 cases), follicular carcinomas (17), purely insular carcinomas (4), Hürthle cell carcinomas (10), anaplastic tumors (7). Four cases were excluded from the study (1 malignant lymphoma, 3 metastases). The mean tumor size was 5.7 cm. In thirty-four cases the tumor extended beyond the thyroid gland. In 41 cases (45%), an insular component was found, which was pure in 4 cases, or associated with a papillary (21 cases), follicular (10), columnar (4) or oxyphilic (2) carcinoma. In the insular subgroup, the mean age was 55.4 years, the mean size 6 cm, and 30 patients died of their disease (73%).
**Conclusions** : the results of our study indicate that older patient age, larger tumor size and extension beyond the thyroid gland are the main characteristics of thyroid carcinomas developping DM. Furthermore, the presence of an insular component in an otherwise differentiated carcinoma should be carefully searched since it is a poor prognostic factor that should lead to a more agressive therapy.

## P-102

MPM2 EXPRESSION OF THE FOLLICULAR CELLS IN FOLLICULAR TUMOURS OF THYROID

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**Aims:** The aim of this study was to determine expression of MPM 2 in follicular cells of thyroid in neoplastic and non neoplastic follicular tumours.
**Methods:** The patients were divided into four groups: Group I (n=10) - patients with follicular carcinoma, group II (n=10) - with follicular adenoma, group III (n=10) - with follicular hyperplasia and group IV (n=10) patients with nodular goiter. MPM 2 was determined by immunohistochemical techniques. Immunohistochemical studies were prformed on formalin fixed, paraffin-embedded tissue with Streptavidin-Biotin Complex using the DAKO LSAB kits and DAB as a chromogen. Mitotic activity of follicular cells was defined as the percentage of antibody-positive cells compared to the total number of follicular cells. Statistical analysis was performed using a Mann-Whitney test.
**Results:** Statistical significant differences were found in nuclear expression of MPM 2 in follicular cells between follicular carcinoma and follicular adenoma, follicular hyperplasia and nodular goiter.
**Conclusion:** The results of this study indicate that expression of MPM 2 maybe useful in diagnosis of folliculat tumors of thyroid.

## P-103

**PAPILLARY MICROCARCINOMA OF THYROID: Clinical and evolutive characteristics.**

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**Aims:** To review the incidence of papillary microcarcinoma of thyroid (PMT) between the patients controlled by the Pamplona Thyroid Pathology Unit in the last 10 years.
**Results:** In this time we have diagnosed 26 cases of PMT which represent the 20% of the total number of papillary carcinoma of thyroid (n=128), the 7.6% of thyroid carcinoma (n=341) and the 2% of the thyroid operated n=1264).
In 5 cases the diagnosed was made after the appearance of a metastatic lymph node without palpable goiter (group 1). One patient died with lung metastasis.
In 21 cases the PMT was a finding in specimens removed by other causes (group 2). No one had associated metastatic adenopathyes, nor local infiltration. After a 5,2 year follow-up all the patients are free of disease.

| | N | AGE | F/M | TALL C. | MULTICENTRCITY | DEAD |
|---|---|---|---|---|---|---|
| Group 1 | 5 | 51.8±18 | 4/1 | 3 (60%) | 1 (20%) | 1 (20%) |
| Group 2 | 21 | 46,7±11,7 | 17/4 | 1 (3,8%) | 6 (28,6%) | 0 |

**Conclusions:** Papillary thyroid microcarcinomas are frequently discovered in surgical removed thyroids, but the overall prognosis is generally excellent even if the tumour is multicentric.
The PMT with inaugural metastasis have worse prognosis and are frequently of the tall cell variant.

## P-104

**NULL CELL ADENOMAS AND ONCOCYTOMAS OF THE PITUITARY GLAND. AN ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDY**

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**Aims**: The aim of this study is to examine by electron microscope and immunohistochemistry 116 pituitary adenomas unassociated clinically and biochemically with excessive hormone ptoduction and to verify the existence of two morphological entities – null cell adenoma and oncocytoma.
**Methods:** For light microscopy hemalaun-eosine, Mallory–Heidenhain's, Gomori's, and Grimelius'stains and PAS and PAS-orange G procedures are used. Ultrastructurally the small tumour pieces have been undergoing the routine technique starting in the operation room. For immunohistochemistry anti-h GH and h-Prolactin, anti-h FSH and h-LH, anti-h ACTH, anti-beta endrophin, anti-gastrin and anti-CEA are testet by immunoperoxidase and imunofluorescent techniques.
**Results**: By light microscopy all adenomas in our series are chromophobic by Mallory–Heidenhan's and PAS-orange G stains and lack agryrophylia by Grimelius'procedure. The cells are mostly monomorphic composing most often diffuse pattern of chromophobic adenoma and more rarely sinusoid or trabecular ones. Ulrtastructutally all tumours show lucent cytoplasm containing relatively few, poorly developed organelles and rare secretory glandules measuring 100–150 nm in diameter. Focal hormone immunoreactivity for prolactin, FSH and LH has been found in 24 cases, the rest being totally negative. The most important result in our study is the oncocytic transformation found in the cytoplasm of 64 adenomas which we designate as pituitary oncosytomas.
**Conculsions**: Null cell adenoma and oncocytoma are two different morphologic entities comprising the non-functioning pituitary adenomas based on electron microscopic and immunohistochemic findings.

## P-105

CHROMOSOME 11Q LOSSES IN SPORADIC ENDOCRINE PANCREATIC TUMORS: ANOTHER TUMOR SUPPRESSOR GENE TELOMERIC OF MEN1 ?

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**Aims:** Endocrine pancreatic tumors (EPTs) occur sporadically and rarely in association with multiple endocrine neoplasia type 1 (MEN1). Many EPTs reveal loss of heterozygosity (LOH) at chromosome 11q13, containing the MEN1 tumor suppressor gene, but only a part of these tumors also harbor MEN1 gene mutations (Am J Pathol 154:429-436, 1999). Hence, it has been postulated that an additional tumor suppressor gene might exist at 11q13 in endocrine tumors (Genes Chromosomes Cancer 22:130-137, 1998). To identify allelic losses distal of the MEN1 locus (11q13-qter), we examined 30 sporadic EPTs with different molecular techniques.

**Methods:** DNA extracted from 30 frozen EPTs was examined for allelic losses of 11q with comparative genomic hybridization (CGH) and PCR-based microsatellite analysis using markers PYGM, D11S4946 (MEN1), D11S4936 and D11S913 (all 11q13). Touch preparations of EPTs were analyzed by fluorescence in situ hybridization (FISH) using cosmid probes for MEN1, F213 and INT2 (11q13) combined with a centromere 11 probe.

**Results:** Losses of 11q were detected in 17/30 (57%) EPTs. Fifteen (50%) of theses tumors harbored deletions of the MEN1 gene locus, including 6 tumors with isolated losses and 9 tumors with losses of the MEN1 gene locus plus distal 11q13 markers. Of these 9 EPTs five showed 11q losses as revealed by CGH, four of which exhibited monosomy by FISH. Interestingly, CGH detected in two EPTs regional losses of 11q13-q22 not involving any of the tested MEN1 and telomeric 11q13 markers.

**Conclusion:** Our results suggest the existence of a second tumor suppressor gene located telomeric of the MEN1 gene, which might play a role in the pathogenesis of EPTs.

## P-106

GONADOTROPIN-RELEASING HORMONE RECEPTOR DISTRIBUTION IN NORMAL PITUITARY CELLS AND PITUITARY ADENOMAS

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**Aim:** The gonadotropin-releasing hormone (GnRH), which is known as a regulator of gonadotroph cells, has been recently indicated as a biological modulator of other pituitary cells, including somatotroph cells. Its activity depends on binding to a specific receptor (GnRHR), which is known to be expressed by FSH- and LH-cells. GnRHR mRNA, however, has been recently detected in normal pituitaries and also in various pituitary adenomas, including FSH-LH cell, GH-cell and ACTH-cell tumors. However, studies indicating specific cell localization of GnRHR in normal pituitary cells have never been reported. Our aim was to evaluate the immunohistochemical expression of GnRHR in normal pituitary cells and related adenomas.

**Methods:** Formalin fixed and paraffin embedded normal pituitaries and 50 pituitary adenomas (12 FSH/LH-, 10 GH-, 3 GH/PRL-, 5 PRL-, 2 TSH-, 8 ACTH-, and 10 α-subunit/null cell tumors) were immunostained with specific antibodies directed against GnRHR (clone A9E4, Novocastra,UK) and various pituitary hormones. Colocalization studies were performed using double label immunostains.

**Results:** GnRHR immunoreactivity was found in gonadotroph (FSH-LH) cells, and, unexpectedly, also in GH- and TSH-cells. Among tumors, GnRHR was detected in 7/12 FSH/LH-, 8/10 GH-, 2/3 GH/PRL-, and 6/10 α-subunit/null-cell adenomas, whereas was lacking in TSH-, ACTH-, and PRL-cell tumors.

**Conclusions:** This study represents the first demonstration of GnRHR protein expression in normal non-gonadotroph cells and related pituitary adenomas. Since GnRH is known to be expressed by normal and adenomatous pituitary cells, on the basis of our results, it may be concluded that interaction between GnRH and GnRHR might play a role in modulation of several pituitary functions, through a paracrine and/or autocrine fashion.

## P-107

**CONTRIBUTION OF THE INTRAOPERATIVE BIOPSY IN THYROID PATHOLOGY. ANALYSIS OF 104 CASES.**

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**Aims:** The use of intraoperative biopsy (I.B.) in the diagnosis of malignancy in thyroid tumors remains controversial. In order to asses it's benefit we have evaluated the results achieved during 1998.

**Material and methods:** We have examined 104 surgical specimens of unilateral or bilateral total thyroid resections, and subtotal thyroidectomies where our Pathology Thyroid Unit Protocol indicated to practice a I.B. Every surgical specimen was thoroughly sampled during the I.B. When traslucent nodules were identified, the diagnosis of Hyperplasia was made on macroscopic grounds; if the nodules were firm, touch imprints and frozen sections of the nodule were made. Cuts of at least two levels were stained with toluidine blue and H&E. A conclusive diagnosis could not always be achieved. In those cases a provisional diagnosis was given.

**Results:** 45 Nodular hyperplasias ("nodular or multinodular goiter"), 15 Adenomas, 8 Diffuse Toxic Goiter (Graves disease), 34 well differentiated Ca., 2 Medullary Ca. and 1 Anaplasic Ca.Anaplásico. From amongst the 29 specimens with no histological intraoperative diagnosis, in 26 cases the diagnosis of benignity was confirmed; in 3 cases carcinoma was found. In 66 cases of the 75 with histologic I.B., a conclusive diagnosis was achieved and confirmed later in all of them; in the remaining 9 a provisional diagnosis of Carcinoma was given because the frozen section was not conclusive.

**Conclusions: 1.-**The intraoperative diagnosis of Thyroid Pathology has demonstrated to be useful in 86,5% of the cases. Twenty-five out of 37 Carcinomas were diagnosed in I.B. **2.-** False negative cases in I.B., were found to be 2 Papillary Microcarcinomas and 1 Hürthle cell Ca. **3.-** These results have been achieved using a Protocol for the diagnosis and treatment of Thyroid Pathology.

## P-108

**OXYPHILIC PAPILLARY CARCINOMA OF THE THYROID WITH LYMPHOID STROMA. A report of 7 cases.**

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**Aims:** Papillary carcinoma (PC) is the most common type of primary thyroid malignancy. Several morphologic variants of PC have been described, some of them with prognosis different from the common PC. Oxyphilic variants of any thyroid neoplasms are assumed to portend generally more aggressive biological behavior. We report herein the clinicopathological and immunohistochemical features of 7 cases of recently recognized entity - thyroid oxyphilic papillary carcinoma with lymphoid stroma (Warthin-like PC).

**Methods:** All presented cases were retrieved from the surgical pathology files of our departments. Immunohistochemical examination and in situ hybridisation (ISH) for EBV-encoded RNA using an EBV-ISH detection kit was performed in all cases.

**Results:** All 7 patients were women aged from 45 to 85 years. Associated Hashimoto thyroiditis was present in one case only. All tumors showed papillary arrangement. The tumor cells had nuclear features of PC, their cytoplasm was oxyphilic; diffuse or focal stromal lymphocytic infiltration within the tumor was prominent. Immunohistochemistry demonstrated positivity of tumor cells for keratin, thyroglobulin, Leu-M1 and antimitochondrial antigen. S-100 protein positive dendritic/Langerhans' cells were uniformly present. MIB1 labelling index was low. EBV/ISH revealed no positive signal in neoplastic cells.

**Conclusions:** This variant of PC is relatively rare and occurs predominantly in elderly women. The characteristic histologic features are distinct and well recognizable. Indolent behavior of these tumors seems to be consistent with presence of dendritic/Langerhans' cells and with low proliferative activity. We did not prove a role of EBV in pathogenesis of this lesion.

## P-109

**ANALYSIS OF THE CYCLIN $D_1$/ $p16^{INK4}$/ pRB PATHWAY IN PARATHYROID ADENOMAS**

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**Aims:** Cyclin $D_1$/$p16^{INK4}$/ pRb pathway controls G1→S ckeckpoint and is frequently altered in human neoplasias. Parathyroid adenomas (PTA) are benign clonal cell proliferation's in which cyclin $D_1$ (CD1) is frequently overexpressed. In some cases, a chromosomal 11 inversion involving PRAD1 gene is present. Information concerning cell cycle regulation in PTA is scarce. This prompted us to explore the expression of related proteins in PTA and in normal parathyroids (NPT).

**Methods:** A total of 46 patients with PTA and 11 NPT were included in this study. They were immunostained with antibodies against CD1, $p16^{INK4}$ and Rb. The results were quantified using CAS200 nuclear protein software and statistically analysed using non-parametric methods (Mann-Whitney test).

**Results:** While Rb and CD1 showed a parallel and intense expression in at least 25-50% of the PTA cells, $p16^{INK4}$ was expressed in a small percentage (<25%) of the tumour cells. Surprisingly, Rb and CD1 showed similar results in NPT and PTA ($p>0.5$). In contrast, $p16^{INK4}$ was completely absent in NPT ($p<0.05$).

**Conclusions:** Our findings confirm the presence of alterations in the cyclin $D_1$/$p16^{INK4}$/ pRb pathway in PTA. $p16^{INK4}$ expression appears to be a reliable discriminative marker. Absence of significative results concerning CD1 expression is in contradiction with previous literature reports and must be probably placed in the context of our findings in NPT. CD1 upregulation seems to be physiologic phenomena in NPT. Its rare occurrence in other normal tissues and benign tumors merits a deeper exploration of the corresponding regulatory pathways.

## P-110

**AGGRESSIVE SUBTYTE OF FOLLICULAR VARIANT OF PAPILLARY CARCINOMA**

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**Aims:** The follicular variant of papillary carcinoma is associated with indolent biological behavior and good prognosis. Occasionally, cases with aggressive behavior and distant metastases are known to occur, but the true biological behavior of these lesions remains to be determine.

**Method:** Twelve cases of follicular variant of papillary carcinoma (FV of PC) with aggressive behavior (AFV) and 15 classical variants were studied. Clinical and histological parameters were evaluated as well as the proliferative marker (MIB-1), and p53 expression. Genomic DNA was extracted from microdissected tumor and normal cells, and LOH at specific loci 17p13.1 (p53) was correlated.

**Results:** Patients range in age from 23 to 71 years. Three patients with AFV presented with cervical masses, and two with distal metastases involving lung and Kidney. Tumor size was larger in aggressive forms (4 cm) that in classical forms (2.5 cm). Capsular penetration was seen in all cases of AFV but only in 40% of classical cases. Cervical nodal involvement was seen in 92% of AFV, and paratracheal nodes in 8%. Histologically, both tumor types were composed of follicles lined by cuboidal cells in which the characteristic "clear nuclei" was present. Eightynine (89%) of aggressive cases stained positive for Mib-1, and 78% for p53. In contrast, none of the classic cases showed staining for any of these markers.

**Conclusions:** We report an aggressive subtype of follicular variant of papillary carcinoma of thyroid characterized by aggressive behavior and distant metastases. Immunostaining for Mib-1 and p53, can be useful for recognizing this aggressive subtype of Papillary Cancer.

## P-111

PRIMARY MALIGNANT TERATOMA OF THE THYROID GLAND: A CASE REPORT

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**Aims:** Malignant teratomas of the thyroid gland are rare We report a case occurred in a 37 year old female, 34 weeks pregnant, with a 4 weeks history of slight tender enlarging left neck mass.

**Methods:** Four days after delivery, emergency surgery was needed because of suddenly compression of the upper respiratory tract. Total thyroidectomy was performed. Microscopically, the tumor showed cysts covered with different epithelia, cartilague and immature neural tissue. She developed local recurrence and local lymph node involvement two months after surgery. The sections of the metastatic tumor showed predominantly immature neural tissue.

**Results:** Reviewed 4746 thyroid glands in our department files, containing one or more nodules, 603 primary carcinoma were found and only 2 of them (0,3%) were teratomas, one mature in a 11 year old patient and the case report. The cases left followed this distribution: 407 were papillary carcinoma (67,5%); 137 follicular carcinoma (22,7%); 25 medullary carcinoma (4,2%); 16 Hürthle cell carcinoma (2,7%); 11 anaplasic carcinoma (1,8%); 5 epidermoid carcinoma (0,8%).

**Conclusions:** Although extragonadal immature teratomas are uncommon, their behaviour is very aggressive and their prognosis is worse than other primary thyroid carcinomas. A combination of surgery, chemotherapy, and radiotherapy is recomended for treatment. This is the second immature teratoma of thyroid gland reported in a pregnant woman.

## P-112

IS ELECTRON MICROSCOPY STILL REQUIRED FOR THE DIAGNOSIS OF PITUITARY ADENOMAS ?

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**Aims** : to evaluate the diagnostic contribution of electron microscopy (EM), of some proliferation indices (PI) and of immunohistochemistry (IHC), respectively.

**Methods** : 105 cases were analysed by EM, PI (nucleolar organizers-AgNORs, PCNA, MiB1-Ki67) and IHC : GH, prolactin (PRL), ACTH (24-39 and 1-24), ßFSH, ßLH, ßTSH, α sub-unit.

**Results** : the series included 27 GH, 38 FSH and/or LH, 13 PRL, 4 mixed (GH + PRL + ACTH or α sub-unit), 16 ACTH, 5 undifferentiated cell, 1 acidophilic stem-cell adenomas and 1 oncocytoma. AgNORs were neither correlated with clinical and biological data, nor with immunohistochemistry and ultrastructure. PCNA index was high (73 %) in the 3 recurrent adenomas ; MiB1 index value was high (1,4 %) in 38/72 macroadenomas (> 10 mm) and in the stem-cell adenoma (2,4 %). IHC alone diagnosed 3 cases (2 FSH-LH, 1 GH + PRL adenomas) in which necrosis was extensive. EM alone diagnosed 8 cases (1 GH, 1 PRL, 1 oncocytoma and 5 undifferentiated cell adenomas) for which IHC was entirely negative. IHC and EM as cumulated methods were necessary to the diagnosis of undifferentiated cell adenomas (5) and plurihormonal tumors (11/27 GH, 3/13 PRL, 4/4 mixed, 6/16 ACTH and 1 acidophilic stem-cell adenomas i.e. : 25 cases). Among the silent adenomas, GH and FSH-LH adenomas were correctly classified by IHC and EM, but types 2 and 3 ACTH silent adenomas were characterized only by ultrastructural features.

**Conclusion** : of a total of 105 cases, cumulated IHC and EM were necessary to the precise diagnosis of 37 cases i.e. more than one third, demonstrating that EM is still required for the diagnosis. It is important that for each case different fixatives adapted to the panel of antibodies used for IHC and specific fixatives for EM be used ; ideally, some samples should be even frozen for an eventual additional study by molecular pathology. The proliferation indices we investigated are of little value in the diagnosis of pituitary adenomas.

## P-113

DENDRITIC CELL RECRUITMENT IN PAPILLARY CARCINOMA OF THE THYROID

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**Aims:** Papillary carcinoma of the thyroid is often associated with a striking chronic inflammatory reaction and may have a high content of intratumoral dendritic cells. The possibility that recruiment of DC is achieved through release of chemotactic factors by tumor cells was investigated.

**Methods:** Primary cultures of papillary carcinoma cells and of normal thyroid cells were established in 9 cases. Chemotactic activity was investigated in supernatants of 25-35 days culture using 48-well chemotaxis chambers and monocyte-derived dendritic cells. Chemokines potentially responsible for DC migration were identified through an RNase protection assay or RT-PCR.

**Results:** Normal and tumor thyroid cells released chemotactic activity for dendritic cells and showed high levels of IL-8, MCP-1 and MIP-3α mRNA. Since >95% cases of papillary carcinoma are characterized by high expression of Met protein (the high affinity receptor for HGF), we have tested the ability of HGF to regulate chemokine release. HGF stimulation of thyroid cells induced a 5 fold increase of chemotactic activity and RNA transcription for RANTES, IP-10, MIP-1β and MIP-1α. We have then correlated production of chemotactic activity by tumor cells with distribution of dendritic cells in tissue sections. It was found that tumor associated dendritic cells had a zonal distribution at the outer surface of the tumor where Met protein is more intensely expressed by tumor cells.

**Conclusion:** HGF-receptor-positive papillary carcinoma cells are capable to releasing chemotactic factors active on dendritic cells. Moreover, chemokine release is upregulated by HGF, raising the possibility that this meccanism is effective in recruiment of dendritic cells into the tumor.

## P-114

**p53, bcl-2 AND Bax EXPRESSION IN THYROID CARCINOMAS**

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**Aim**: Apoptosis represents a critical pathway whose abnormalities are linked with carcinogenesis. p53, a tumor suppressor gene and inducer of apoptosis, bcl-2 which prevents apoptosis and Bax, which acts to promote programmed cell death are important regulators whose abnormal expression or mutation is correlated with several human cancers. To investigate their relationship to thyroid carcinogenesis we analyzed their expression in thyroid carcinomas (Cas).

**Materials and Method**: Paraffin sections from forty thyroid Cas (29 papillary, 2 follicular, 3 medullary , 1 Hurthle cell and 5 undifferentiated) were examined with the monoclonal antibodies anti-p53 (DO7) and anti-bcl-2 and the polyclonal anti-Bax, using streptavidin-biotin immunohistochemical technique. For p53 >5% nuclear staining was estimated as positive. Bcl-2 and Bax were evaluated as follows: 0, negative; +,<10%; ++,10-50%; +++,>50% of tumor cells positive.

**Results**: p53 protein was detected in 5 papillary (17%), 4 undifferentiated (80%), and one medullary (33%) carcinoma and was undetectable in the two follicular and the Hurthle cell Cas. Bcl-2 immunoreactivity was noted in 27 papillary (93%), 2 undifferentiated (40%) as well as in all follicular, medullary and Hurthle cell Cas. Moderate or intense Bax immunostaining was noted in all papillary, follicular, medullary and Hurthle cell Cas, and in 4 out of five undifferentiated Cas.

**Conclusions**: Elevated p53 expression in undifferentiated carcinomas as compared to papillary ones, suggests that p53 genetic alterations are a late event in thyroid carcinogenesis and could be linked to their reported worse prognosis. The increased co-expression of bcl-2 and Bax in papillary and follicular Cas suggests a possible genetic deregulation of apoptosis which may contribute to their pathogenesis.

## P-115

**p53/MDM2 PATHWAY ABERRATIONS IN PARATHYROID PATHOLOGY: $p21^{WAF1}$ AND MDM2 ARE FREQUENTLY UPREGULATED IN PARATHYROID ADENOMAS.**

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**Aims**: Parathyroid (PT) adenomas (PTA) are the main cause of primary hyperparathyroidism. Typically, PRAD-cyclin D1 (CD1) gene rearrangements are known to be present in at least some sporadic PTA. Surprisingly, in spite of their benign nature, CD1 overexpression is a frequent finding. However, cell cycle regulation in normal parathyroid tissue (NPT) and PTA remains largely unknown. We have systematically explored several proteins involved in the $p53/MDM2/p19^{ARF}$ pathway in PTA and compared the results with NPT.

**Methods:** We immunostained 49 PTA and 12 NPT with anti $p21^{WAF1}$, MDM2, p53, $p27^{KIP1}$ antibodies. The proliferative cell fraction was assessed with Ki-67 Ab. The slides were processed by cytometry (CAS200) and the results statistically analysed using non-parametric methods (Mann-Whitney test)

**Results:** $p21^{WAF1}$ ($p<0.05$) and MDM2 ($p<0.05$) expression as well as the cell proliferation rate (Ki-67) ($p. <0.05$) were significantly higher in PTA compared with NPT. The opposite results were found for $p27^{KIP1}$ ($p<0.05$). Only some PTA showed occasionally positive staining for p53, albeit no significant difference ($p<0.05$) was found in comparatione with NPT.

**Conclusions**: MDM2 and $p21^{WAF1}$ were the more discriminative markers in our hands in order to differentiate PTA of NPT. This overexpression is surprising taking in account the benign nature of PTA, and makes them suitable candidates for further molecular analysis.

## P-116

**THE ARMANNI-EBSTEIN LESION REVISITED**

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**Aims**: It was the aim of the present investigation to examine the contents of the vacuolated tubular cells in the kidneys in diabetic coma - the Armanni - Ebstein phenomenon.

**Methods**: Frozen sections were made of kidneys in a medico-legal material of diabetics. The sections were stained with the "fettrot" method for neutral fat. Sections were also stained with haematoxylin-eosin and periodic-acid-Schiff (PAS).

**Results**: The vacuolated cells were strongly stained for lipids in their cytoplasm. The red staining was uniform and extensive in the proximal tubules. The PAS stain was negative.

**Conclusions**: The proximal tubules in the kidneys in hyperglycaemia contain neutral lipids and only smaller amounts of glycogen. This is consistent with the known hyperlipidaemia in diabetics. The observation may have consequences for the prevention of diabetic kidney disease and other late manifestations.

## P-117

**TYPE II DIABETES MELLITUS DUE TO HORMONE-RELATED AMYLOIDOSIS OF THE ISLETS OF LANGERHANS IN RHEUMATOID ARTHRITIS**

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*Type II diabetes mellitus (DM)* of elderly patients is a chronic disease of multiple etiologies. Probably the most important etiologic factor of adult type *(DM)* is *hormone-related (isolated) amyloidosis localized to the islets of Langerhans (hIAI).* *(hIAI)* amyloidosis is a progressive cumulative process, and may be accompanied by clinically latent or manifest *(DM).*

**Aims**: The prevalence and relationship of *(DM), (hIAI),* and *fatal septic infection (FSI)* were investigated in a randomized autopsy population of 141 in-patients with *rheumatoid arthritis (RA),* to determine the relationship between *(hIAI)* and *(DM),* and the influence of *(hIAI)* on *(FSI).*

**Methods:** The formaldehyde fixed and paraffin-embedded tissue were stained with HE or Congo red according to Romhányi. *(hIAI)* and *(FSI)* was determined by retrospective histological investigation. *(DM)* was clinically diagnosed. The correlations were analysed by $\chi^2$-test.

**Results:** *(hIAI)* was observed in 16 (11.3%) of 141 RA patients. In nine of 16 patients *(hIAI)* was accompanied by manifest (DM) (contingency coefficient=0.7818 - $p<0.0001$). In one of 9 patients *(hIAI)* and manifest *(DM)* were accompanied by sepsis (contingency coefficient=-0.2307 - $p<0.9762$ not significant). In one of 7 patients *(hIAI)* and clinically latent *(DM)* were accompanied by sepsis (contingency coefficient=-0.0819 - $p<0.7070$ not significant). Twenty-six (18.4%) of 141 RA patients suffered of clinical manifest *(DM),* in 5 patients it was accompanied by sepsis (contingency coefficient=0.1239 - $p<0.6555$ not significant). Fatal septic infection was found in 23 (16.3%) of 141; it was accompanied by *(DM)* in 5 and by *(hIAI)* in 2 patients (contingency coefficient=-0.1713 - $p<0.9379$ not significant).

**Conclusions**: Hormone-related amyloidosis localized to the islets of Langerhans is associated with clinically manifest (controlled), or latent (uncontrolled) adult type diabetes mellitus. Controlled and treated diabetes mellitus does not correlate with the frequency of fatal sepsis. Uncontrolled clinically silent stage of adult type diabetes mellitus might be accompanied with a higher risk of *(FSI),* but this correlation could not be confirmed in this autopsy population.

## P-118

AUTOINMMUNE THYROIDITIS: IMMUNOLOGICAL AND IMMUNOMORPHOLOGICAL CORRELATION

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**Aim:** Comparison of immunoserological and immunohistochemical features of chronic autoimmune thyroiditis (AT).

**Methods:** Laboratory data of serum of the T and B lymphocytes, triiodothyronine (T3) and thyroxine (T4) levels, and immunoglobulins M,G,A were used. Histology of operated thyroids from the same patients was made with subsequent morphometry and immunomorphological analysis with primary antibodies to T and B lymphocytes (CD45RO), CD45RA), thyroglobulin, immunoglobulins M and G (Shandon & Daco).

**Results:** AT classic form (72% cases) and so-called "nonspecific" focal thyroiditis (28% of cases) were studied. A positive correlation between T activated and B lymphocytes rates in both serum and tissue ($P<0,01$) was found. Also a strong positive correlation was noted between T lymphocytes levels in serum of AT patients and expression of CD45RO in histologic preparations ($P<0,001$). In paraffin sections of thyroid from patients with classic AT T/B lymphocytes index was 2 times lower than in cases of "nonspecific" focal thyroiditis. No significant difference between levels of Ig M,G,A in group of patients and control group without thyroid pathology was revealed. Severity of lymphoid cell infiltration correlated negatively with level of T-lymphocytes (including activated subsets), with T3 and T4 thyroid hormones and thyroglobulin expression of tissue. Reverse correlation was found between thyroid hormones levels and area of sclerotic changes ($P<0,001$), while levels of B and T-activated cells were higher in cases with more prominent sclerosis. Interestingly to mark strong negative correlation between B and T-activated lymphocytes levels and thyroid hormone production. Pre-, intra- and postoperative evaluation in serum of T and B lymphocytes indicated increasing of their levels in 6 and 12 month after operation.

**Conclusion:** This data allow to select valuable diagnostic and prognostic criteria of autoimmune thyroiditis.

## P-119

**RECURRENCE IN PARATHYROID HYPERPLASIAS IS PREDICTED BY THE AgNOR QUANTITY**

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**Aims:** recurrence in parathyroid hyperplasia has been yet investigated analyzing histological parameters and proliferative fraction, although no data about proliferation rate are available in this condition.

**Methods:** nineteen cases of parathyroid hyperplasia, with a well known clinical history (9 recurrent and 10 non-recurrent), were studied; nineteen cases of parathyroid tumours were also tested as pathologic controls. Clinical and pathological data, including age, sex, preoperative calcium, phosphorus, parathyroid hormone serum levels, weight and size of resected glands and follow-up informations were available. From the corresponding formalin-fixed paraffin-embedded tissue blocks, 4μm thick sections were submitted to the AgNOR technique according to guidelines of the Committee on AgNOR Quantification. By image analyzer and specific softwares, the mean area ($\mu m^2$) of AgNORs per nucleus (NORA) was evaluated at one focal plane in at least 100 nuclei per specimen; differences of mean NORA values among considered groups of patients were assessed by Analysis of variance and the Newman-Keuls' test.

**Results:** the mean NORA value encountered in recurrent hyperplasias was $3.600\pm0.317$ $\mu m^2$; this value was significantly higher ($P<0.001$) in comparison to that found in non-recurrent hyperplastic glands ($2.261\pm0.276$ $\mu m^2$). Both hyperplastic groups showed significant lower ($P<0.001$) mean NORA values in comparison to that found in tumours; the difference was significant even when tumours were compared with hyperplasias considered as a whole ($P<0.001$).

**Conclusions:** the proliferation rate, expressed by AgNOR analysis, is able to determine the higher risk of recurrence in parathyroid hyperplasias; this standardized parameter may represent an additional tool in the predictive biological behaviour of hyperplasias, similarly to that reported for nodular pattern of growth and elevated Ki-67 values.

## P-120

CHROMATIN TEXTURE FROM HEMATOXYLIN STAINED THYROID LESIONS

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**Aims**: Quantitative aspects of cytology and histology should be considered in diagnostic standardisation processes.

**Methods**: Cells stained with progressive hematoxylin and taken from paraffin blocks were overlaid with the extracted texture. This technique was based on the lineal detection of the grey level gradient developed from the common logarithmus of the integrated optical density (IOD) of each individual nucleus.

**Results**: Diffuse and nodular goiters (36) have "salt and pepper" appearance. Adenomatous goiters (2) and adenomas (26) cells have "blurry or smudgy" chromatin, while atypical adenomas with capsular invation(4) have a "woodworm" nuclear appearnce. Encapsulated folliculo-papillary carcinomas (3) have large nuclei with "empty grappe skin".

**Conclusion**: This findings enforce the suitability of computerized textural techniques to recognize objective chromatine textural aspects, particularly the used technique which is a mathematical function of the DNA contact of the nuclei.

## P-121

GENETIC HETEROGENEITY OF BENIGN THYROID LESIONS
Statitc and flow cytometry, karyotyping and " in situ" hybridization analysis

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**Aims**: Analysis of the genetic instability of thyroid tissue
**Methods** : 75 thyroid lesions ( 38 goiters, 30 adenomas, 3 folliculo-papillary encapsulated carcinomas and 4 normal thyroid) were studied by static and flow cytometry. Four cases were also analized by " in situ" hybridization (centromeric probes for chromosomes 1 and 17) and ten cases by G-banding cytogenetics.
**Results** demonstrate a polymorphysm and genetic instability in the thyroid tissue that may be related with the spontaneous polyploidization of their cells. The most consisted finding in citometry was the presence of two clones associated with clinical or histological hyperactivity (46% bersus 23% in non-functioning cases: Chi-square distribution with a $p<0.005$). Chromosomal anomalities were detected in two out of ten cases: (46,XX,t(5,19)) in 87% of cells of a diffuse hyperplastic goiter and 49,XX,+7,+17,+22 in 19% of cells of a thyroiditis case. Finally, the " in situ" hybridization technique showed hidden trisomies of clonal origin in all of the studied cases.
**Conclusion**:Genetic instability of thyroid tissue even in benign and normal tissue have to be considered in order to evalute prognostic factors in thyroid neoplasias..

## P-122

**MICROADENOMA FORMATION IN THE INTESTINE OF TRANSGENIC MICE EXPRESSING AN ACTIVATED β-CATENIN MUTANT.**

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**Aims**: Mutations in the *Adenomatous Polyposis Coli* (APC) gene or activating mutations in the β-catenin gene itself cause aberrant post-translational accumulation of β-catenin which is thought to be responsible for the excessive β-catenin signaling involved in the activation of oncogenesis.
**Methods**: We have generated transgenic mice that express a mutant β-catenin with an NH2-terminal truncation (Δ N131 β-catenin) in their intestinal villi in order to analyse the oncogenic potential of activated β-catenin in the small intestine. This mutant lack both the GSK-3b phosphorylation domain involved in the control of β-catenin stability and the α-catenin-binding domain necessary for β-catenin adhesion.
**Results**: Transgenic mice synthesising large amounts of the activated β-catenin mutant show resumption of proliferation in the small intestinal villi, increased apoptosis and abnormal epithelial cell migration leading to dysplasia and micro-adenoma. Progression to adenocarcinoma was not demonstrated, probably because of the early death of the animals as a result of abnormal kidney development.
**Conclusion**: Our findings demonstrate that in vivo expression of an activated β-catenin mutant in the intestine is sufficient to stimulate epithelial cell proliferation and to lead to microadenoma, which supports the idea that deregulation of the β-catenin signaling pathway may be the main oncogenic consequence of APC mutations in intestinal neoplasia

## P-123

**Regulation of MMP-2 Expression and Tumor Invasion of Malignant Melanoma by p38 Mitogen-activated Protein Kinase**

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**Aims:** Proteolytic enzymes, such as matrix-metalloproteinase (MMP)-2, are involved in invasion of malignant melanoma. P38 mitogen-activated protein kinase (p38MAPK) is a stress-induced member of the mitogen-activated protein kinase family. Concerning similarities between inflammatory tissue and tumor tissue, we investigated the function of p38MAPK in invasion of malignant melanoma.
**Methods:** Immunohistochemistry, Imunoblotting, Northern Blot, Zymography, Invasion assay
**Results:** p38MAPK was found to be constitutively phosphorylated at low levels in MEWO cells. Inhibition of MMP-2 mRNA was observed after treatment of cells with SB 203580, a specific inhibitor of p38MAPK, for 24h as well as 48h. Parallel to the changes observed in mRNA levels of MMP-2 there was a reduction of gelatinase activity of MMP-2 observed in zymogram. Invasion of cells through a matrigel basal membran was reduced after inhibition of p38MAPK by SB 203580.
**Conclusions:** This study shows that p38MAPK stimulates expression of MMP-2 and invasion of malignant melanoma cells in-vitro.

## P-124

S-ADENOSYLMETHIONINE IN THE TREATMENT OF ACETAMINOPHEN OVERDOSE.

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N-acetylcysteine (NAC) is the treatment of choice for acetaminophen (APAP) overdose.The SAM-synthetase is the enzyme that catalyses the synthesis of S-adenosylmethinone (SAM), route connected with the synthesis of glutathione.
**Aims**: 1) To confirm the effectiveness of NAC after APAP overdose. 2) To investigate the efficacy of SAM as hepato-protective agent. 3) To investigate if the association of SAM plus NAC enhance the hepatoprotective effect.
**Material and Methods:** Male, 20 week-old mice weighing 25 g were treated with APAP (350 mg/Kg) ip. The groups studied were 1) APAP+ saline.2)APAP+SAM 3)APAP+ NAC 4) APAP+SAM+NAC. In each group the treatment was administred at intervals of 0,3,4,5 hs. Mortality was determined at 24 hs. At that times it was studied in all the groups: 1) Blood levels of AST and ALT.2) Hepatocelular necrosis was determined in histological sections and graded from 0 to 3. 3) Intracellular levels of SAM-Synthetase
**Results:** 1) Early and late administration of SAM and NAC showed a significative improvement in survival in respect to the APAP group ($p<0,01$ at 0h) and ($p<0,005$ at 3, 4 and 5 h). 2) A significant reduction of AST and ALT plasma levels was demostrated in SAM and NAC groups. 3) The extension of the liver necrosis was reduced in the NAC group as compared to APAP and SAM groups. 4) There was an improvement of SAM-synthetase activity in the SAM group as compared to the APAP and NAC groups.
**Conclusion:** The survival and biochemical results show that the SAM can be an alternative in the treatment of acute failure after acetminophen overdose.

## P-125

**NASA DESIGNED ROTATING WALL BIOREACTOR SYSTEM AS A SURGICAL PATHOLOGY TOOL**

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Aims: NASA Engineers recently designed a rotating wall bioreactor instrument that simulates conditions of space flight. The purpose was to permit experimentation on tissue responses and behavior of tissues under the microgravity microenvironment of space flight. The instrument operates computer-assisted to provide nutritional balance, simultaneously providing for metabolic wastes and required pO2 tensions.

Method: We considered whether the system would support human tissue samples and undertook a comparison of neoplastic and benign samples. Tissues tested included discarded tonsils, lymph nodes, joint synovia, skin, spleen, general soft tissue, bone, prostate, lung, kidney, brain, and carcinomas of prostate, GYN and lung. Histologic examination was done at baseline, 10-14 days, and up to 6 weeks of cultivation.

Results: Growth of the following was successful with preservation of epithelia, ductular histoarchitecture, stroma and microvessels at Day 10-14 and again at day 30-36: skin, prostate, spleen, tonsils, lymph nodes and lung. Intermediate success was found with adipose, breast, and synovia. It was different to maintain brain and kidney. Large cell carcinoma of lung was easily maintained while prostate carcinoma at base line showed progressive differentiation to more normal acini. Tumors of mesenchymal origin also were maintained with limited experience.

Conclusions: The NASA bioreactor has potential as an adjunctive research tool for use in human pathology investigation. It may be an inducer for tumor differentiation.

## P-126

Orientation of collagen fibers and presence of silicone particles in the capsule of textured silicone mammary implants. A study in rabbits.

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**Aim.**-The most common complication of the silicone mammary implants is the capsular contracture. The deposition of collagen fibers and the presence of silicone particles in the capsule of mammary implants were studied. The two factors have been associated with this complication.

**Material and methods.**-Each one of the 30 adult female white rabbits received 4 silicone mammary implants: subcutaneous smooth and textured, and submuscular smooth and textured. A total of 120 implants were used. The orientation of fibers of collagen and the presence of silicone particles were studied in each specimen (4 for each capsule-implant) at 5, 16, and 30 weeks after implantation.

**Results.-** The capsule formed around subcutaneous and submuscular smooth implants was thin, with layers of arranged collagen fibers, parallel to the implant surface. No silicone fragments were identified.The capsule of the textured implants was thicker, with layers of collagen fibers aligned parallel to the overall implant surface. The capsule-implant interface showed irregular fibrous projections, resulting from tissue ingrowth into the poros of the textured surface. For this reason the textured implant was fixed to the capsule. In contrast, the capsule of the textured implants showed silicone particles in 89,28% and 96,55% of the subcutaneous and submuscular implants, within foreing-body giant cells. These results were observed at 5, 16, and 30 weeks after implantation.

**Conclusion:** Biocell textured surface doesn't prevent the linear deposition of collagen fibers. Silicone particles may shed when the textured surface is exposed to mechanical forces and may become incorporate with the capsule. We believe that prevention of capsular contracture, clinically observed with Biocell implants, is owed to the capsule-implant adherence, which counteracts contractile forces of the linearly arranged collagen fibers and stimulus from silicone particles.

## P-127

**COMPARISON OF THE EFFECTS OF TAMOXIFEN AND TOREMIFENE ON APOPTOSIS IN RAT LIVER**

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**Aims:** The role of apoptosis during hepatocarcinogenesis was studied in tamoxifen-induced hepatocellular carcinoma in the rat. Comparisons were made with toremifene. Tamoxifen and toremifene are antiestrogens which are used in the treatment of breast cancer.

**Methods:** Equimolar doses of the antiestrogens (tamoxifen 45 mg/kg and toremifene 48 mg/kg) were given by oral administration to six week old female Sprague-Dawley rats for 12 months including a 3-month recovery period. Glutathione S-transferase P (GST-P) was detected immunohistochemically from paraffin-embedded sections with a specific antibody. Apoptotic cells were either visualized by haematoxylin eosin staining or with *in situ* detection by labeling 3'-ends of DNA by a digoxigenin-ddUTP/ddATP with terminal transferase and anti-digoxigenin antibody. The expression of GST-P-positive foci is a marker of hepatocyte initiation and their induction is predictive for carcinogenic potential.

**Results:** Tamoxifen increased the incidence of GST-P-positive foci significantly after 3 months treatment whereas toremifene induced only a few GST-P-positive foci. Hepatocellular carcinoma was found in 4 out of 5 rats after 12 month's tamoxifen treatment but not in toremifene treated rats. The detection of apoptotic cells with haematoxylin eosin staining was the more sensible method. After 6 month's antiestrogen treatment the number of apoptotic hepatocytes increased significantly. Both antiestrogens caused a time-dependent increase in the number of apoptotic hepatocytes. In toremifene treated rats the number of apoptotic hepatocytes was reduced to control level after the 3-month recovery period. The highest incidence of apoptotic hepatocytes was found in tamoxifen-induced liver tumors. In the preneoplastic foci no increased number of apoptotic cells was observed.

**Conclusions:** Apoptosis may play a role in the mechanism of promotion of tamoxifen-induced liver tumors.

## P-128

MONOCYTES AS REGULATORS OF TGF-β1 ACTIVITY IN EXTRACELLULAR MATRIX PRODUCTION.

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Tissue regeneration and repair proceed in cascade fashion beginning with inflammation and finally extracellular matrix (ECM) deposition. Cytokines produced by both resident cells and monocytes/macrophages (Mϕ) infiltrating zone of injury regulate ECM production.

**Aim:** Influence of Mϕ on development normal tissue reparation and scar formation was studied.

**Methods:** Distribution of ECM components, bFGF and TGF-β1 by immunohistochemical methods was studied in granulation tissue at 12, 21 d after wounding of scald in patients. Levels of bFGF and TGF-β1 in skin organ culture were measured.

**Results:** Granulation tissue formation accompanied by intensive Mϕ infiltration. In granulation tissue intensive accumulation of fibronectin, laminin and collagen types I, III and weak deposition collagen type IV were found. Level of matrix-associated form of bFGF was significantly increased but levels of soluble and matrix-associated forms of TGF-β1 were like in control. In the same time level of cell-associated TGF-β1 was increased. The abundance of Mϕ in zone of injury prevented of wound healing.

Inhibition of Mϕ infiltration induced collagen type IV accumulation, decrease of laminin and collagen types I, III deposition, appearance of matrix-associated, and increase of soluble form of TGF-β1, and decrease of matrix-associates bFGF and start of tissue repair. The total absence of Mϕ leaded to disappearance of matrix-associates bFGF and TGF-β1, and increase of soluble TGF-β1, and hypertrophic scar.

**Conclusions:** Thus, Mϕ induced ECM components formation by change of TGF-β1 activity are of key regulators by tissue reparation.

## P-129

**TREATMENT WITH NON-HYPERCALCEMIC ANALOGS OF VITAMIN D AND GONADAL STEROIDS AUGMENTS ANABOLIC CHANGES IN RAT BONE.**

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Introduction. Vitamin D analogs upregulate biochemical responses and sensitivity to estradiol ($E_2$) of rat bone due to increased cellular receptors of $E_2$.

Aim: The present study was designed to correlate histological changes with biochemical anabolic effects due to sequential treatment with vitamin D analogs and $E_2$.

Methods: " Less-calcemic" side chain analogs of vitamin D CB 1093 (CB) and MC 1288(MC) (from Leo Pharmaceutics) were injected daily into prepubertal female or male rats on days 1-5 and 8-11 followed by $E_2$ on days 6 and 12 sacrificed on day 13. Paraffin embedded bone sections were stained for PCNA, using the avidin biotin method. Methyl methacrylate bone sections were stained for giemsa for morphology study.

Results: Increased number of proliferating osteoblasts was detected by anti PCNA, after sequential treatment with CB or MC together with $E_2$. This increase was to a greater extent compared to the effect of each of the hormones administrated separately,. These results are in accordance with anabolic morphological and biochemical changes.

Conclusions:These skeletal changes due to sequential treatment with gonadal steroids and "less-calcemic" analogs of vitamin D suggest that a form of combined treatment might be useful against metabolic bone loss such as in post menopausal osteoporosis.

## P-130

**ANGIOGENESIS INDUCED IN MUSCLE BY A RECOMBINANT ADENOVIRUS EXPRESSING FUNCTIONAL ISOFORMS OF BASIC FIBROBLAST GROWTH FACTOR.**

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**Aims:** The present work studies the angiogenic effects of a replication-deficient adenovirus (Ad), Ad-RSVbFGF, bearing the human basic fibroblast growth factor (bFGF) cDNA, either mixed with Matrigel and injected subcutaneously or directly injected in the muscle of mice.

**Methods:** We evaluated angiogenesis in mice 14 days after injection of Matrigel plugs containing Ad-RSVbFGF. The recombinant virus was also injected into the hindlimb muscles of *mdx* mice. The myofibre necrosis-regeneration process in this animal model of Duchenne's myopathy results in the presence of numerous foci of immature fibres that are more efficiently infected by Ad vectors than are mature fibres. Moreover, this process mimics the situation observed in an ischemic muscle where necrosis is followed by muscle regeneration. Tissues were excised, fixed and embedded in paraffin. Semi-serial 5 µm-thick sections were prepared. Immunohistochemical detection methods were used to document expression of the human bFGF proteins and to quantify vessels in the injected tissues.

**Results:** Along the interface between hypodermis and the Matrigel plugs, an abundant granulomatous tissue was observed including neoformed capillaries, fibroblast proliferation and increased production of stromal matrix. Numerous fibroblasts and several foci of hyperplastic capillaries were also found exclusively within Matrigel plugs. 6 days after injection in the muscle, we observed an increase in the vessel number within the gastrocnemius injected with Ad-RSVbFGF compared with the contralateral hindlimb. Furthermore, arterioles and venules of the perimysium showed some degree of dilation of their lumens.

**Conclusions:** The data presented here demonstrate that a recombinant adenovirus harboring bFGF gene is capable of expressing this growth factor in a localized manner and during a short period of time in myofibres. Moreover, Ad-mediated transfer of the human bFGF gene can induce angiogenesis in muscle, making this vector a potential treatment for therapeutic angiogenesis of ischemic diseases.

## P-131

TUMOR HYALINE GLOBULES: MATURATION AND RELATIONSHIP TO PLASMA MEMBRANE PERMEABILITY

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Aim: We have previously studied the relationship of tumor hyaline globules (HG) to apoptosis, and found a potentially causal relationship. The multiplicity of proteins found within or around HG led us to investigate the association between HG and alteration of membrane permeability.

Methods: We studied histological material from 68 malignant epithelial and mesenchymal tumors. The sections were stained by H&E, PAS±diastase and Masson's.Ten cases were studied by electron microscopy. Immunoperoxidase stains for for kappa, lambda, alpha 1-antitrypsin, C3 and ferritin were also performed.

Results: All HG stained with PAS±diastase. Two types of HG were identified. Type I stained dark red on H&E and Masson's stains and Type II stained pale pink on H&E and green on Masson's stains. Ultrastructurally Type I HG consisted of clearly recognizable secondary lysosomes, whereas Type II HG represented large homogeneous lipid droplets. The latter were often associated with fibrin fibrils that permeated them and connected them to the extracellular space. This phenomenon was also clearly demonstrated with Masson's stain, staining red fibrin fibrils transversing the green Type II HG. Whereas all cells containing HG stained for kappa, lambda, alpha 1-antitrypsin, C3 and ferritin, only cells with Type II HG showed deposits consistent with fibrin.

Conclusions: HG represent degenerative cellular components, related to apoptosis. The current study indicates that HG exhibit a maturation process that is linked to the plasma membrane permeability. Thus, Type I HG represent an early phase of cell disintegration, when the plasma membrane is permeable to small proteins only. Type II HG are end products of cell death, when the plasma membrane is widely permeable creating a continuity between the intra- and extracellular spaces, which are at that point linked by fibrin fibrils.

## P-132

THE EFFECT OF NITROGEN OXIDE ON HEALING OF WOUNDS

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**Aims**: to study the possibility and mechanism of nitrogen oxide (NO) effect on healing of wounds.

**Methods**: the effect of nitrogen oxide obtained chemically and plasmochemically was studied on experimental models of aseptic and infected rat skin wounds (linear and 300 $mm^2$ in size) and human skin fibroblast (Fb) culture. Light and electron-microscopic studies as well as the inclusion of $^3$H-thymidine and $^3$H-uridine were used.

**Results**: cell proliferation, DNA, RNA, and protein synthesis in the Fb culture increased 1.5 – 2.6-fold on exposure to NO in the dose-dependent manner. The wounds, notably infected ones, revealed normalization of microcirculatory disorders, abatement of inflammation, intensification of phagocytosis, activation of macrophages, acceleration of Fb proliferation, collagen synthesis and fibrillogenesis, and epithelization. The mechanical strength of the scars at 7 days increased 2.5 – 2.7-fold. The area of large wounds at 10, 14, 18, and 21 days reduced 2 – 3-fold depending on the degree of wound infection, the dose and source of NO. The maximum effect was achieved with plasmochemical NO. High NO doses lead to an inhibition of the wound process and a decrease in DNA, RNA, and protein synthesis in the Fb culture. The use of plasmochemical NO for treating chronic and sluggish wounds, ulcers, and burns in clinical setting proved to be markedly efficacious.

**Conclusions**: gaseous NO stimulates the healing process affecting vascular and nervous trophism, phagocytosis, and Fb proliferation. The effect is dose- and NO source-dependent.

## P-133

EFFECTS OF BPC 157 ON MICROVASCULAR REACTION AFTER THERMAL INJURY

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BPC 157, a gastrointestinal tract pentadecaptide described firstly few years ago, showed so far a vast variety of organoprotective effects in different type of injury. One of the most prominent actions was antiinflammatory and antiedematous activity. Also a prominent effect on healing of different wound type was described. Angioprotection was one of the common denominators in many so far described BPC 157 effects. In this study we investigated the effect of BPC 157 on microvascular reaction after thermal injury. **Methods.** For experiments male albino Wistar rats, 5 animal per group, were used. After animal preparation a probe heated on $62^0$ C was applied to their skin during different time periods (20 , 30 , 40 , 50 and 60 seconds respectively). BPC 157 and control substance were applied locally immediately after injury induction. Colloidal carbon was applied intravenously after 45 , 60 , 75 minutes and after 2 and 3 hours respectively. The animals were sacrificed 5 minutes after carbon administration. After routine processing tissue samples were assessed on light microscopy by scoring or using image analyser system (SFORM, VAMS Zagreb) and on electron microscopy, for different parameters. The obtained results were statistically analysed using Mann - Whitney and Kruskall - Wallis tests. **Results.** BPC 157 treated animals showed significantly lower oedema and vessel congestion, less extravasation of erythrocytes and colloidal carbon as well as less reactive changes of endothelial cells. **Conclusion.** Our results support the concept of direct endothelioprotective action of BPC 157 which should be further investigated. Literature: *Seiwerth, S. et al., 1997. BPC 157 effect on healing. J. Physiol. (Paris) 91, 173-178.*

## P-134

QUANTITATIVE AND MORPHOLOGICAL CHANGES IN PERITONEAL CELLS AFTER rhTNF-α INDUCED SEPTIC SHOCK IN PREGNANT RABBITS

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**Aims:** The aim of the study was to examine the hypothesis that TNF-α is an important local immunomodulator of immunity cell function at the sites in the peritoneal cavity of pregnant rabbits in the course of septic shock induced by i.p. rhTNF-α administration.

**Methods:** The study used pregnant and non-pregnant white Dutch rabbits, c. 3kg b.w. The rhTNF-α ($4x10^7$U/mg) was injected in a single i.p. dose of 100μg/kg b.w. as the stimulus to induce experimental shock. Control animals received i.p. PBS. The peritoneal cavity was rinsed with PBS after 12h following i.p. injections and cytological study were made. Examination of the cytosmears staining by HE and MGG, and AS-D naphtol esterase were performed in a light microscope. Cellular changes were evaluated in SEM and TEM microscope.

**Results:** The studies found no significant qualitative and quantitative differentiation in the population of peritoneal cells in pregnant and non-pregnant animals. Features of increased phagocytic activity and apoptotic-type changes were observed in neutrophils and macrophages. Most of evaluated eosinophils show characteristic ultrastructural degranulation changes.

**Conclusions:** The presence of TNF-α not only activates the permanent set of immunocompetent cells of the peritoneal cavity but also contributes to the rapid flow of other haemogenous cells involved in the process of local delimitation of an inflammatory process. No features of a decrease in cell reactivity were observed in pregnant animals.

## P-135

**PATHOMORPHOLOGY OF THE EXPRESSION OF E-SELECTIN AND VCAM-1 AFTER COINCUBATION OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELL CULTURES WITH A CAUSATIVE AGENT OF LYME DISEASE *BORRELIA BURGDORFERI sl.***

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**Aims:** To visualize and assess the expression of E-selectin and VCAM-1 by human umbilical vein endothelial cell (HUVEC) cultures when stimulated with causative agent of borreliosis, different species of *Borrelia burgdorferi sensu lato (Bb sl.)*.

**Methods:** The consequence of direct contact of HUVEC cultures with *Bb sl.* is upregulation in the expression of different adhesion molecules (cell receptors), e.g. E-selectin and VCAM-1, regularly expressed by stimulated HUVEC cultures. In our experiments, we used primary HUVEC cells, isolated from fresh umilical cords by modifying the methods of Jaffe (1973) and Sellati (1995), and HUVEC cell lines. Harvested HUVEC cells were growing in flasks, containing M199 or EMEM growth medium, and plated into 24-wells microplates with round cover-slips on the bottom of each well. After the incubation with different amounts and species of *Bb sl.*, TNF-α as positive and EMEM as negative controls, for the different period of time, we gently washed all wells, fixating cells with methanol, and performed indirect immunohistochemical reactions (IHR) with monoclonal antibodies against E-selectin and VCAM-1.

**Results:** On light microscopy (LM) the products of IHR were seen as different amounts of tiny brown granules, but usually only on the surface of the HUVEC cell monolayers, limited to the borders of cytoplasm membranes. There were also different time-and dose-dependent intensities of positive reactions. EMEM controls were negative or slightly positive.

**Conclusions:** The morphology and the locations of IHR products on the confluent monolayers of HUVEC cultures, seen by LM, can be different from the usually seen IHR products. So, it is sometimes very difficult to assess, whether the IHR is real or it is an artifact.

## P-136

**CATHEPSIN B PARTICIPATION IN THE COLLAGEN DEGRADATION DURING THE REGRESSION OF EXPERIMENTAL HEPATIC FIBROSIS**

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**Aims: 1. To reveal the participation of cathepsin B in the intracellular and/or extracellular collagen degradation; 2. To determine what cells of fibrotic liver participate in this process.**

**Methods: The liver fibrosis in the rat was induced by CCl4 injection. The activity of cathepsin B in fibrotic liver in the 10 and 21 days after the injection cancellation was investigated electron-histochemically. Z-Ala-Arg-Arg-MBNA(Bachem) served as the substratum for cathepsin B. We used Smith and Van Frank (1975) technique.**

**Results: The reaction product was revealed as a fine granular sediment in the Kupffer cell and fibroblast phagolysosomes containing fragments of collagen fibrils. We found extracellular localization of cathepsin B in both periods of investigation. The reaction product having the shape of small electron-dense granules was noted on the hepatocyte, Kupffer cell and fibroblast plasmalemma and on adjacent collagen fibrils.**

**Conclusions: The detected extracellular activity of cathepsin B during the fibrosis regression suggests that in addition to the intracellular proteolyzis, cathepsin B is secreted by hepatocytes, macrophages and fibroblasts in the intracellular space and can take part in the extracellular collagen degradation.**

## P-137

NON-GENOMIC ANTIC-PROLIFERATIVE EFFECTS OF PROGESTINS: DOSE-DEPENDENCY AND CELL CYCLE SPECIFICITY.

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**Aims**: Characterize the antiproliferative effects of progesterone and other progestins on transformed human cell lines from cancers of the uterine cervix.

**Methods**: Cell culture with serum supplemented media without phenyl red, flowcytometric DNA content analysis was performed after ethanol fixation and propidium iodide staining, steroid receptors were assayed by immunochemistry.

**Results**: Progesterone, medroxy-progesterone and megestrol acetate caused a concentration-dependent growth inhibition in C4-1, C33A and Me-180 cells. The C4-1 cell line was studied further since it was devoid of genomic receptors for progesterone, estrogen and testosterone. The antiproliferative effect of progesterone was not reversed by blockers of genomic receptors. The present study showed a half-maximal inhibition at about 10μg/ml and that progesterone caused an increased cell fraction in $G_1$ phase.

**Conclusion**: The present study indicates that progesterone has a growth inhibitory effect conveyed by a specific non-genomic mechanism of action which arrests the C4-1 cells in the $G_1$ phase.

## P-138

BASIC FIBROBLAST GROWTH FACTOR PRODUCTION IN HUMAN PERITONEAL MESOTHELIAL CELLS: INDUCTION BY INTERLEUKIN-1ß

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**Aims:** Clinical end experimental evidence suggest that human peritoneal mesothelial cells (HPMC) act as a bioactive cellular membrane which is involved in the regulation of peritoneal inflammation and fibrosis. We have recently shown that HPMC produce transforming growth factor-beta (TGF-ß) when activated by the proinflammatory cytokine interleukin (IL)-1-ß (Am J Pathol 148:1679-88). In the present study we defined the production of basic fibroblast growth factor (bFGF) by HPMC.

**Methods:** bFGF was detected in situ by immunohistochemistry in normal and inflamed omental tissue. HPMC were isolated from human omentum majus and bFGF production was analyzed by specific immuno-assay and quantitative RT-PCR.

**Results:** Immunohistochemical analysis of peritoneal tissue revealed constitutive expression of bFGF by HPMC that was markedly increased in serosal inflammation. Cultured HPMC produced large amounts of bFGF. Almost 80 % of bFGF (1,547 ± 173 pg/$10^5$ cells) was localized intracellularly and approximately 20 % (357 ± 27 pg/$10^5$ cells) was associated with extracellular matrix components on the cell surface. Treatment of HPMC with IL-1ß resulted in a time- and dose dependent increase of the production and the release of bFGF. The intracellular content of the protein was increased by 41 % (2,136 ± 138 pg/$10^5$ cells; $P< 0.05$). Cell surface bound bFGF was elevated 58 % (564 ± 52.4 pg/$10^5$ cells; $P< 0.01$) above control values. Quantitative RT-PCR analysis revealed a 3.5 fold increase of bFGF-specific mRNA transcripts within 12 hours of stimulation with IL-1ß.

**Conclusions:** bFGF and TGF-ß are regarded as key players in the regulation of wound healing and tissue repair. Our data suggest that HPMC, by the release of both cytokines are crucially involved in peritoneal inflammation and healing which may also result in peritoneal fibrosis.

## P-139

COMPARATIVE STUDIES ON THE ULTRASTRUCTURE OF THE RAT LUNGS AFTER INTRATUMOR TREATMENT OF MORRIS HEPATOMA WITH rhTNF-α AND ITS MUTEINS

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**Aims:** The aim of the study was the comparative analysis of changes found in the lungs of Buffalo rats in the course of Morris hepatoma 5123 after i.t. treatment with rhTNF-α and its muteins.

**Methods:** TNF-α has been shown to interact with two receptors: p55R and p75R. Mutein V binds selectively with p55R. Mutein VI fails to recognize either TNF-R. The cytokines were applied in a dose of 10μg in a cycle of 8 days. The control group consisted of animals which were given i.t. PBS. Ultrastructural examinations were based on TEM.

**Results:** Mutein VI-receiving animals showed enhanced changes of cytotoxic nature. Severe damage to endothelial cells was observed. Blood vascular lumen showed accumulation of neutrophils and monocytes. Focally, within pulmonary alveoli conglomerates of fibrin and fragments of damaged cells were found. The epithelium of pulmonary alveoli showed signs of considerable damage, including necrosis. The lumen of pulmonary capillaries in rhTNF-α-treated animals showed a predominance of eosinophils and monocytic cells. Features of endothelial stimulation were observed, although without a tendency to form microthrombi. Much less pronounced changes both in the lung capillary bed and in the alveolar epithelial cells were noted in the mutein V-given animals.

**Conclusions:** Our findings confirm the possibility of peripheral activation of cells involved in the cytokine-induced antitumor response. Mutein V with the smallest effect on the lung tissue rebuilding seems to be a rhTNF-α derivative which can delimit the undesirable symptoms in the course of antitumor therapy reduced to i.t. injections.

## P-140

THE EFFECT OF TENASCIN ON FOCAL ADHESION PLAQUES ADDED TO CELL CULTURES

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**Aims**: The role of tenascin (TN) in the organization of filamentous actin cytoskeleton and focal adhesion complexes was examined in highly invasive MDA-MB-435 breast and DU-145 prostatic cell-lines based on the high stromal expression of TN in invasive ductal carcinoma of the breast.

**Methods**: The effect of TN on focal adhesion plaques used in soluble form added to cell cultures or using TN as a coating substrate was studied. Double labeling for actin filaments and vinculin plaques was used. Stained specimens were analysed on laser scanning confocal microscopy.

To assess the stromal expression of TN formalin fixed, paraffin emedded specimens were examined immunohistochemically.

**Results:** Soluble TN added to well spread cells had no significant effect on focal adhesion plaques nor on phalloidin stained F-actin stress fibers. A slight tendency in reduction of the number of vinculin plaques was observed at a high concentration (100 μg/ml) of TN.

On TN substrate the cells were slightly able to attach but remained rounded and spread out weakly only after longer incubation.

**Conclusion**: The role of TN in the ineraction of cell-cell and cell-matrix must be more complex and must be influenced also by other extracellular components.

## P-141

MEROSIN DISTRIBUTION AND ANGIOGENIC FACTOR GENE EXPRESSION IN ANGIOGENESIS OF SUPRAGLOTTIS, LUNG AND BREAST CARCINOMAS.

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**Aims:** To investigate the role of angiogenesis in tumor growth by evaluating the relationship among vascularity, matrix proteins and angiogenic factors in solid tumors.

**Methods:** Distribution of vessels, fibronectin, type IV collagen, tenascin, laminin and merosin was evaluated by immunohistochemistry, and gene expression for VEGF, $FGF_2$, TGFβ1, Flt-1 and KDR by in situ hybridization, in 10 supraglottis squamous carcinomas, 10 non-small cell lung carcinomas and 9 classic (NOS) invasive ductal breast carcinomas.

**Results:** Independently of histology and vascularity of tumors, merosin was observed in basal membranes of a limited proportion of vessels. Merosin expression in endothelial cell lines suggested that the presence of merosin in vessels may be related to early angiogenesis. Stromal and parenchymal vascularity was associated with merosin distribution only in supraglottis carcinomas, whereas in lung and breast carcinomas merosin was expressed only in stromal vessels. It is worth of note that the highest proportion of merosin positive vessels in stroma and parenchyma was constantly associated with the highest number of cells producing angiogenic factors. In supraglottis carcinomas the majority of cells producing angiogenic factors were VEGF positive neoplastic cells, whereas in lung and breast carcinomas they were $FGF_2$/TGFβ1 positive stromal macrophages and fibroblasts.

**Conclusions:** In vivo ed in vitro expression of merosin in vessels may be related to an early phase of angiogenesis. Evaluation of distribution of early developing vessels and of local production of angiogenic factors contribute to a better understanding of the role of tumor-angiogenesis in the potential growth and spread of solid tumors. Furthermore in tumors of various histogenesis, different cells might be responsible for the production of angiogenic factors and for stimulating tumor angiogenesis.

## P-142

**TIMING OF ENDOMETRIAL BIOPSY AND INTER AND INTRA-OBSERVER VARIABILITY IN LUTEAL PHASE DEFECTS.**

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**Aim:** To determine the magnitude of inter- and intra-observer in dating endometrial biopsies performed in different parts of the luteal cycle (mid- and late luteal phase) and its impact on the diagnosis of luteal phase defects (LPD).

**Material and methods:** 55 infertile women (mean age 31.6, range 24-40) underwent two endometrial biopsies during a single menstrual cycle. A midluteal biopsy was done on postovulatory days 6-8 (7.33±0.67), and a late biopsy 4 days later (postovulatory days 10-12, mean 11.17±0.69). Biopsy specimens were evaluated by two different pathologists on H&E stained slides following the criteria of Noyes et al, using a single day reading. Two blinded evaluations were performed by each pathologist, six months apart. An out-of-phase biopsy (LPD) was defined as ≥3 days lag between the chronological and the histological day.

**Results:** Overall intra-observer variation was –0.09±0.94 in the midluteal and –0.57±1 in the late luteal biopsy. Interobserver variation was -0.13±1.11 and –0.18±1.04 respectively. At least one reading of LPD was observed in 23/55 (41.8%) of midluteal biopsies and 5/49 (10.2%) of late biopsies. However, exact agreement in the diagnosis of LPD was observed only in 6/23 (26%) of these cases (10.9% of all midluteal biopsies) and in 0/5 late biopsies. Thus, clinically significant inconsistencies between evaluators or evaluations were detected in 30.9% midluteal and 10.2% of late luteal biopsies. No correlation was found between the diagnosis of LPD in mid- and late luteal biopsies.

**Conclusions:** Histological dating of endometrial biopsies is subject to a small but highly significant variability with an important impact on clinical management. Differences between chronological and histological day are more frequent in the midluteal phase, but its significance is unclear.

## P-143

EXPRESSION OF MUC-4 IN NORMAL, METAPLASTIC AND DYSPLASTIC CERVICAL EPITHELIUM.

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Normal ectocervix displays a typical stratified squamous epithelium with a basal layer where the proliferating cells are confined, and a differentiated keratinocyte layers in the upper compartment. The carcinogenesis of cervical mucosa is a well characterised multistep process that has been associated to the infection with HPV. Several degrees of dysplasia have been identified as preneoplastic lesions, and are characterised by higher proliferayion rate associated with as unfinished maturation process. Until now, nine human genes coding for humans mucins have been identified. From them, MUC4 has been initially partially cloned from a tracheobronchial cDNA and it has been detected in the normal epithelia of the endorecvix as the other locations. The aim of this study was to analyse, by immunohistochemistry with specific antibodies, the expression patten of MUC4 in normal ectocervix and in samples with metaplasia and several degrees of dysplasia. Results show that in normal ectocervical epithelium, MUC4 is only detected focally in the basal layer of 4/19 samples. In squampus metaplasiaMUC4 is found in 7/8 tissues with a range of positive basal cells between 10-50%. In dysplastic lesions, MUC4 is higly expressed: in 15/18 mild, 7/7 moderate and 9/9 severe dysplasia, with a high degree of positive cells (more than 75%).
In conclusion, the activation of MUC4 in preneoplastic lesions of ectocervical epithelium may be interpreted as conservative properties of basal layer, and may be used as marker for dysplastic cervucal epithelium.

## P-144

MALIGNANT MIXED MÜLLERIAN TUMOR OF THE FEMALE GENITAL TRACT. A CLINICOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY.

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Malignant mixed müllerian tumors (MMMT) of the female genital tract are uncommon neoplasms with a poor prognosis. They are composed of a mixture of carcinomatous and sarcomatous elements, and have generally been regarded as a type of sarcoma. Recent evidence suggest that they should be more closely related to carcinoma.

In order to verify this hypothesis, we performed a clinicopathologic and immunohistochemical study of 6 cases. Three monoclonal antibodies were used to detect epithelial antigen (AE1/AE3, CAM 5.2, EMA). Various monoclonal antibodies against mesenchymal antigens (vimentin, desmin, actin, and osteonectin) were also used. The patients'age ranged from 68 to 83 years (mean, 77). The origin of primary tumor was: uterus (4 cases), cervix (1 case), ovary (1 case). Metastases were found in 3 cases (lymph nodes, 2; lung, 1; bone, 1). The epithelial component was endometrioid carcinoma (3 cases), and a mixture of endometrioid and serous carcinoma in the other cases. Heterologous sarcomatous component was present in all cases. The sarcoma cells expressed not only mesenchymal antigens but also focally epithelial antigens. The carcinoma was positive for epithelial antigens, vimentin and negative for other mesenchymal antigens. B-HCG was focally detected in one carcinoma. Five patients died from tumor and one of them is alive and remained tumor free 6 months after diagnosis.

Our study supports the evidence that malignant mixed tumors of the female genital tract should be classified as carcinomas rather than sarcomas.

## P-145

### TAMOXIFEN AND ENDOMETRIAL CHANGES : REPORT OF 13 CASES

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Tamoxifen is a synthetic, non-steroidal anti-estrogenic compound, successfully used in the therapy of breast cancer. However, in recent years, this therapy has been associated with an increased incidence of various endometrial changes, both benign and malignant.
We present 13 cases concerning patients aged 51-79 yrs, who underwent mastectomy for invasive breast carcinoma and were treated with tamoxifen, for a period ranging from 2 to 9 years. All patients presented with abnormal uterine bleeding.
Of the above, 7 women developed malignant endometrial neoplasms (4 endometrioid adenocarcinomas, 1 adenosquamous adenocarcinoma, 1 serous papillary carcinoma and 1 müllerian adenosarcoma). Benign endometrial neoplasms (polyps) were found in 3 patients, while histology revealed simple or complex endometrial hyperplasia in the remaining 3 cases.
Our work emphasizes the role of tamoxifen on the endometrium, as has already been supported by other studies and stresses the need of gynecological examination before, during, as well as after tamoxifen therapy.

## P-146

### ASSESMENT OF THE PROLIFERATIVE RATIO AND THE PATTERN OF CELULAR DIFFERENTIATION IN LESIONS OF THE UTERINE CERVIX ASSOCIATED WITH HPV INFECTION.

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**AIMS:** Recents studies suggest that Ki-67 antigen quantification can offer useful information in order to classify pre-malignant uterine cervix lesions, produced by human papillomavirus (HPV). However, virus replication in some lesions could determine and exceeded value of this parameter. The aim of this work is to test this hypothesis, using a terminal differentiation marker like involucrin.

**METHODS**: A double and simultaneous immunostaining to Ki-67 (Mib-1 antibody) was carry out. Involucrin was used on histological sections of condylomatous lesionsfrom positive cervix for immunohistochemical techniques to a structural HPV common antigen.The percentage of the Ki-67 positive nuclear area was quantified by image analysis.

**RESULTS:** All the sample showed numerous positive cells both to Ki-67 and involucrin, mainly placed in the upper middle of the epithelium.

**CONCLUSIONS:** In our opinion, the intense viral replication in non-malignant lesions produced by HPV determine and exceded estimation of the celular proliferation ratio expressed as the percentage of the Ki-67 positive nuclear area. So, this parameter must be used with caution in order to classify pre-malignant lesions.

## P-147

### OVARIAN TERATOMAS. A CLINICOPATHOLOGIC STUDY OF 48 CASES.

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Germ cell tumors constitute about 20% of all ovarian tumors. Most of them are found in children and young adults. About 95% of these tumors are bening cystic teratomas.
Forty-eight primary ovarian teratomas were obtained from the files of Pathology Department, Hospital General de Vic (Barcelona), from 1982 to 1998. They ocurred in females aged between 5 to 92 years old (mean, 42) and they had presented symptoms of abdominal swelling or pain. The ovarian tumors can be cystic or solid and cystic. The two ovaries were involved in 18% of the cases. On microscopic examination, 45 cases were mature cystic teratomas (MCT), 1 case was immature teratoma (IT) with foci of yolk sac tumor (hepatoid subtype), and 2 cases of mature cystic teratomas with malignant transformation (one squamous cell carcinoma and one adenocarcinoma). In 100% of 48 teratomas were found ectodermal elements, in 60% endodermal and in 46% mesodermal elements. There was thyroid tissue in 19% of cases. All the MCT were treated only by surgery, but the patient with IT also received adjuvant chemotherapy. The two patients with malignant change in ovarian mature cystic teratomas were treated by surgery, radiation and chemotherapy. All the patients with MCT and IT are alive and disease free. The two patients with malignant teratoma are alive, and apparently disease free, 4 and 2 years after diagnosis.

## P-148

### CARCINOMA OF ENDOMETRIUM: COMPARATIVE IMMUNOHISTOCHEMICAL STUDY OF 65 CASES WITH OESTROGEN AND PROGESTERONE RECEPTORS WITH Bcl-2 AND p53 EXPRESION.

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**AIMS:** Carry out a comparative study of 65 cases of Endometrial Carcinoma, and the type of endometrium adjacent to the tumor, relating clinical findings with immunohistochemical studies (bcl-2, p53, oestrogen and progesterone receptors) and mitotic and apoptotic indexes.
**METHODS:** We are investigating 65 cases of Endometrial Carcinoma, classifying them by their histologic type; registering the type of residual endometrium which accompanies the tumor. The recorded clinical data are age, staging, treatment and evolution. Immunohistochemistry was performed using a combination of the microwave-oven heating and the standard streptavidin-biotin-peroxidase complex methods on 4um thick sections. The antibodies used were antihuman bcl-2 mouse monoclonal antibody (prediluted), p53 (prediluted), anti-oestrogen receptor mouse monoclonal antibody (prediluted) and anti-progesterone receptor mouse monoclonal antibody (prediluted). Scoring of the immunohistochemistry results was performed according to the methods described by Sinicrope et al. Apoptotic index (AI) and Mitotic index (MI) were examined in haematoxylin and eosin (H&E)-stained sections under high-power (x40 objective and a 10 ocular) magnification. Statistical analysis of data for AI, MI and immunoreactive scores was performed using Mann-Whitney U-test.
**RESULTS AND CONCLUSIONS:** This study of 65 cases confirms that a high level of bcl-2 expression plays a central role in the inhibition of apoptosis in endometrial carcinomas, being more closely associated with Progesterone Receptor than Oestrogen Receptor status.

## P-149

HIV LOCALIZATION IN HPV-RELATED, HIGH GRADE SQUAMOUS INTRA-EPITHELIAL LESIONS OF THE CERVIX IN WOMEN WITH HIV INFECTION

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**Aims.**To evaluate a possible mechanism of HIV and HPV interaction, we have identified the tissue compartments in the cervix which harbor HIV. **Methods.** 39 paraffin embedded, cervical conization specimens with high grade Cervical Intraepithelial Neoplasia (CIN III) occurring in HIV-infected women were studied. From selected intraepithelial HPV-positive (confirmed by in situ hybridization), non ulcerated specimens, we obtained serial 4-5 μ thick sections that were stained with Hematoxylin and Eosin, anti S100 protein and anti CD4. The presence of intra-mucosal Langerhans' or dendritic cells and/or CD4 positive cells was registered. Three consecutive, non microdissected, full thickness sections of the same specimens were used for Polymerase Chain Reaction (PCR) analysis (group A). Other uncovered three, consecutive sections from the same blocks were examined with an inverted microscope and full-thickness specimens of mucosa were dissected from the underlying cervical stroma, gently removed and used for PCR (group B).The quality of DNA was checked by HLA-DQα amplification; then a nested PCR for HIV proviral DNA was performed. **Results.** 5/39 (12.8%) cases of the group A were positive whereas HIV was not detected in the microdissected sections of the group B, with or without intraepithelial Langerhans' or CD4 cells. **Conclusions.** HIV does not affect cervical epithelium. The lack of infected Langerhans' and/or dendritic cells indicates a migration to the proximal lymph nodes of the infected cells. The absence of HIV proviral DNA in the CIN infiltrated by CD4 cells could be due to the low number or absence of infected CD4 cells.

## P-150

PARAVAGINAL FEMALE ADNEXAL TUMOR OF PROBABLE WOLFFIAN ORIGIN. A CASE REPORT

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Female adnexal tumor of probable Wolffian origin (FATWO) is a rare neoplasm originating from mesonephric remnants. Approximately 50 cases have been reported, predominantly arising in broad ligament, mesosalpinx, ovarian hilus, and periadnexal region.

**Aims:** The aim of this report is to present the clinical and pathological features of a FATWO occurring in paravaginal area. So far, there have been only two cases reported to occur at this site.

**Case report:** The patient is a 23-year old, nuliparous female, presented with a painless fixed paravaginal tumor. In August 1995, explorative laparatomy was performed. Encapsulated ovoid, tumor, located between urinary bladder and vagina, tightly fixed to vaginal wall connective tissue, was found. The uterus and both adnexa appeared unremarkable. The tumor was removed and biopsy sample from left ovary was taken. The patient had had an uneventful post-operative course and she remained well, without evidence of recurrence, 42 months after laparatomy.

**Results:** The tumor was a solid ovoid mass with smooth surface, measuring 6.7 x 5 x 3.7 cm. On sectioning, the neoplastic tissue was solid, soft, butter yellow in colour; multiple cysts varying up to 3 cm in diameter and hemorrhagic foci were also present. Microscopically, it was well circumscribed and surrounded by fibrous capsule, composed of tumor cells arranged in different patterns: solid, closely packed tubules, and microcystic. The histochemical and immunohistochemical features of the tumor were identical to other cases of FATWO reported in the literature.

**Conclusions:** Although the majority of these tumors are benign, a few cases have shown definite low malignant potential, with metastases and recurrences developing after at least 6 to 16 years following radical surgery. The presented case indicates that in spite of the unusual location, FATWO must be recognized, ensuring a careful and prolonged follow-up.

## P-151

**VILLOGLANDULAR ADENOCARCINOMA OF THE UTERINE CERVIX. A CASE REPORT.**

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**Introduction:** Villoglandular adenocarcinoma of the uterine cervix (VCC) is a distinct histologic type of cervical cancer, which occurs primarily in young women and has an unusual excelent prognosis.
**Case report:** A 33 years-old woman, with no history of previous oral contraceptive treatment, presented with postcoital vaginal bleeding. Colposcopic examination revealed an exophitic and friable cervical lesion. Patient was diagnosed by cervical cytologic smears and cervical and endometrial biopsy material. Radical hysterectomy and bilateral pelvic lymphadenectomy, with intraoperative consulting, was performed.
**Results:** Cervical smears revealed long villous fronds, and papillae lined by columnar cells with intact citoplasmic borders and mild atypia. Gross examination of the hysterectomy specimen showed an exophytic cervical tumor of 20mm. as its greater diameter. Histologic examination of both, cervical biopsy and surgery specimen, revealed an exophytic tumor with a complex papillary architecture. The epithelium lining papillary tufts showed mild atypia and the core of the papilla presented moderate chronic inflamatory infiltrate. The infiltrating component was superficial and presented minimal desmoplastic response. The tumor was confined to the cervix. Vascular involvement was not seen.
**Discussion:** Although deep invasion with extension into the uterine corpus may occur, VCC are mostly superficial and restricted to the cervical portion. It represents, by definition, a well-differentiated adenocarcinoma. For all these reasons, some authors propose a more conservative treatment (simple excisional biopsy or conization), a therapeutic option of particular importance for young women who wish to preserve reproductive capability.

## P-152

**KI-67 EXPRESSION IN ENDOMETRIA FROM LONG TERM TAMOXIFEN TREATED PATIENTS.**

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**Aims:** To demonstrate proliferative activity in apparently atrophic endometria of long term tamoxifen treated patients (LTTTPs) obtained during regular check ups during tamoxifen administration.
Methods and materials: 67 endometria from postmenopausal patients. Of these , 47 corresponded to LTTTPs (TX group). The control groups were 6 were endometria from breast cancer patients untreated by tamoxifen (CN1 group) and 14 from postmenopausal patients without breast cancer or tamoxifen treatment (CN2 group). Percentage measurements of marked nuclei were performed in: surface epithelium (SE), non-dilated glands (GLA), dilated glands (DIL) and stroma (ST). For statistical analysis, Kruskal-Wallis test was applied.

**Results:** LTTTPs (TX group) showed a higher percentage of Ki67+ nuclei than SE ($p<0.001$); GLA ($p<0.001$);ST($p<0.001$) than both control groups; dilated glands did not show any significant differences. See table

| GROUP | SE | GLA | DIL | ST |
|---|---|---|---|---|
| TMX | 10.5 | 12.9 | 0.5 | 2.6 |
| CN1 | 1 | 0.8 | 0 | 0.8 |
| CN2 | 1.2 | 1.4 | 0.2 | 0.1 |
| SIGNIFICANCE | P<0.001 | P<0.001 | NO. | P>0.001 |

**Conclusions:** There is a significantly increase of Ki67 expression in the endometria of LTTTPs. This expression is present in surface epithelium, glands and stroma and clearly demonstrates the proliferation induced by tamoxifen in apparently atrophic endometria.

## P-153

ATYPICAL OVARIAN ENDOMETRIOTIC CYSTS (AOEC). AN IMMUNOHISTOCHEMICAL STUDY OF p53, MIB-1 (Ki-67), AND ESTROGEN (ER) AND PROGESTERON RECEPTORS (PR).

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**Aims:** To investigate the possible precancerous significance of marked atypia in the lining epithelium of non-hyperplastic endometriotic cysts of the ovary.

**Methods**: Five cases of AOEC were immunostained for p53, Ki-67, ER, and PR. Results were scored by a combined system based on both the percentage of positive cell nuclei and the staining intensity (range 0 to12), and compared with those of cases of non-atypical, non-hyperplastic endometriosis (NANHE) and cases of clear cell carcinoma of the ovary (CCC).

**Results:** The values for p53 in AOEC (median ± SE 4.0 ± 1.44, range 1-6) were similar to those of CCC (median ± SE 4.0 ± 1.73, range 0-6) and higher than in NANHE (median ± SE 1.0 ± 1.15, range 0-4). ER and PR tended to be lower in atypical areas of AOEC than in NANHE and were negative in CCC. Proliferation index was low in both types of endometriosis, though some isolated Ki-67 positive atypical cells were consistently found in each case of AOEC.

**Conclusions**: Marked atypia in the lining epithelium of nonhyperplastic endometriotic cysts appears not to be a degenerative change, and might represent a precursor lesion for nonendometrioid types of ovarian carcinoma.

## P-154

**PROGNOSTIC SIGNIFICANCE OF SPONTANEOUS APOPTOSIS, p53 STATUS AND bcl-2 EXPRESSION IN OVARIAN CARCINOMAS.**

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**Aims:** To look for possible associations among apoptotic index, p53 status, and bcl-2 expression and prognosis in ovarian carcinoma.

**Methods:** We have studied 20 cases of primary epithelial ovarian tumors (5 serous, 6 endometrioid, 3 mucinous, 2 clear cell carcinoma, and 4.tumors of low malignant potential). We reviewed clinical stage, histologic type and grade, according to FIGO considerations.We also evaluated the nuclear atypia, necrosis and peritumoral grade of inflammation. Apoptotic cells were counted on H&E sections in 10 high power fields. P53 and bcl-2 expression were detected immuno-histochemically.

**Results:** Moderate or high AI were detected in 13 cases, p53 protein accumulation in 12 cases and diffuse Bcl-2 expression in 8 cases. Most of the cases with high AI belonged to the group of serous (5/5) and endometrioid (4/6), whereas tumors of low malignant potential and mucinous carcinomas mostly showed low AI (3/4 and 2/3 respectively). P53 protein was often demonstrated strongly in serous carcinomas and mildly in endometrioid once. Strong bcl-2 immunoreactivity was also seen in tumors of low malignant potential (3/4), and endometrioid and clear cell carcinomas (4/7 and 2/2 respectively). A correlation was found between high AI, and other prognosis factors. We also found some association between high AI and a high accumulation of p53 protein and an inverse relation with expression of bcl-2.

**Conclusions:** Although we saw a relative variability in the extent of apoptotic cell death, there was a correlation to certain histologic types. The bcl-2 and p53 protein seems to be implicated inhibiting and inducing apoptosis, respectively. As shown by the results of the present study, frequent apoptosis could have a poor prognostic sign in ovarian carcinomas.

## P-155

PROGNOSTIC SIGNIFICANCE OF p53, CA19-9 AND M3SI EXPRESSION IN OVARIAN CARCINOMAS

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**Aims:** In recent years, some factors influencing prognosis of ovarian carcinoma (OC) have been studied, but at present the stage can be considered the only reliable prognostic indicator. Among markers, p53 seems to be associated with aggressive behavior in OC, even if its value as independent prognostic factor needs to be established. The authors investigated the prognostic significance of clinicopathological, histological features and of expression of p53, gastroenteropancreatic epithelial cells antigens, tumor and proliferation markers.

**Methods:** We evaluated 87 OCs, including 25 mucinous, 29 serous, 12 endometrioid, 9 clear cell and 12 undifferentiated carcinomas. Sections were immunostained using following antisera and mAb: p53, pepsinogen II (PgII), gastric foveolar antigen M1, cathepsin E, intestinal antigen M3SI, colorectal antigen CAR-5, pancreatico-biliary antigen Dupan-2, CEA, TAG-72, CA19-9, CA125, MIB1. Grade and FIGO stage were assessed. The mean follow-up was 64 months (range 1-238). Statistical analysis was performed using Logrank test and Cox's regression model.

**Results:** Univariate analysis revealed that M3SI ($p<0.001$) and CA19-9 ($p<0.005$) using a >30% cut-off point, MIB1 ($p<0.001$) and p53 ($p<0.001$) using a >20% cut-off point were significant predictors of poor survival. In addition negativity for M1, DUPAN-2, BD5 and cathepsin E correlated with poor outcome. On multivariate analysis serous and undifferentiated histologic types ($p=0.0434$), advanced stage ($p=0.0140$), p53 overexpression ($p=0.0014$), CA19-9 ($p=0.0290$) and M3SI ($p=0.0153$) immunoreactivity were the only independent variables predictive of poor outcome.

**Conclusions:** Our results confirm that advanced stage is a reliable prognostic indicator of poor outcome in ovarian carcinomas. p53, CA19-9 and M3SI seem to be independent prognostic markers of aggressive behavior.

## P-156

**METALLOPROTEINASES (MMP) AND THEIR TISSUE INHIBITORS (TIMP) IN CERVICAL SQUAMOUS CELL CARCINOMA (SCC).**

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**INTRODUCTION:** MMPs are a family of enzymes which participate in extracellular matrix degradation and remodelling. They are believed to play a role in tumor invasion and metastasis. All MMPs are negatively regulated by TIMPs, whereas MT-MMP can activate MMP-2. Recently, their expression has been analyzed in cervical adenocarcinomas.[1] In the present study we have evaluated their presence and potential role in the progression of cervical SCC.

**METHODS:** Frozen-sections of 5 CIN-III lesions, 15 cervical SCCs and 5 controls were immunohistochemically evaluated for the expression of MMP-2, -3, -9, TIMP-1, -2 and MT-MMP.

**RESULTS:** MMP-2 was expressed by tumor cells in 9/15 tumors and by normal epithelium (4/5). MMP-3 was variably expressed by inflammatory cells present in CIN-III, tumors and controls. In contrast to controls, MMP-9 and MT-MMP were upregulated in inflammatory cells surrounding tumor and CIN. TIMP-1 staining revealed stromal expression in 12/15 tumors, in 1/5 CIN-III and in none of the controls. Strong TIMP-2 protein expression was found along tumor invasion margins and along basal membranes of both CIN and normal epithelium.

**CONCLUSION:** The variable expression of MMPs and TIMPs in tumor and tumor stroma of cervical SCC, suggest a crucial role in the carcinogenesis of cervical SCC. Extension of case numbers is needed to assess a possible relationship between MPP/TIMP expression and tumor behaviour.

1. Davidson , B et al. Expression of metalloproteinases and their inhibitors in adenocarcinoma of uterine cervix. Int. J. Gynecol. Path.1998; 17(4): 295-301.

## P-157

ADENOCARCINOMA OF THE CERVIX.

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**Aims:** A clinicopathologic analisis of 105 patients with cervix adenocarcinoma.

**Methods:** Using formalin-fixed and parafin-embedded cervical tissues, we examined CEA,PCNA,Cerb-B2,ER,PgR,P53,Bcl-2, high and low molecular weight cytokeratines (CK-H,CK-L),synaptophisin, intestinal oncofoetal antigene (B1MA), D1-cyclin by immunohistochemistry.

**Results:** The age ranged from 21 to 76 with a median age of 53 years. Histological variants: carcinoma of endocervical type - 25, adenosquamous - 27, serous - 13, mesonephral - 6, glassy cell - 1, endometrioid -14, clear cell - 6, poorly differentiated adenocarcinoma - 6, adenocystic - 4, intestinal type - 1, adenoid basal - 1, adenoma malignum - 1. In some cases without metastasis in regional lymphatic nodes distant metastasis were developed after period from 1 till 9 years: lung - 3, liver - 1, peritoneum - 3, omentum - 1, ovari - 1, pleura - 1, backbone - 1, subclavicular lymphatic nodes - 4. There were 6 cases of synchronous primary - multiple tumors. The positive staining with CEA was observed in all cases of adenosquamous and endometrioid cancers, 20% of serous and endocervical carcinomas. In cases of mesonephral and clear cell types - reaction with CEA was negative, CK-L was low expressed, CK-H was strongly erexpressed. CK-H was strongly positive in reserve cells of atypical cervical glands and squamous part of adenosquamous carcinoma. The overexpression of P53 gene was found in 18% of the whole group of adenocarcinomas. ER and PgR expression were positive in 13 and 15% respectively and were observed mainly in endometrioid and endocervical carcinomas. PCNA expression changed from 11 to 83% of cells and there was straight correlation with a stage of disease.

**Conclusion:** Immunohistochemical study revealed some differences in markers expression between variants of cervix adenocarcinomas. There were also specifications in sites of metastasis.

## P-158

IMMUNOHISTOCHEMICAL STUDY OF P-170 (MDR1) IN ENDOMETRIAL CANCER.

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**Aims**: Evaluation of P-170 expression in endometrial cancer and its correlation with histological type, grade of differentation, stage of disease and behavior.

**Methods**: 20 cases of endometrial cancer have been reviewed; half of them were early stages. A minimal follow up of three years was made. The P-170 expression was perfomed by immunohistochemical procedure with a polyclonal antibody: P-glycoprotein (P-gP) and the positivity was analyzed as membranous or cytoplasmic. The results were compared with clinical and other pathological prognostic factors.

**Results**: 10 cases were I FIGO stage (50%); 7 cases II (35%) and 3 cases III stage (15%). The histological diagnosis were endometrioid carcinoma 13 cases (65%), serous papillary carcinoma 5 cases (25%), adenosquamous carcinoma 1 case (5%) and mixed carcinoma 1 case (5%). The histological grade was G1 and G2 in 13 cases (65%) and G3 in 7 cases (35%). P-gP was negative in 9 cases (45%); membrane expression in 6 cases (30%) and strong cytoplasmic expression in 5 cases (25%). The last group corresponded to patients with serous papillary carcinoma that four of them died during the first year after diagnosis, despite adjuvant radiumtherapy and chemotherapy.

**Conclusions**: The P-gP expression in low grade of differentiation of endometrioid and serous papillary carcinomas seems to have some correlation with accelerated course and poor response to chemotherapy or radiotherapy. In this sens, the P-gP immunohistochemical valoration could bring an additional information about the prognosis of these tumors.

## P-159

LACTOFERRIN EXPRESSION, RECEPTOR STATUS AND PROLIFERATIVE ACTIVITY IN STAGE I AND II ENDOMETRIAL ADENOCARCINOMAS

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**Aims:** lactoferrin (LF) is a major estrogen-inducible protein and its expression has been directly correlated with the epithelial cell proliferation in the mouse uterus; in human endometrium few data are available about LF immunolocalization, while its possible relationship with proliferative activity as well as receptor status has not been extensively investigated.

**Methods:** forty-two formalin-fixed paraffin-embedded surgical samples of stage I and II endometrial adenocarcinomas (EA) were obtained from an equal number of patients (age range 38-84 yrs.; mean age 63.8). On 3μm thick serial sections the following procedures were carried out: polyclonal antibody for LF (Dako); monoclonal antibody against Ki-67 antigen (MIB-1, Immunotech); monoclonal antibodies for estrogen and progesteron receptors (ER, 1D5 DBA; PgR-ICA, Abbott); finally, one section was submitted to the AgNOR technique according to guidelines of the Committee on AgNOR Quantification. Statistical analysis was performed by Analysis of variance, Newman-Keuls' test and Fisher's exact test.

**Results:** a variable expression of LF was revealed in 26 cases of EA; immunopositivity (> 10% of neoplastic elements) for ER and PgR receptors was found in 28 and 27 cases respectively; high expression of Ki-67 antigen (> 22% of neoplastic elements) was encountered in 19 cases; high AgNOR quantity (argyrophilic intranuclear precipitates > 4.2 $\mu m^2$) was evident in 17 cases. No relationships were demonstrated among LF immunoreactivity and all other considered parameters.

**Conclusions:** in I and II stage EA, our preliminary data appear to be different from those reported in experimental studies, since no correlation has been found between LF expression and receptor status or proliferative activity; therefore, it may be suggested that LF evidence in EA could be determined by other mechanisms such as defective or functionally impaired cellular LF receptors.

## P-160

MESENCHYMAL TUMORS OF THE UTERINE CORPUS ASSOCIATED WITH TAMOXIFEN THERAPY.

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**Background:** Although the association between Tamoxifen use and endometrial adenocarcinoma is recognized the Tamoxifen has been also associated with endometrial polyps, hiperplasia, mesenchymal and mixed epithelial mesenchymal tumors of the uterus.

**Case report**: We reviewed the clinical history and pathology material of two patients, 55 and 72 years old, who developed malignant neoplasms of the uterine corpus after being treated for breast carcinoma with Tamoxifen. Both patients received adjuvant Tamoxifen theraphy for five years.

The treatment was stopped when the diagnosis was made of uterine Mullerian adenosarcoma in the 55 years old and stromal endometrial sarcoma in the other patient. Both tumors were poypoid endometrial masses that superficially invaded myometrium.

**Comments:** Prolonged exogenous or endogenous hyperestrinism may lead to the development of these tumors. Tamoxifen may have played a role in the ocurrence of mesenchymal and mixed Mullerian tumors, although it is well known that these types of tumors may arise de novo in elderly women.

## P-161

### IMMUNOHISTOCHEMICAL STUDY OF OVARIAN SERTOLI-STROMAL CELL TUMORS

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**The aim** was to describe the immunohistochemical profile of ovarian Sertoli-stromal cell tumors (SSCT).
**Material and methods:** Slides of ovarian SSCT from our files were stained immunohistochemically with the following primary antibodies: Cytokeratin (CK)-7, -8, -18, Ber-EP4, EMA, CA-125, Vimentin, Desmin, α-Smooth Muscle Actin (α-SMA), S-100 protein, CD99, Inhibin α, Melan A, Calretinin, Estrogen- and Progesterone receptor.
**Results:** One retiform, two well-differentiated and 4 Sertoli-Leydig cell tumors of intermediate differentiation were included.
As to the Sertoli cells, all tumors were positive for CK8, CK18, Inhibin α, Melan A and Progesterone receptor while negative for EMA, CA-125, and α-SMA. Except for the retiform type, all the tumors were positive for Vimentin, S-100 protein, CD99 and negative for CK7.The reactivity for Ber-EP4, Desmin, Calretinin and Estrogen receptor was variable.
Leydig cells in all the tumors were positive for Vimentin, Inhibin α, Melan A, Calretinin, CD99 and negative for CK7, CK8, Ber-EP4, EMA, CA-125, Desmin, α-SMA, S-100 protein, Estrogen- and Progesterone receptor. Except for af few cells in one tumor, none of the Leydig cells stained with CK18.
**Conclusions:** SSCTs are characterized immunohistochemically by positive reaction of both cell types for Inhibin α and Melan A, and negative reaction for EMA, CA-125 and α-SMA. The retiform tumor differs from the others, as the Sertoli cells reveals a focal positive reaction for CK7 and negative reaction for Vimentin, S-100 protein and CD99.
In the differential diagnosis of SSCT from primary ovarian carcinomas a panel of antibodies including CK7, EMA, CA-125, Inhibin α, Melan A, Calretinin, S-100 protein and CD99 is valuable.

## P-162

### MUCIN PROFILE AND THE PCNA AND Ki 67 IMMUNREACTIVITY OF THE OVARIAN TUMORS

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Intestinal type mucins were demonstrated in many of ovarian tumors, especially proliferative ones like borderline, and malignant.

The aim of this study was to determine the correlation between tumor proliferative activity and the mucin profile of the mucinous ovarian tumors.

The material of this study consist of fourteen benign, eight malignant, and three borderline ovarian mucinous tumors. Alcian blue pH 2.5/ Periodic acid Schiff (AB/ PAS) stain was used to identify the intestinal type mucins. For the demonstration of the cell proliferative activity, PCNA and Ki 67 labeling indices were used.

All of the malignant and borderline tumors were stained with AB/ PAS(100%). In benign ovarian neoplasms, nine cases were stain with AB/ PAS (64 %). Both antibodies especially PCNA showed significantly higher labeling indices in the malignant ovarian neoplasms. Among the benign neoplasms only one case showed positive PCNA immunreactivity (7%).

Our results confirm that coexistence of AB/ PAS positivity and PCNA/ Ki 67 immunreactivity are mostly seen in malignant and borderline ovarian mucinous tumors with respect to benign ones.

## P-163

### BREAST CARCINOMA PRESENTING AS AN OVARIAN MASS: A STUDY OF 14 CASES

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**Aims:** Breast carcinoma is the most common metastasis to the ovary, but presentation as an ovarian mass is extremely uncommon. We present our experience with this phenomenon to highlight it and associated problems in differential diagnosis.
**Methods.** We reviewed the clinico-pathological features of 14 cases, mostly seen in consultation, in which a previously undiagnosed breast carcinoma presented as an ovarian mass. Immunohistochemical stains were evaluated in some cases but the diagnosis was usually made on hematoxylin and eosin-stained slides.
**Results:** The patients ranged from 33 to 72 years (average of 48.4 years). Two-thirds of the tumors were bilateral. They ranged from 3 to 18 cm. The tumors were typically solid but were occasionally cystic. On histological examination, 9 (64%) of the tumors were ductal adenocarcinomas and five (36%) were lobular. 11 cases (79%) were composed of multiple discrete nodules, 7 (50%) showed ovarian surface involvement, and four (29%) contained angiolymphatic invasion. The most common morphological patterns observed were: cords (86%), diffuse masses (71%), clusters (64%), and islands (36%). 7 tumors displayed at least focal signet ring cells. The differential diagnosis included numerous primary and metastatic tumors, including adult granulosa cell tumor, Sertoli-Leydig cell tumor, small cell carcinoma of hypercalcemic type, serous adenocarcinoma, metastatic gastric carcinoma, metastatic malignant melanoma, and metastatic alveolar soft part sarcoma. Many of the cases were initially misdiagnosed.
**Conclusions:** Undiagnosed breast carcinoma presenting as an ovarian mass is rare, and when it occurs it is frequently misdiagnosed. Knowledge of the wide morphological spectrum of metastatic breast carcinoma aids in its identification.

## P-164

### PLACENTAL PATHOLOGY IN MALARIA: HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND QUANTITATIVE STUDY

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**Aim:** To characterize the histological changes in malarial placentas and their relationship with parity and maternal and cord parasitemias.
**Methods:** Histological study on 1179 placentas from Ifakara, Tanzania, an area with intense and perennial malaria transmission. Immunohistochemical and quantitative studies for CD45 and fibrin were done in 60 cases.
**Results:** 415 placentas showed parasites (active infections); in 303 of them parasites and pigment coexisted (chronic infections). 475 cases showed hemozoin deposition without parasites (past infections). 46.3% of women with parasitized placentas did not show parasites in the peripheral blood. Basal membrane thickening ($p=0.002$), fibrinoid necrosis ($p=0.004$) and prominence of sincytial knots ($p=0.031$) were associated to active malarial infection. No quantitative differences for intervillous fibrin or villous area were found. The most significant association with active malarial infection was intervillous inflammation ($p<0.001$). Chronic infections were associated with the most severe changes, specially inflammation (OR 28.7, 95% CI=16.0-51.5, $p<0.001$). Past infections did not show differences with non-infected placentas. Primiparas showed chronic infections more frequently than multiparas (52% vs. 15%, $p<0.001$). They also showed significantly higher placental parasitemias and intervillous inflammatory infiltrate.
**Conclusions:** Placental histology is more sensitive than peripheral blood examination in detecting malarial infection during pregnancy. Most malarial infections recover during pregnancy leaving few residual changes in the placenta. Intervillous inflammation is the most frequent finding associated to malaria and is specially severe in primiparas suggesting that mechanisms other than immunosupression are responsible for the high susceptibility in this group.

## P-165

**IMMUNOHISTOCHEMICAL EVALUATION OF ESTROGEN AND PROGESTERONE RECEPTOR CONTENT IN ENDOMETRIAL CARCINOMA**

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**Aims**: To evaluate the estrogen and progesterone content by performing immunohistochemical stains for ER/PR in tissue sections obtained from routinely processed (formaline-fixed and paraffin-embedded) material, with a direct comparison versus the biochemical method and studing the correlation with clinicopathologic parameters.
**Methods**: A series of sixty-six primary endometrial carcinomas consecutively surgically treated during the period 1989-1990, were immunohistochemically stained for ER and PR using formalin-fixed, paraffin-embedded sections, with monoclonal antibodies 1D5 and PGR-1A6. Biochemical analysis of ER was done on a homogenate of tumor. The ER and PR content in the malignant and benign components was evaluated separately.
**Results**: There was a significant correlation between the immunohistochemical and the biochemical results. The ER and PR positivity of the malignant component correlated with each other. There was a significant inverse correlation between tumor ER/PR positivities and nuclear grade. The free survival of the patients was significantly dependent on the PR status of the miometrium.
**Conclusions**: The immunohistologic methods for determining the sex steroid receptors for endometrial carcinoma can be performed on formaline-fixed, paraffin-embedded tissue and may provide prognostic information.

## P-166

EXPRESSION OF METALLOTHIONEIN IN MALIGNANT TRANSFORMATION OF CERVICAL NEOPLASIA: AN IMMUNOHISTOCHEMICAL STUDY

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**Aims**: Metallothioneins (MTs) are cytosolic proteins rich in cysteine appearing to play a physiological role in the absorption, transport and metabolism of trace elements, mainly of zinc and copper. Recent reports have linked over-expression of cellular MT with the progression of malignant tumors. The aim of this study was to investigate the possible significance of MT expression in malignant transformation of cervical neoplasia.
**Methods**: Ten CIN 1, 14 CIN 2, 19 CIN 3, 26 invasive squamous carcinomas and 18 adenocarcinomas consisted the group of this study. Sections of paraffin embedded tissues were stained immunohistochemically by the streptavidin-biotin peroxidase technique, using a mouse ($IgG_{1k}$) monoclonal antibody (Clone E9, Zymed, San Francisco, Calif., USA) that recognized a common epitope for both MT isoforms (I,II).
**Results**: MT expression was prominent in cells of the basal cervical layer of normal and CIN 1 patients. In the progression of CIN 2 to CIN 3, enhanced positivity for MT was evident in CIN 3 patients. Intense MT expression was observed in 22 of 26 invasive squamous cell carcinoma cases. The distribution of immunoreactivity was diffuse and MT expression was also prominent in isolated stromal cells. A statistically significant difference ($p<0.05$) was observed between MT intensity and histological grade, whilst the distribution of MT did not correlate to tumor size and lymph node status. None of the adnocarcinomas examined presented positive staining for MT, except of two adenosquamous carcinomas with positive staining in the squamous element.
**Conclusion**: The expression of MT during the progression of cervical neoplasia may provide important information about the biological mechanisms underlying the carcinogenic transformation.

## P-167

**LOSS OF HETEROZYGOSITY ON CHROMOSOME 17q IN EPITHELIAL OVARIAN TUMORS.**

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**Aims**: Loss of heterozygosity (LOH) on chromosome 17q is frequent in epithelial ovarian tumors (EOT), but its clinicopathologic significance remains to be elucidated.
**Methods**: DNA of 50 patients with EOT was extracted from blood and from fresh-frozen and paraffin-embedded tissue (14 benign, 7 borderline, and 29 malignant). Six microsatellite loci were amplified by PCR (D17S250, TRHA1, D17S800, D17S855, D17S579, D17S513). LOH was scored by the absence or reduction of the signal to less than 50% of one of the alleles in tumor DNA compared with normal DNA.
**Results**: LOH was identified on chromosome 17q in at least one locus in 12 tumors (24%), all of them carcinomas (12 of 29 tumors, 41.3%). It occurred more frequently among high grade serous carcinomas (8 of 14 tumors, 57%) and mixed endometrioid-serous carcinomas (2/5, 40%). LOH was detected in all informative markers of 10 tumors, suggesting the complete loss of an entire chromosome 17 homologue. Patients with LOH+ carcinomas were older than those with LOH- malignant tumors (mean ages 67 and 49).
**Conclusions**: The results support the hypothesis that LOH on chromosome 17q may be associated with the development of ovarian malignant tumors in elderly patients, and particularly with high grade serous or mixed endometrioid-serous carcinomas.

## P-168

MICRIONVASIVE CARCINOMA OF THE CERVIX

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**Aims**: Microinvasive carcinoma of the uterine cervix (MC) represents an early stage of the disease and preservation of the uterus in patients who desire conservation of fertility can be achieved by means of conization.
**Methods**: In the last 9 years, thirteen patients have been diagnosed of MC at our Institution. The mean age of these patients was 36.5 years (ranging from 26 to 46 years). In all cases depth of invasion, superficial extension, the presence of dysplasia in the adjacent epithelium, HPV infection, confluence of the infiltrating nests, margin status and FIGO staging were considered.
**Results**: The cytologic study revealed high grade squamous intraepitehial (HG-SIL) lesions in all patients. The biopsies guided by colposcopy or microcolpohisterescopy were diagnosed as HG-SIL in eight patients and as MC in the remaining five. Cervical conization was the surgical procedure for treatment in 12 and hysterectomy in one. In 11 patients diagnosis of MC was confirmed; in one cone and in one hysterectomy specimen only HG-SIL was seen. The mean depth invasion was 1.6 mm (0.5 - 3.2 mm) and the superficial extension was 2.1 mm ( 0.3 - 5.2 mm). Surgical margins were involved by HG-SIL in three patients, and none of them has shown a residual lesions after 1 to 3 years of follow-up. The lesions showing deeper infiltration revealed a more prominent chronic inflammatory response, and the lesions with larger superficial extension were more often confluent. All the patients are alive and without evidence of recurrence after a follow up period ranging from 1 to 5 years.
**Conclusion**: Microinvasive carcinoma of the uterine cervix has a very good prognosis and in most cases, conization is enough as a therapeutic procedure. For those patients with the desire to preserve fertility, local treatment seems to be a valid alternative provided that a close control can be assured.

## P-169

**STEROID RECEPTORS: OESTROGEN AND PROGESTERONE RECEPTORS IN ENDOMETRIAL CARCINOMAS**

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The female genital tract is a primary target organ for steroid hormones, oestrogen and progesterone. Steroid receptors are represented in the whole reproductive tract, the hormonal dependence being maximal in endometrium, with high expression of both receptors in pre-menopausal women.

Immunohistochemical techniques have made it possible to study the expression of oestrogen and progesterone receptors (ER and PR) in relationship with tumour pathology.

We used a sample of 93 endometrial carcinomas for our study (60 grade I, 26 grade II, and 7 grade III, according to FIGO). These cases underwent an immunohistochemical and statistical study, showing ER and PR expression.

The mean percentage of ER expression was 41, 42% for grade I, 30,23% for grade II and 24,29% for grade III. The mean percentage of positivity for PR expression was 45,12% for grade I, 27,88% for grade II, and 7, 86% for grade III. The expression of both receptors in mucosae adjacent to the tumour was very high in all cases. Statistically significant differences were obtained when the expression of the receptors in the tumoural zone and adjacent mucosae was compared.

This study shows that endometrial carcinomas usually have a high expression of both types of receptors and that this expression is inversely related to the FIGO classification.

## P-170

PROGNOSTIC FACTORS IN ENDOMETRIAL CARCINOMAS

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The purpose of this study was to define prognostic parameters for endometrial carcinomas. Tumour type, grade of differentiation, depth of invasion and stage have all been linked to survival. Recently, DNA-content and S-phase fraction have been proposed as valuable prognostic factors in endometrial carcinomas. In other tumours bcl-2 (a suppressor of apoptosis), Ki-67 (a marker of cell proliferation) and mean nuclear volumen (an objective and unbiased estimator of particle size) have provided prognostic information.

Tumours from 265 hysterectomy specimens were evaluated histologically according to the recommandations from WHO and FIGO (tumour type, grade of differentiation, depth of invasion and stage). Bcl-2, Ki-67, mean nuclear volumen and mitotic index were determined using immunohistochemistry or stereology. DNA-content and S-phase fraction were determined by flow cytometry. The patients were followed for a median of 1625 days (4½ year) and postsurgical treatment, recurrence and death were registrated. The histological parameters, Ki-67, mean nuclear volumen and DNA-content were all significantly correlated to survival and recurrence. Mitotic index was significantly correlated to survival and S-phase fraction to recurrence. Bcl-2 was neither correlated to survival nor to recurrence.

## P-171

COMPUTERIZED MORPHOMETRIC MEASUREMENTS OF ENDOMETRIAL HYPERPLASIA WITH PROGNOSTIC EVALUATION OF RISK OF MALIGNANT DEVELOPEMENT

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Aims: Women with endometrial hyperplasia have traditionally been overtreated. Computerised image analysis has shown to be a help in estimating the individual risk of cancer development. We wanted to check whether a discriminant factor, D-score, could separate the patients with good prognosis from those with a later cancer development. To evaluate the method, the results from two centres are compared. At one of the centres the method is used for a long time, while the other recently established it.

Methods: Histologic D+C archival material from 70 women with endometrial hyperplasia was investigated and reclassified. 28 women had developed cancer, and for the rest no cancer had been diagnosed during a follow-up of more than 10 years. Median age of the patients was 48,6 years (28-83). The most atypical area was marked on each section and analysed on a computer with QProdit software program from Leica. D-score was calculated from the outer surface density of the glands, the volume percent of stroma and the SD of the shortest nuclear axis. D-score above 1 indicates low probability of cancer development, and values below 0 indicates increased risk.

Results: In the group of 38 women where we predicted a good prognosis, only one developed cancer (after 17 years). Of the 18 women with D-score values below 0, 12 developed cancer.

The material was also investigated by trained technicians in a centre in the Netherlands, and the results showed high agreement.

Conclusion: The method of D-score can be used for individual prognostic advice for patients with endometrial hyperplasia. The method can be reproduced and is useful also for centres which are inexperienced.

## P-172

**β-CATENIN EXPRESION PATTERN IN STAGE I AND II OVARIAN CARCINOMAS. RELATIONSHIP WITH β-CATENIN GENE MUTATIONS, CLINICOPATHOLOGIC FEATURES AND CLINICAL OUTCOME.**

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**Aims:** To correlate β-catenin (BC) immunohistochemical expression pattern with BC gene mutations, clinicopathological features, and disease outcome in 69 stage I and II ovarian carcinomas.

**Methods:** Imunohistochemical analysis of BC expression pattern, PCR amplification and direct sequencing of BC gene exon 3, and relapse free and overall survival univariate and multivariate analyses.

**Results:** BC expression was localized in the nuclei in 11 tumors (16%), 9 endometrioid carcinomas with widespread nuclear expression and 2 serous carcinomas with focal nuclear expression. The remaining 58 carcinomas (84%) only had membranous BC expression. All but one of the endometrioid carcinomas with nuclear BC expression had considerable squamous metaplasia and five of these cases had large areas of endometrioid tumor of low malignant potential.There were oncogenic mutations in the phosphorilation sequence for GSK-3beta in exon 3 of the BC gene in seven endometrioid carcinomas with BC nuclear expression. Three mutations affected codon 32 (D32G, D32Y and D32Y), one affected codon 33 (S33C), two affected codon 37 (S37C and S37F), and one affected codon 41 (T41A). In the univariate and multivariate survival analyses, BC nuclear expression was selected as an indicator of good prognosis, since no patient whose tumor expressed BC in the nuclei showed relapses or died, in contrast with the 19 relapses and deaths among patients with tumors which only had BC membranous expression.

**Conclusions:** Oncogenic BC mutation is characteristic of a group of endometrioid carcinomas with a good prognosis, most of which originate from previous benign or borderline lesions. Endometrioid carcinomas with exclusively membranous expression of BC seem to represent a different subgroup of carcinomas that probably have a worse prognosis.

## P-173

**PREVALENCE OF HPV16 E-350G-VARIANT IN SQUAMOUS INTRAEPITHELIAL LESIONS (SIL) OF THE UTERINE CERVIX.**

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**Aims:** To study the prevalence of HPV16 variants in SIL of the cervix and the possible differences according to HIV status.

**Methods:** We analized 50 formalin-fixed and parafin embeded biopsies from 39 HIV(-) and 11 HIV(+) patients with SIL. HPV16 was identified in all the cases by the polimerase chain reaction (PCR) using the consensus primers MY09/MY11 and posterior analysis with Rsa I. The complete E6 sequence was amplified and directly sequenced.

**Results:** Distribution of the prototype and variant was as follows:

| | Prototype (E-350T) | Variant (E-350G) |
|---|---|---|
| HIV(-) (n=39) | | |
| Low grade SIL (n=16) | 12 (75%) | 4 (25%) |
| High grade SIL (n=23) | 11 (52%) | 12 (48%) |
| HIV(+) (n=11) | | |
| Low grade SIL (n=2) | 2 (100%) | 0 |
| High grade SIL (n=9) | 6* (67%) | 3** (33%) |

*4 cases (66%) with mixed infection (HPV16+31).
**All the cases with HPV16 only.

**Conclusions:** The HPV16 E-350G variant is more frecuently associated with high grade SILs in HIV(-) patients. This supports the hypothesis of an increased oncogenetic potential of this variant. In contrast, in HIV(+) patients the prototype is more prevalent in these lesions. These results can be explained by the impairment of the immunological surveillance in these patients, or by the interaction of multiple virus in mixed HPV infections.

## P-174

WELL-DIFFERENTIATED VILLOGLANDULAR ADENOCARCINOMA OF THE UTERINE CERVIX. CYTOHISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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**Introduction:** Cervical adenocarcinomas account for approximately 8-26% of all invasive cervical cancers and in general have been associated with a relatively aggressive course, that is considered to be worse than that of squamous cell carcinoma. However, in the last decade a form of well-differentiated villoglandular adenocarcinoma (VGA) has become recognized as a distinct histologic subtype of cervical cancer often associated with an excellent prognosis.

**Case Report:** A 35 year-old Spanish female, gravida 2 para 1, presented an abnormal vaginal bleeding with cervical cytologic smears positive for malignant cells. Colposcopic examination showed a polypoid exophytic and friable mass with bleeding appearance. Cervical biopsy revealed numerous thin papillary projections lined by an epithelium with mild cytologic atypia showing often an intestinal appearance. Immunohistochemical study showed reactivity against AE1-3, CEA, RE and negativity for VM, ENS and chromoganine at epithelial level with 90% of nuclei Ki-67 +. Stromal papillae resembled normal cervical stroma but containing acute and chronic inflammatory infiltrate with scattered nests of clears cells of macrophagic appearance (CD68+). Following cervical biopsy management options were discussed with the patient and as desired to preserve fertility, a cold-knife conization was performed one month later. Cervical conization revealed a VGA showing a depth of stromal invasion of 3 mm and a horizontal spread of 11 mm, with a free ectocervical margin, but the endocervical limit was positive for malignancy and a simple hysterectomy with pelvic lymphadenectomy was then performed. No residual invasive adenocarcinoma was noted in the surgical specimen. There were no postoperative complications and on surveillance the patient remains without clinical evidence of recurrent disease after 6 mo of follow-up.

**Conclusion:** VGA is a distinct histologic type of cervical cancer with an excellent prognosis. In spite of this well-known favorable prognosis careful study of the histologic and immunohistochemical characteristics of this tumors is needed to establish a correct differential diagnosis of these histological entity above all to decide if such patients can be managed with a conservative therapy (cone alone versus simple hysterectomy).

## P-175

MULLERIAN ADENOSARCOMA OF THE CERVIX: REPORT OF A CASE AND REVIEW OF THE LITERATURE.

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Mullerian adenosarcoma (MA), a variant of mixed mesodermal tumor of the uterus, is a rare tumor composed of benign epithelial and malignant stromal elements. Its location in the cervix is very infrequent. We present a case of an endocervical heterologous MA, diagnosed in a nulliparous 25 year old woman , admitted to the hospital on four occasions in a period of two years, because of metrorrhagia and recurrent cervical masses, considered initially as endocervical polyps. The last curettage performed, revealed several irregular polypoid masses, measuring 3,5x2,5x1cm. Histologically the composition of the masses was both epithelial and mesenquimal. The epithelial elements were glandular, lined by a single layer of benign columnar cells. The stromal component was highly celular, with atipical cells, periglandular cuffing and more than 3 mitoses per 10 HPF. Cartilaginous elements were also present. A diagnosis of endocervical heterologous MA was reached, and a total hysterectomy and bilateral salpingo-oophorectomy were performed. The histological examination confirmed the preoperative diagnosis. The neoplasm showed aditional rhabdomioblastic differentiation. Neither chemotherapy nor other adyuvant therapies have been administered. There has been no evidence of recurrence two years after the surgical treatment. To our knowledge, forty five more cases of cervical mullerian adenosarcoma have been reported in the English literature, and only in fifteen of those heterologous elements were present.

## P-176

**HISTOMORPHOLOGY OF CHORIONIC VILLI DURING PREGNANCY**

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The evaluation of chrionic villi during pregnancy could be suggestive of fetal and/or placental abnormalities. Sampling of chorionic villi is generally performed for genetic surveillance in women with echographically suspected fetal malformation. The same procedure can be applied to study villous histomorphology.

Twenty women with known fetal malformation and candidate to fetal abortion underwent to villous sampling for genetic evaluation. In this occasion, with no effective additional risk of fetal mortality (rate < 3%), placental material was removed and immediately fixed in an original mixture of alchool and formalin with citrate as anticoagulant, for two hours at room temperature. This fixative solution was prepared in order to obtain adequate hemolysis. Briefly, the samples were dehydrated in graded alchools, diaphanized in xylol and, finally, paraffin embedded. The sections were stained with hematoxylin-eosin and Giemsa methods and observed at light microscopy.

In the first samples, the villi eventually present in the solution, were concentrated by cytocentrifugation at different r.p.m.. In these cases, the histological examination revealed a poor preservation of cytological details. In the remaining cases, concentration was obtained by simple precipitation and no artefacts of cytological details were observed. In all cases, no less than 30 chorionic villi were available for the histological examination. Mostly, the villi resulted immature-intermediate and, rarely, terminal, according to the age of gestation.

In conclusion, we think that this technique could be a reliable method for the *in vivo* evaluation of placental diseases.

## P-177

NON-ASSOCIATION OF EPSTEIN-BARR VIRUS (EBV), HUMAN PAPILLOMA VIRUS (HPV) AND SIMIAN VIRUS (SV-40) IN 4 CASES OF LYMPHOEPITHELIOMA-LIKE CARCINOMA OF UTERINE CERVIX.

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AIMS: Lymphoepithelioma-like carcinoma of uterine cervix is an infrequent tumor in western countries. The presence of EBV and HPV has been documented, in most cases. We estudied 4 cases seeking for the presence of viral sequences of EBV, HPV and SV-40.

METHODS: We studied clinical and histopathological findings of 4 patients with lymphoepithelioma-like carcinoma of uterine cervix. The polymerase chain reaction was used to detect sequences of EBV, HPV and SV-40 from DNA paraffin embedded tumors. TCR-IgH gene rearrangement was also performed.

RESULTS: All 4 tumors showed a typical syncitial growth pattern of undifferentiated cells with lymphocytic infiltration. Immunohistochemically tumoral cells expressed low molecular weight cytokeratins (AE1-AE3,CAM 5,2) and the lymphocytic infiltration was predominantly T (CD45 RO, CD3),the TCR-IgH gene rearrengement being negative. The detection of EBV, HPV and SV-40 sequences by PCR was negative in all tumors.

CONCLUSIONS: Lack of association between EBV, HPV and SV-40 and concurrence of cervix lymphoepithelioma-like type tumor does not support the hypothesis that these viruses were involved in the pathogenesis of this peculiar tumor. Further, the absence of clonality in T predominant lymphocytic infiltrate supports their reactive character.

## P-178

"Intermediate differentiated ovary Sertoly-Leydig tumor".

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Background Sertoli- Leydig cell tumors (SLCT) are rare tumors of young patients (average age 25 years), composed of a mixture of cells resembling male Sertoli and Leydig cells. SLCT are classified into 3 categories: well, intermediate and poorly differentiated tumors. Heterologous elements are present in intermediate and poorly differentiated tumors.

Case report: A 17 year-old girl with previous history of amenorrea presented with a solid well circumscribed mass of the left ovary measuring 9 cm in the abdominal sonography. Serum levels for testosterone, androstenedione, 17-Hydroxiprogesterone and AFP were elevated. A left salpingo-oophorectomy and a right ovarian biopsy were performed. Light microscopy examination revealed small spindle shaped gonadal stroma cells arranged in small solid nests solid cord-like structures and open tubules. Additionally, heterologous elements presenting as glands lined by columnar and Goblet cells were observed. Immunohistochemestry Gonadal stroma and Sertoly cells showed positiveness for Vimentin. Cytokeratin was more strongly expressed in Sertoly-like cells of tubular areas and the epithelial component of the heterologous elements. S-100 was also positive and focally AFP. Steroid hormones were negative. The tumor was diagnosed as an intermediate differentiated SLCT. Areas with different histological features were microdissected and clonality analysis was performed using the HUMARA clonality assay.

Discussion: The therapy of SLCT mainly depends on tumor stage and differentiation. Other factors such as ovarian rupture, patient's age and desire for preservation of fertility should be also considered. The prognosis of these tumors is controversial. Well differentiated SLCT are almost always benign, whereas poorly differentiated are most likely to be malignant. The presence of mucinous elements is a favourable feature, whereas cartilage or skeletal muscle represent a worse prognosis.

## P-179

INTRAVENOUS LEIOMYOMATOSIS: REPORT OF A CASE WITH EXTENSION INTO THE PULMONARY ARTERY, AND REVIEW OF THE LITERATURE

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**Aims:** We report a case of intravenous leiomyomatosis (IVL), a very rare uterine neoplasm, with pulmonary artery involvement in a 40-year-old woman presenting with inferior vena cava thrombosis. Up to date 150 cases of IVL have been reported in the world literature, 30 with intracardiac extension and only two with tumor extension into the pulmonary artery.

**Methods:** The surgical specimens (total hysterectomy with bilateral salpingo-oophorectomy and intravascular tissue from heart and veins) obtained by simultaneous sternotomy and laparotomy, were routinely processed and stained immunohistochemically.

**Results:** The uterus was enlarged, with multiple myomas and the adnexal veins were filled with soft and rubbery masses. The intravascular tumors were lobulated and worm-like, extending through inferior vena cava to the heart and expanding to the pulmonary artery.

Microscopic examination of the specimens revealed smooth muscle tumors without atypia or mitoses. Immunohistochemically the tumors stained positively for vimentin, desmin and estrogen-receptors.

**Conclussions:** The diagnosis of IVL, because of its rarity, is not often considered and the differential diagnosis of IVL should include low-grade endometrial stromal sarcoma and smooth muscle tumors either benign or malignant.

Our case was diagnosed before histerectomy, so that a complete surgical approach was performed. Patients with positive estrogen-receptors are candidates for treatment with anti-estrogenic agents such as tamoxifen. The patient, one year later, is alive and free of disease.

## P-180

Endometrial Adenocarcinoma in Pre and Post Menopause: Evaluation of the Reactivity to bcl-2, p53, Mib 1 and Correlation with ER and PR expression.

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To point out the factors which affect the development and growth of endometrial adenocarcinoma, we performed an immunohistochemical assay with bcl-2, p53, Mib 1 and with antibodies for sexual steroids ( anti ER and anti PR) in two different groups each consisting of 25 and 29 patients in pre and postmenopausal age respectively. The intentions was to analyze eventual correlations between antigen expressivity and clinico-pathologic features. Bcl-2 was expressed in 61% of the premenopausal cases in G1 and 57% of postmenopausal cases in G1, in 33% of premenopausal cases in G2 and 30% of postmenopausal cases in G2 and lastly in 20% of the premenopausal cases in G3 and 19% of postmenopausal cases in G3. We noted that bcl-2 tends to be expressed with a higher frequency and intensity in those neoplasms positive for estrogens and progesterone receptors. Estrogens were infact expressed in 73% of the premenopausal cases in G1 and 65% of the postmenopausal cases in G1, in 22% of the premenopausal cases in G2 and 20% of the postmenopausal cases in G2 and finally in 1% of the pre and postmenopausal cases in G3 and it is also clear that percentage of positivity is more prominent in more differentiated forms.The results obtained with p53 were opposite to those of bcl-2 and with anti-ER and anti-PR; the percentage values of neoplastic cells increased progressively with increasing aggressivity and loss of differentiation of the neoplasm.Even lymphnode metastasis seem to be correlated with high values of p-53. The data for Mib1 is aligned to that of p53 with progression in receptor positivity of the neoplastic cells which increases with the increasing histologic grade.The results obtained lead to the conclusion that the expression of bcl-2 in endometrial carcinomas is without doubt affected by hormonal influence.Besides, bcl 2 is more often evident in carcinomas which express estrogens in well differentiated endometrial carcinomas and in those less invasive while rarely expressed in the less differentiated and metastasizing forms.On the contrary, p53 overexpression is seen with a higher frequency in endometrial carcinomas which do not express or express a low reactivity to ER and PR, in poorly differentiated carcinomas and in those which are highly aggressive and in forms with lymphnode metastasis.

## P-181

**GASTROINTESTINAL STROMAL TUMOR.- SEQUENTIAL EVOLUTIVE REVIEW & IMMUNOHISTOCHEMICAL PROFILE**

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**Aims.-** The gastrointestinal stromal tumors (GISTs) are a heterogeneous group of neoplasms that can reveal differentiation towards smooth muscle, periferal nerve sheath, or neural, or can only show undifferentiated mesenchymal cells. The clinical outcome is closely related to several gross and microscopical parameters. We report a case of GIST, intially called low grade leiomyosarcoma, which recurred after 5 year, with the purpose of contributing to the knowledge of this controversial entity.

**Methods.-** A 77 year old female presented with an abdominal mass, causing bleeding and severe intestinal occlusion. At laparotomy, a large mass, involving small and large intestinal wall was detected. A paliative surgical resection was performed. The patient died at the postoperative course. Five years before an ileal tumor was resected, that was interpretated, histologically, as a low grade leiomyosarcoma. 30 tumor sections were taken for histology and the surgical slides of the original tumor were reviewed Immunohistochemical study in both tumors was made, using the ABC method, including: Vimentin, smooth mucle actin, desmin, cytokeratins, LCA, NSE, S-100, and CD 34.

**Results.-** Grossly a large tumor mass was detected, measuring 10 cm., infiltrating largely intestinal wall of ileum and right colon, but overlying mucosa was free of tumor. Extensive necrosis and hemorrhage were seen. Microscopically, the tumor was composed of densely proliferation spindle cells, with mild to moderate nuclear pleomorphism and a low mitotic count (less than 5 X 50 HPF). The neoplastic cells from both tumors only showed difuse positivity for CD 34 and focally for NSE.

**Conclusions.-** The pathological diagnosis of these tumors is made chiefly by immunohistochemistry. The present case has been interpretated as a malignant undifferentiated GIST. It is a paradigm from the sequential pathological approach and behaviour of GIST.

## P-182

STAGE INCIDENCE AND STATISTIC SURVIVAL IN TUMORS OF THE LARGE BOWEL.

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**1) Aims:**The aim of the present work is to study the Stage Incidence and statistical survival of big bowel adenocarcinomas in a 20-year period of Cancer Registry of the Oncologic Hospital of Gipuzkoa, Basque Country, Spain.

**2) Methods:** We have taken the last informatized 20 years of the Registry of Cancer of San Sebastian, separating the data in five year periods, We studied the distribution in time, localisation, incidence by UICC Stages, and survival of different groups by the method of Kaplan-Mayer and Logrank test.

**Results:**The total number of studied cases was 310. The total cases of the colon were 112, and of rectum were 198. The total of Staged cases were 182: to the colon corresponded 77 and to the rectum 105. So the incidence was almost double in the rectum (198 cases from 310: 63,9 %) as in the colon (112 from 310: 36,1 %). There is no difference between the two periods: in 1.980-88, rectum 105/158, 66,5%; colon 153/158, 61,2%. In the period 1.989-93: rectum 93/152, 61,2%, colon 59/152, 38,8 %. The greatest differences among colon and rectum were in Stage II, with 28,57 % vs. 37,14%, and in Stage IV, the opposite, 16,19% of cases in the rectum vs. 24,67 cases in the colon. In the Stages I and III, the differences were irrelevants.

The survival of tumors in the total series of the large bowel, with the restriction of the different times of the periods, was of 28,89 % of cases at 5 years, 18,87 % at 10, and 11,01 % at 15 years. The survival of separated colon and rectum were of 37,89 % vs. 24.00 % at 5 years, 25,60 % vs. 15,34 % at 10 years, and 0 vs. 11,05 % at 15 years. There is a Logrank of $p<0,049$ favorable to the colon. The differences in survival by Stages in the total series were absolutely significant, with a Logrank of $p<0,0000$. Considering the paucity of cases of Stage I, we take the survival by Stages at 10 years: Stage I, 66,3 %, Stage II, 22,4 %, Stage III, 19,8 %, and Stage IV, 0%, (8,3 % at 5 years).

**Conclusions:** There are differences in the incidence of the tumors of rectum and colon, but not in the survival. The differences of survival in each Stage, studied separately in colon and rectum, in the first and second period, are not significant. Survival is good in Stage I (11 cases, 6,04 %), bad in Stages II and III (135 cases, 74,18%), and catastrofic in Stage IV 36 cases (19,78 %).

## P-183

*IN VITRO* ANGIOGENETIC ACTIVITY OF PANCREATIC CARCINOMA CELL LINES:THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND BASIC FIBROBLAST GROWTH FACTOR.

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**Aims:** Aim of this study was to determine and compare the angiogenetic activity of pancreatic carcinoma cell lines (PCCLs) in order to improve the understanding of the regulating mechanism of angiogenesis in human pancreatic cancer. We examined the significance of the two most potent angiogenetic growth factor, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).

**Methods:** 16 well characterized PCCLs were grown under normoxic (5% $CO_2$, 95% air) and hypoxic (5% $O_2$, 10% $CO_2$, 85% $N_2$) conditions. Serum free conditoned media of the PCCLs were investigated by sandwich enzyme linked immunoadsorbent assay (ELISA) with anti-VEGF and anti-bFGF antibodies. *In vitro* angiogenetic activity of CM of PCCLs was determined by $^3$HThymidine incorporation assay of human umbilical vein endothelial cells (HUVECs) and of dermal microvascular endothelial cells (DMVECs) with and without neutralizing anti-VEGF and anti-bFGF antibodies.

**Results:** Measurable levels of VEGF were detected by ELISA in 12 of 16 conditioned media of PCCL (0.3-10.3 ng/$10^5$ cells). Basic FGF were detected in 5 CM of 16 PCCLs (0.7-7.3 pg/ml). Proliferation of HUVECs cultured with CM could be inhibited by neutralizing anti-bFGF antibody in 5 of 16 conditioned media under serum free conditions. CM of hypoxic conditions exert a strong stimulation of HUVECs in the majority of cases.

**Conclusions:** Both VEGF and bFGF were produced by PCCLs. The angiogenetic activity of CM of PCCLs varies in a wide range and did neither correlate with the grade of differentiation of PCCLs nor with the p53 and Ki-ras status.

## P-184

PROGNOSTIC SIGNIFICANCE OF Ki-67 AND P53 ANTIGEN EXPRESSION IN CARCINOMAS OF GALLBLADDER

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Aims: To analyse p53 protein and Ki-67 antigen immunoreactivity in 41 cases of gallbladder carcinoma (GC) and its influence in the prognosis.

Methods: The immunohistochemical study was performed using the Biotin-Streptavidin-Alkaline Phosphatase method. The primary antibodies were anti p53 (DO-7; Dako) and anti Ki-67 (MIB-1; Biogenex).Nuclear staining was evaluated by two independent pathologists and a minimum of 200 cells were counted. The p53 overexpression was scored by a semiquantitative method evaluating the intensity and the incidence of positive stained cells. The intensity was graded as absent(0), mild(1), moderate(2) and intense(3); the incidence was categorized as absent(0), <10% (1), 10 to 50%(2), and > 50% of positive cells(3). A final score was obteined by adding the values of both variables. A score ≥3 was considered as positive for overexpression. The MIB index was calculated as the percentage of positive tumor cell nuclei. The statistical analysis was evaluated by the chi-squared test; criterion for significance was $p<0.05$. Survival was evaluated by Kaplan-Meier curves.

Results: The MIB index was increased in most GC. The p53 protein was expressed in 68.4% of cases (58.8%, 83.3% and 72.4% of well, moderately and of poorly differentiated, respectively) ($p>0.05$). Concerning the grade of invasiveness, 58% of the Tis-T1-T2 GC and 72,4% of the T3-T4 GC overexpressed p53 ($p>0.05$). Five-year survival of patients with GC that overexpressed p53 was 17,2% while survival of patients with GC that did not was 30% ($p>0.05$).

Conclusions: MIB1 index and the p53 protein expression are increased in gallbladder carcinomas.The p53 overexpression is not related to cell degree differentiation, level of gallbladder wall invasion or with patient survival.

## P-185

**INTRAMUCOSAL CYSTS IN THE GASTRIC BODY OF PATIENTS WITH ZOLLINGER-ELLISON SYNDROME**

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**Aims:** To ascertain the frequency and the clinico-functional correlations of intramucosal cysts in the gastric body of patients with the Zollinger-Ellison syndrome (ZES) and to clarify the relevant mechanism of development.

**Methods:** A total of 106 consecutive ZES patients (58 M, 48 F; mean age: 53 yrs, range 19-93 yrs) were investigated with a mean of 7.2 biopsy specimens of the body mucosa per patient proved to be suitable for the study. Biopsies of endoscopically detectable polypoid lesions were not considered. Cystic changes were evaluated with respect to their severity by assessing the cyst grade (0, absent, 1; <30%, 2; 30-60%; 3 >60% of the mucosal area of the biopsy specimen of individual patients showing the most pronounced finding, respectively) and to their intragastric distribution by assessing the ratio of biopsy specimens showing cystic changes over the total number of biopsies examined in each patient.

**Results:** Intramucosal cysts were found in biopsies of non-polypoid gastric body mucosa in 71.7% of 106 patients with Zollinger-Ellison syndrome (ZES) and showed grade 2 and 3 severity in 22 and 8 cases, respectively. The severity of cystic changes correlated with the gastrin levels ($p=0.0005$) and was more advanced in patients with active than in those with cured disease ($p=0.037$). In the former group, furthermore, advanced cystic changes correlated with age ($p=0.03$) male gender ($p=0.014$), years of disease from onset ($p<0.02$), years of omeprazole treatment ($p=0.033$), basal acid output ($p<0.02$), severity of ECL cell proliferative changes ($p=0.028$), and absence of previous gastrinoma resection ($p=0.039$) whereas they did not correlate with MEN-1 status, gastritis, maximal acid output, total duration of any antisecretory drug treatment, daily doses of omeprazole (>20mg vs 20 mg), years from surgery, duodenal localization of gastrinoma(s), presence of gastric carcinoid tumor(s) and of liver metastases. In groups of patients subdivided according to three levels of serum gastrin, the duration of omeprazole treatment was not related to the severity of cystic changes.

**Conclusions:** Intramucosal cysts in non polypoid gastric body mucosa of ZES patients are by far more common than the already reported fundic gland polyps, to which they likely give raise. Circulating levels of gastrin have an important independent role in their development.

## P-186

**APOPTOSIS IN VARIOUS TYPES OF COLITIS**

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**Background:** Apoptosis of individual crypt epithelial cells is noted in several immune and non-immune associated intestinal disorders. Its presence and pathologic implication in various types of colitis, were investigated in this study.

**Design:** The study comprised 1200 endoscopic biopsy specimens obtained from 1200 patients with various types of colitis. Included were: 632 normal or with mild non-specific colitis, 218 biopsies with infectious colitis, 53 with collagenous colitis, 72 biopsies with ischemic colitis, and 175 biopsies with active ulcerative colitis (UC). Several histologic features were assessed in the routine stain, using a semiquantitative scale from 0-3+, and the number of apoptotic bodies in 10 architecturally successive crypts (apoptotic body count (ABC) was tallied.

**Results:** Overall, the ABC's ranged from 0-6, but were ≤ 1 in 65% of specimens. ABCs of the 632 biopsies that showed no or only mild histologic abnormalities displayed a mean of 0.65 (range 0-5), as compared to the mean of 1.48 for the 618 histologically abnormal specimens ($p<0.01$). ABC's ≥3 (mean 3.4) were noted in all 175 specimens that displayed features of active ulcerative colitis. With infectious colitis, the ABC averaged 1.72, and with ischemic 1.75, but exceeded 3 in only 2 and 3 of the 218 and 72 specimens respectively. All 175 UC specimens with ABC≥3, exhibited more active inflammation and greater degree of mucous depletion with adenomatous changes than did abnormal biopsies with ABCs < 3 ($p<0.05$).

**Conclusions:** Mild apoptosis accompanies several types of colitis including infectious and ischemic colitis. More prominent apoptosis is present in cases of active ulcerative colitis and is associated with higher degree of mucous depletion, active inflammation and adenomatous epithelial changes. Thus it may serve as a marker in the differential diagnosis between other types of colitis and active ulcerative colitis indicating the immunologic features of the latter.

## P-187

**PROGNOSTIC SIGNIFICANCE OF HISTOLOGIC PARAMETERS IN PREOPERATIVELY IRRADIATED PATIENTS WITH LOCALLY ADVANCED RECTAL CANCER.**

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**Aim** : To analyse the importance of pathological factors as predictors of outcome in patients with locally advanced rectal cancer (LARC) treated with preoperative hyperfractionnated and accelerated radiotherapy (HART).

**Patients and Methods** : 98 resected specimens from patients with LARC were examined. A quantification of a relationship between disease free survival (DFS) and confounding clinical and pathological variables was performed using a Cox proportional hazards model. Survival curves were estimated according to the Kaplan-Meyer method.

**Results** : Tumors were staged pT3-T4 in 77% of cases vs 98% of initially cT3-4. The median infiltration of the rectal wall was 10mm and the median clearance was 2mm. A tumoral vascular invasion was observed in 22% of cases. Adenocarcinomas were well, moderately and poorly differentiated in 24%, 63% and 11%. RRM (radial resection margin) was negative in 79% of cases. Local recurrence was observed in 6 patients and distant metastasis in 17 patients. The overall actuarial DFS was 64%. By multivariate analysis we found that following pathological factors among all studied variables, were independent predictors of outcome: rectal wall involvement, RRM, pT and pN status (<0.05). A p value of 0.055 was obtained for the clearance illustrating the increased risk for recurrence if the distance between the tumor infiltration and RRM decreases.

**Conclusion** : In this series of patients with LARC treated with preoperative HART, we demonstrate the importance of infiltration of the rectal wall, clearance, and absence of tumor at the radial resection margin in addition to classical prognostic factors such as pT and pN.

## P-188

**DUODENAL-CONTENT REFLUX ESOPHAGITIS INDUCES THE DEVELOPMENT OF GLANDULAR METAPLASIA AND ADENOSQUAMOUS CARCINOMA IN RATS**

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**Aim:** To study the development of glandular metaplasia as a precursor lesion of esophageal carcinomas in a time course experiment of duodenal-content reflux (DCR) without carcinogen.

**Methods:** Thirty-two male Sprague-Dawley rats, weighing 250g each, underwent an esophagojejunostomy (EJ) under ether anesthesia in order to divert bile and pancreatic secretions into the esophagus. Groups of 5 or 6 animals were sacrified at 10, 15, 20, 25, 30 and 35 weeks after EJ. The esophagus was fixed in 10% buffered formalin and embedded in paraffin blocks. Sections were stained with hematoxylin and eosin , and PAS/ alcian blue. Histological changes in the squamous epithelium were classified as reactive changes (RC) including hyperkeratosis, basal cell hyperplasia and ulceration, as glandular metaplasia (GM) and as adenosquamous carcinoma (ASC).

**Results:**

| | Time (weeks) | | | | | |
|---|---|---|---|---|---|---|
| Histological findings | 10 n=6 | 10 n=5 | 20 n=6 | 25 n=5 | 30 n=5 | 35 n=5 |
| RC | 6 | 5 | 6 | 5 | 5 | 5 |
| GM | 0 | 0 | 1 | 1 | 2 | 3 |
| ASC | 0 | 0 | 2 | 1 | 2 | 2 |

GM and ASC were found mainly in the lower 2/3 of the oesophagus.

**Conclusions**: Chronic DCR in the rat induces mucus cell differentiation and provides a morphological substrate for the development of carcinomas with glandular and squamous phenotypes.

## P-189

### LYMPHOCYTIS GASTRITIS AND HELICOBACTER PYLORI INFECTION

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**Aims.-** Our aims was to perform a comparative study between lymphocytic gastritis (CG/LG+) with associated infection by Helicobacter pylori (HP) and chronic gastritis HP+ but without intraepithelial lymphocytosis (CG/GL-).
**Material and Methods.-** From 130 consecutive gastric biopsies with HP infection studied in our Department, only 2 were considered as CG/GL+. Antral and corpus biopsies from these 2 cases were compared to 5 cases corresponding to GC/LG-, using monoclonal antibodies to leukocyte subsets (CD45, CD45RO, CD3, CD4, CD8, CD20) and HP. In addiction, intraepithelial lymphocytes (IEL) were counted and expressed as number of IEL per 100 epithelial cells.
**Results.-** In CG/LG+ cases, we observed an increased number of IEL (30-60), most of them with CD8 phenotype , wich was higher in the corpus than in the antrum; neither glandular atrophy nor lymphoid follicles were seen. In CG/LG- cases, intraepithelial lymphocytosis was absent (4-10 IEL) but lesions were more intense and the antrum was more severely involved than the corpus.
**Conclusions.-** The morphological differences between CG/LG+ and CG/LG- along with the low incidence of the former, suggest that they represent different ways of response to aggression. In CG/LG+, HP may cause antigenic changes in gastric epithelium either acting by itself or along with other factors ( such as viruses or genetic predisposition), inducing intraepithelial lymphocytosis as occurs in other diseases caused by immune mediated mechanisms.

## P-190

### *HELICOBACTER PYLORI*- ASSOCIATED ATROPHIC GASTRITIS IS RELATED TO *VACA* AND *CAGA* GENOTYPES

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**Aims:** To assess the relationships between the *H. pylori vacA, cagA* and *iceA* genotypes and the presence of atrophic gastritis.
**Methods:** Gastric biopsies were obtained from a total of 372 *H. pylori*-infected patients from Portugal ($n = 194$) and Colombia ($n = 178$). Cases were classified for the presence of atrophy according to the updated Sydney system. Presence of *cagA*, *vacA* and *iceA* genotypes was determined directly on DNA from gastric biopsies by PCR and LiPA™.
**Results:** *cagA* was present in 117 (60.3%) of the Portuguese patients. 61 (31.4%) of the cases contained multiple *vacA* genotypes. Among the 133 cases with a single *vacA* genotype, s1a/m1, s1a/m2, s1b/m1, s1b/m2, and s2/m2 were observed in 2.26%, 0.75%, 45.1%, 9.0% and 42.8%, respectively. 64 (33.0%) of the cases contained multiple *iceA* genotypes and in 7 (3.6%) cases the *iceA* genotype could not be determined. Among the remaining 123 cases, 55 (42.3%) were *iceA*1. Atrophic gastritis was associated to the presence of *vacA* s1 ($P < 0.001$), *vacA* m1 ($P = 0.004$) and *cagA* ($P < 0.001$), but not to the *iceA* status. *cagA* was present in 161 (90.4%) of the Colombian patients. 8 (4.5%) of the cases contained multiple *vacA* genotypes. Among the 170 cases with a single *vacA* genotype, s1a/m1, s1a/m2, s1b/m1, s1b/m2, and s2/m2 were observed in 4.1%, 0%, 85.3%, 2.9% and 7.7%, respectively. 39 (21.9%) of the cases contained multiple *iceA* genotypes and in 7 (3.9%) cases the *iceA* genotype could not be determined. Among the remaining 132 cases, 55 (42.3%) were *iceA*1. Again atrophic gastritis was associated to the presence of *vacA* s1 ($P < 0.001$), *vacA* m1 ($P = 0.01$) and *cagA* ($P < 0.001$), but not to the *iceA* status.
**Conclusions:** We conclude that the association between atrophic gastritis and the *H. pylori cagA+/ vacA* s1, m1 genotypes is highly significant. These observations strongly support the hypothesis that long-term clinical outcome of *H. pylori* is related to different bacterial genotypes.

## P-191

### PREVALENCE OF HELICOBACTOR PYLORI IN 106 POSTMORTEM HUMAN STOMACH

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**Aims:** Our purpose was to determine the prevalence and distribution of Helicobacter Pylori infection and its relationship to gastritis.
**Methods:** Stomachs were collected from corpses subject of sudden death brought to our Legal Medicine Department. Who hadn't received prolonged medical treatment before death were included. 106 cases aged 3 to 83 years were investigated. Four tissue specimens were obtained from predetermined sites from each postmortem stomach and examined for histological gastritis. The infection was confirmed by finding Helicobacter Pylori on Giemsa-stained paraffin block sections. Helicobacter Pylori infection, inflammation in the mucosa, ages, sexes, prior symptoms and social status of the subjects were evaluated.
**Results:** 89 % of subjects were infected and 94.7 % showed histological gastritis (p:0.0063).
**Conclusion:** Evaluation of results revealed that; prevalence of infection did not inrease by age, or sex, or social status. Also; frequency and intensity of prior symptoms did not differ significantly between Helicobacter Pylori(+) and Helicobacter Pylori(-) subjects.

## P-192

### MINIMAL REQUIREMENT FOR NODAL STAGING OF COLORECTAL CANCERS

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**Aims:** To define a minimum number of lymph nodes required for reliable staging of colorectal carcinomas.
**Methods:** All specimens from resected pT3 and pT4 primary colorectal carcinomas assessed at our department between January 1996 and December 1998 were retrieved from our archives. Nodal involvement of these cancers was expressed as the number of nodes examined and the number harboring metastases. The distribution of metastatic nodes was analyzed in relation to the number of lymph nodes recovered.
**Results:** From a total number of 232 patients (126 males and 106 females) selected from the archives 190 pT3 and pT4 tumors were analyzed. 73 were node negative and 117 had metastases in the regional lymph nodes; 39 of these latter had more than 3 nodes involved. Dividing cancers as node negative or node positive, it seemed from our data that examining 6 or fewer nodes increases the risks of false negative staging. On the other hand, the cumulative percentage of nodal involvement in relation to the number of nodes required for the detection of node positivity implies the investigation of 15 nodes for the identification of at least 90% of pT3-4 tumors with any nodal involvement, and 13 nodes for identifying at least 90% of cancers with more than 3 metastatic nodes.
**Conclusions:** Node-negative colorectal carcinomas with less than 6 lymph nodes examined have a higher chance of being understaged, while those with 15 or more nodes examined seem to be reliably staged. However qualitative features of the lymph nodes might be included in the mathematical analysis to permit lowering the limits for reliability of staging.

## P-193

EXPRESSION OF MUCINS MUC1, MUC2, MUC5AC AND MUC6 IN INTESTINAL METAPLASIA OF THE STOMACH

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**AIMS:** To investigate expression of mucins in the sub-types of IM using MAbs to: MUC1 (HMFG1 and HMFG2), MUC2 (PMH1), MUC5AC (CLH2), and a novel MUC6 MAb (CLH5).

**METHODS AND RESULTS:** The MAb to MUC6 was produced using a peptide from the MUC6 tandem repeat. MAb CLH5 was tested with mucin extracts by Western blot, and with gastric mucosa in immunohistology. We found MUC 6 in glands of the antrum and mucopeptic cells of the body, gallbladder, Brunner glands, and acinar cells of the pancreas. The immunocytolocalization was cytoplasmic or perinuclear. These observations and the stronger reactivity of CLH5 with TFMSA-treated mucins suggested that the epitope of CLH5 may be affected by glycosylation. To evaluate the interference of GalNAc glycosylation with the epitope of CLH5, *in vitro* time course glycosylation of peptide MUC6 was performed with 1 to 5 mol of GalNAc. MAb CLH5 reacted with the generated glycopeptides. In contrast to normal gastric mucosa, the complete form of IM/type I exhibited little/ no expression of MUC1, MUC5AC and MUC6, and expression of the intestinal mucin MUC2 in goblet cells. The incomplete forms of IM, types II and III, showed expression of MUC1, MUC5AC and MUC6 as well as the intestinal mucin MUC2.

**CONCLUSIONS:** Decreased levels of expression of "gastric" mucins (MUC1, MUC5AC, MUC6) and expression of MUC2 in IM type I suggest a differentiation towards intestinal phenotype. Our observations that incomplete IM (type II and III) maintains expression of "gastric" mucins with *de novo* expression of MUC2 support that complete and incomplete IM represent divergent differentiation programs.

## P-194

**IMMUNOEXPRESSION OF ANGIOGENIC FACTORS VGF AND bFGF IN COLORECTAL ADENOMATOUS POLYPS.**

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In the sequence adenoma-carcinoma of he colon, angiogenesis seems to be essential for tumoral and growth progression.

**Aim:** To study the immunoexpression of angiogenic factors and their relationship with COX-2 and iNOS intratumoral activity in colorectal adenomas.

**Methods:** 43 colonic adenomas were studied. Angiogenesis was assesed by intratumoral microvessel density (IMD) in the connective axis of the polyps (VWF imunostaining, 100X) and by quantitative immunohistochemical study of VEGF and bFGF in vessels. COX-2 was determined by generation of PEG2 in polyp homogenates incubated ith $10^{-4}$ aspirin; iNOS was determined by conversion of $C^{14}$ arginine to citrulline in presence of EDTA.

**Results:** 35 adenomas were tubulo-villous, 7 tubular and 1 villous. Foci of carcinoma was detected in 9 cases. Strong immunoexpression of VEGF (64±6%) and bFGF (38±6%) was observed in all cases. Higher size and IMD was found in samples with carcinoma (27±4 and 44±4) as compared with remainder adenomas (13±1 and 27±4), $p<0.05$. There was a positive correlation between IMD and COX-2 activity ($r=0.46$, $p<0.01$).

**Conclusions:** The high microvessel density and COX-2 activity in adenomas with cancer as well as the strong immunoexpression of vascular angiogenic factors in all colonic adenomas , suggest a significant role of the angiogenesis in their tumoral progression.

## P-195

**FUNDIC GLAND POLYPS AND PARIETAL CELL HYPERPLASIA: AN IMMUNOHISTOCHEMICAL COMPARISON STUDY**

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**Aims:** Fundic Gland Polyps (FGPs) are small sessile (2-5 mm) usually multiple polyps arising in the gastric, acid-secreting mucosa. They are histologically characterized by superficial and deep cystic dilatations, with shortened gastric pits, incospicuous lamina propria. A possible role for omeprazole therapy has been suggested. Parietal cell hyperplasia (PCH), characterized by hypertrophy of parietal cell and a "serrated" contour of glands, is known to be higly characteristic of omeprazole therapy. As PCH may display deep cystic dilatations, it can be confused with FGPs, and rarely coexist.

**Methods:** We performed an immunohistochemical study on 2 sporadic, 22 syindromic FGPs and 8 PCH, using monoclonal antibodies (MoAbs) against Ck7 and 20, EMA, chromogranin A, CEA and oncofetal epitopes, and proliferation antigens, aimed to show any possible difference in the antigenic profile of the two lesions.

**Results:** Ck 20 showed a normal surface distribution in controls, FGPs, and PCH; EMA highlithed the normal parietal cell population of FGPs, and the enhanced population characteristic of PCH; all FGPs but one (linear hyperplasia) showed endocrine cells in normal number, whereas 7 out 8 PCH showed linear hyperplasia of endocrine cells; both FGPs and PCH showed an abnormal neoexpression of CK7. The surface foveolar mucin M1 showed enhanced deep expression in FGPs, whereas the PCH were alike controls (surface positivity only). FGPs and PCH often expressed CEA, syalil-TN and CA19.9. MIB1 labelling index of sporadic FGPs, PCH and controls did not show differences, whereas syndromic FGPs showed an enhanced surface and deep labelling index.

**Conclusions:** As Ck7, CEA and syalil-Tn are expressed by fetal stomach, FGPs and PCH showed an immature immunophenotype. Consistent differences between FGPs and PCH were the M1 expansion of polyps (foveolar metaplasia), and the frequent endocrine cell hypeplasia of PCH.

## P-196

EXPRESSION OF MICROSATELLITE INSTABILITY AND hMSH2 IMMUNOREACTIVITY IN 25 PATIENTS WITH EARLY ONSET OF COLORECTAL CANCER.

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Aims: To investigate the correlation between colorectal cancer with RER phenotype and immunohistochemical pattern of hMSH2.

Methods: Non neoplastic mucosa and tumor tissues of 25 patients with colorectal carcinoma under 40 years of age were retrieved from the files of the Department of Pathology of Medical School of "Federico II" University of Naples - Italy. Morphological and immunohistochemical analysis of p53, bcl2 and p27 expression was performed and correlated with proliferative index by Mib1, with hMSH2 expression and with microsatellite instability by molecular studies. The expression of immunohistochemical markers was tested using specific monoclonal antibodies and the results evaluated by a semiquantitative analysis.

Results: p53 was expressed in about 40% of poorly differentiated carcinomas, in which high proliferative index was seen. Bcl2 and p27 were expressed in well differentiated carcinomas.

We examined the rate of replication errors (RER) at microsatellite loci by determining the microsatellite markers BAT26, BAT40, and D2S123. We also screened the BAT-RII microsatellite sequences of the TGFβ type II receptor gene, which may be one of the target genes of the defective DNA repair in HNPCC tumors. Furthemore, we have examined the protein expression pattern of hMSH2 by immunohistochemistry in paraffin-embedded tumors with RER phenotype.

Conclusions: Our results suggest that the immunoistochemical examination of protein expression may be a rapid method for prescreening tumors for mutations in the MMR genes. However the utility of immunohistochemistry, as an adjunct to genetic diagnosis, has to be determined on a larger number of patients

## P-197

**IMMUNOHISTOCHEMICAL ANALYSES OF PRIMARY GASTRIC LYMPHOMAS**

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**Aims:** The aim of this study was to review primary gastric lymphoma cases seen at our department and characterize them histopathologically and immunophenotypically.

**Methods:** We studied a series of 41 gastric lymphomas and tested them with a panel of monoclonal antibodies including anti-immunoglobulin light and heavy chains, anti-CD3, CD5, CD20, CD30, CD35, CD45, epithelial membran antigen (EMA) and proliferating Cell Nuclear Antigen (PCNA) in immunohistochemistry.

**Results:** Histologically, 12 cases were classified as low grade (LG) mucosa associated lymphoid tissue (MALT) 20 as high grade (HG) MALT, 9 as other B-cell nonHodgkin's lymphomas. B-cell immunophenotype was confirmed in all cases. Lymphoepithelial lesions, confirmed by EMA staining, were seen in 10 of 12 LG and 18 of 20 HG MALT lymphomas. Lymphoid follicles were present in all LG and HG MALT lymphomas and there were CD35 positive cells in their centers. The same light and heavy chain restriction of centrocyte like cells and plasma cells was observed. Centrocyte like cells were CD3, CD5, and CD30 negative and showed low PCNA index. Large blastic cells had high PCNA index. There were CD30 positive 3 cases diagnosed as anaplastic large cell lymphoma.

**Conclusions:** This study yielded histopathologic and immunohistochemical findings of gastric lymphomas; the results suggest that most of the gastric lymphomas are LG or HG B-cell MALT lymphomas.

## P-198

**GRANULOMATOUS APPENDICITIS**

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**Aims:** Granulomatous appendicitis is an uncommon disease. In differential diagnosis, Crohn's disease (CD), idiopathic granulomatous appendicitis, tuberculosis, infection with Yersinia pseudotuberculotica, sarcoidosis, actinomycosis, parasitic infestation, diverticulitis and foreign body reaction, should be considered. Our aims were to establish the incidence of granulomatous appendicitis, to identify the etiology, and to estimate the long-term prognosis.

**Methods:** A retrospective review of 147 consecutive appendectomy specimens from 1984-1994 was performed. Eight patients with granulomatous appendicitis, histologically diagnosed as CD, were selected for further study. Seven of them had clinical signs of acute appendicitis with no evidence of CD elsewhere. Slides were stained by Kreyberg, Ziehl-Neelsen, Auramin-Rhodamin, Grocott, PAS and Brown-Breen methods. The patients were followed clinically from 5 to 15 years.

**Results:** The median age of eight selected patients with granulomatous appendicitis was 28 years (18 to 48 years). There were five women and three men. Most of appendices were enlarged and firm, thickened due to transmural fibrosis and chronic inflammation. Loosely arranged granulomas were found in all cases, there were also well circumscribed, epitheloid, noncaseating, sarcoid-like tight granulomas in seven, and granulomas with central abscess and some necrosis in three cases. Stains for microorganisms were negative in granulomas in all cases. Crypt abscesses were found in six, fissures in seven, and lymphoid aggregates in all cases. Among our patients, two had recurrence of CD elsewhere.

**Conclusions:** Granulomatous appendicitis can occur as a part of widespread CD. However, it is still not clear, whether isolated granulomatuos appendicitis, with exclusion of other possible causes, with usual benign long-term course, without progression to CD elsewhere, really represent a form of CD, or it is an idiopathic disease.

## P-199

FOLLOW UP OF GASTRITIS AFTER H. PYLORI ERADICATION

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**Aims:** To evaluate sequentially the changes in the histological lesions of gastritis after treatment for eradication of Helicobacter pylori (HP).

**Methods:** Patients (n=314 ) with duodenal ulcer and infection by HP, included in several one week treatments for eradication of HP, underwent gastric endoscopy and biopsies from antrum and corpus before treatment and at 4 weeks, 6 months, 1 year and 2 years after treatment. Histological assessment of gastritis was done following the Updated Sydney System, scoring from 0 to 3 HP density, inflammation (CI), activity (AC), atrophy (AT), and intestinal metaplasia (IM). Surface epithelial damage (ED) and lymphoid follicles (LF). were recorded but non graded.

**Results:** Pretreatment: All patients had histological evidence of chronic gastritis and HP infection (99% of antral and 90% of corpus biopsies colonized). The mean values of gastritis variables and frequency of ED and LF were significantly higher in antrum than in corpus.Post-treatment: At 4 weeks , HP was eradicated in 245 patients (78%) remaining infected 69 (22%). There were 3 reinfections and none spontaneous cure. Patients in whom successfully eradication of HP was achieved, showed resolution of AC and ED since 4 weeks. CI and LF dropped progressively without reaching normal values. In patients with persistent HP infection, there was a transient decrease in HP density, AC and DE at 4 weeks, recovering pretreatment values at 6 months. The other variables remained unchanged. As before treatment, HP predominated in antrum.There were not changes in AT or IM, either in HP eradicated or HP infected patients.

**Conclusions:** 1) After HP eradication there is a rapid an maintained improvement of chronic gastritis with disappearance of AC and ED. 2) AT and IM does not seem to be influenced by germ eradication. 3) In patients with persistent infection, treatment does not produce changes in topographic distribution of HP and gastritis.

## P-200

**β-CATENIN EXPRESSION IN ULCERATIVE COLITIS**

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**Background**: Patients with long-standing ulcerative colitis (UC) have a 10 times greater risk to develop colorectal carcinoma (CRCA) than normal individuals. Data on molecular genetics of UC-associated CRCA are not homogeneous. It remains unclear whether the genetic alterations on tumor suppresor genes such as APC, found in almost 85% of sporadic cases, also occur in UC-associated CRCA. Mutations in APC are probably initial events in colon tumorigenesis: APC regulates β-catenin (βC) cytoplasmic levels by promoting its degradation. βC is located in the membrane and cytoplasm and interacts with E-cadherin, regulating cell-to-cell adhesion. When APC is inactivated, βC is not degraded and translocates to the nucleus where its binding to TCF/LEF activates gene transcription. In normal tissues, nuclear βC has only been described during embryo development associated to cell migration events. This nuclear location can be detected by immunohistochemistry.

**Aim**: Based on the fact that APC mutations could be involved in UC-associated CRCA, and that nuclear localization of βC could be used as a marker of these alterations, we tried to assess possible changes in βC cellular distribution related to the different steps in CRCA development.

**Methods**: Samples from 6 patients with UC-associated CRCA where studied. Representative sections of normal, inflammatory, and displastic mucosa were selected. Immunohistochemistry methods were used to detect βC expression. Heat-based antigen retrieval was performed.

**Results**: In the whole cases studied, nuclear βC was detected in adenomatous foci and heterogenously in areas of carcinoma. Interestingly, in 3 out of the 6 cases nuclear βC was observed in apparently normal crypts according to the usual histologic criteria.

**Conclusions**: Normal crypts showing nuclear βC expression could represent early transformation events not still morphologically traduced. However, since normal mechanisms associated to re-epithelization - which involves cell migration- or reparative changes could explain these findings, further studies would be required.

(*): Colon Cancer Study Group: Batlle, E., Baulida, J., Corominas, JM., Gallén, M., Lloreta, J., Munné, A., Sancho, E. and Real, FX.

## P-201

**EXPRESSION OF bcl-2 AND c-erbB-2 IN THE COLORECTAL NEOPLASIA**

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**Aims**: Various oncogenes and tumor suppressor genes are known to have effects on patients outcome and used as diagnostic,prognostic factors. The aim of this study was to investigate bcl-2 and c-erbB-2 expressions in colorectal carcinomas and correlation between their presence and other clinicopathologic parameters.

**Methods**: Eighty-six colorectal carcinomas and 17 adenomas were stained with bcl-2 and c-erbB-2 immunohistochemically. Staining patterns were assessed semiquantitatively and correlated with tumor size, Duke's classification, tumor differentiation, mucinous characteristic and anatomic locations.

**Results**: We detected bcl-2 expression in 10 of 17 adenomas (58.8%) and 31 of 86 carcinomas(36.04%). Positive staining in normal mucosa was observed only in the compartment of cryptic cells. However neither the difference in the rates of bcl-2 positivity in adenoma and carcinoma groups, nor the correlation with other mentioned clinicopathological parameters,were found statistically significant. bcl-2 expression was found significantly high in mucinous carcinomas. Expression of c-erbB-2 was observed in 12 of 86(13.95%) carcinomas. It was not detected in adenomas and normal mucosa.Although the incidence of c-erbB-2 in nonmucinous carcinoma was higher than that of mucinous carcinoma, it was not significant. In addition we were unable to show any significant relation between c-erbB-2 expression and other clinicopathologic features.

**Conclusion**: Our results suggest that c-erbB-2 protein expression in colorectal carcinomas,is not very frequent event. There is no correlation between c-erbB-2 expression and malignant potential of colorectal carcinomas.Higher expressions of bcl-2 in adenomas than carcinomas suggest us a possible role of bcl-2 in early carcinogenesis of colon. However since we were unable to find any significant correlation between bcl-2 expression and other parameters the impact of this gene on the biological behavior is still unclear for us.

## P-202

**MICROVESSEL COUNT, PROLIFERATING CELL NUCLEAR ANTIGEN AND KI-67 INDICES IN GASTRIC ADENOCARCINOMAS**

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**Aims** : The aim of the present study was to immunohistochemically investigate the prognostic value of neovascularization (expressed as microvessel count- MVC) and tumor cell proliferation (expressed as PCNA labeling index–PLI and Ki-67 labeling index-KLI) in gastric adenocarcinoma. Correlations with clinicopathologic features were also evaluated

**Methods**: Tumor specimens from 74 patients diagnosed as gastric adenocarcinoma were included in this study. Formalin fixed,paraffin embedded tissue sections were stained immunohistochemically with F-VIII, PC10 and MIB-1 monoclonal antibodies. By using an occular grid subdivided into 100 areas, number of microvessels and PC10 , MIB-1 positive and negative cells were counted at X400 magnification. Chi-square test, Kaplan-Meier method and cox regression analysis were used for statistical analysis.

**Results** : The results showed that, MVC and PLI had a significant correlation with invasion and lymph node metastasis. The prognosis was significantly worse in patients with high MVC ( >14 ) and with high PLI (>49 %). However any relationship was not observed between KLI (38 %) and clinicopathologic parameters, so KLI failed to predict the prognosis. Cox model showed that, MVC and PLI were independent prognostic variables.

**Conclusion**: Ki-67 labeling index in gastric carcinomas has no prognostic relevance. However, the evaluation of microvessel count and proliferating cell nuclear antigen index in gastric carcinomas could be reliable indicators of prognosis.

## P-203

**Role of $p27^{kip1}$ and p53 expression as a marker of answer to neoadjuvant terapy in rectal carcinoma**

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**Aims:** The role of neoadjuvant therapy in rectal carcinoma is still debated. The expression of p53 and $p27^{kip1}$ in preoperative biopsies was correlated to histologically determined response to neoadjuvant therapy.

**Material**: 35 patients with rectal adenocarcinoma in preoperative stage T3-T4 underwent to neoadjuvant therapy (31 patients received chemioterapy and RT, 4 only RT) followed by surgery after a median time of 38 days. The expression of $p27^{kip1}$ and p53 was semiquantitatively evaluated by immunohistochemistry on pre-operatory biopsies respectively with the MoAbs p27 (clone57) and 1801. Positive tumors were those with more than 10% positive cells. Cases responsive to therapy were considered those with less than 40% residual neoplasia in the post-operative specimens.

**Results**: p27 nuclear staining was observed in 31/35 cases. None of the negative cases showed response to therapy ; conversely only 8/31 (25.8%) of p27 positive cases did not show tumor reduction (p=0.0095). The expression of p53, detected in 16/35 cases, was not significantly correlated to histologic response, which was observed in 11/16 (69%) of the p53 positive tumors and in 12/19 (63%) of the negative ones (p=0.504).

**Conclusions**: The expression of p27 seems to have a predictive value of positive answer to neoadjuvant therapy and is very promising as a parameter for therapeutic choices.

## P-204

**EVIDENCE SUPPORTING THE POTENTIAL UTILITY OF PRE-REPLICATIVE COMPLEX PROTEINS AS DIRECT MARKERS OF INFLAMMATORY BOWEL DISEASE ACTIVITY**

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Aims: Inflammatory bowel disease (IBD) is an increasing cause of morbidity in the Western World, with incidence doubling in England and Wales over the last forty years, and is usually diagnosed on endoscopy. However, there are no satisfactory markers at present to indicate the severity of disease activity and monitor treatment effects.

We are interested in the potential utility of minichromosome maintenance (MCM) protein expression in exfoliated colonocytes recovered from faecal samples as a direct indicator of IBD activity. MCM proteins are components of the pre-replicative complex essential for initiation of DNA replication in eukaryotic cells. They are present throughout the cell cycle but down-regulated in quiescence and cell differentiation, thus making them specific markers for proliferating cells.

Methods: We have used immunohistochemistry to examine the expression of Mcm 2 and Mcm 5 in histological sections from over twenty colectomy specimens with evidence of active and/or quiescent ulcerative colitis (UC) and Crohn's disease (CD).

Results: In areas unaffected by IBD (and in control sections of normal colonic mucosa), expression of MCM proteins is restricted to the normal proliferative compartment in the lower third of colonic glands. In quiescent IBD there is mild expansion of the proliferative compartment, but surface colonocytes remain negative for Mcm 2 and Mcm 5. In contrast, surface cells adjacent to areas of ulceration in active UC and CD show strong expression of the proteins.

Conclusions: Our data suggests that cells expressing MCM proteins may appear in the faeces during bouts of inflammatory activity in known IBD patients. We are currently investigating the detection of MCM-containing colonocytes in faecal smears prepared by our group from IBD patients.

## P-205

APOPTOSIS AND PROLIFERATIVE ACTIVITY IN PRIMARY GASTRIC LYMPHOMA

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**Aims:** 1. Evaluation of proliferative activity and apoptosis in primary low-grade MALT type and high-grade gastric lymphoma; 2. Estimation of correlation: a) between proliferative and apoptotic indices, b) between them and histological grade and marks of progression (regional lymph nodes metastases, serosa infiltration); 3. Attempt to find features associated with progression;

**Methods:** Material included archival paraffin-embedded surgical specimens from 59 patients with primary gastric lymphoma; slides were stained routinelly with HE, by Giemsa method (to reveal spiral bacteria), with paS and immunohistochemically with MIB-1 (Immunotech), ApopTag *In Situ* Apoptosis Detection Kit-Peroxidase, Oncor, and polyclonal Dako antibodies kappa, lambda (immunoglobulin light chains) to evaluate plasma cell-line differentiation. Apoptotic index was evaluated as the number of apoptotic bodies in randomly choosen 10HPF; proliferative index - as the per cent of MIB-1-positive nuclei. Statistical analysis was performed using Spearman's method, tau Kendall method and $chi^2$ test.

**Results:** 1. The statistically significant difference between apoptotic indices in low- and high-grade primary gastric lymphoma was find ($p=0.0023$). The difference between proliferative indices in these groups was not significant. 2. There was a significant correlation between apoptotic indices and marks of progression; proliferative index did not correlate with these features. 3. The number of plasma cell-line differentiated neoplastic cells correlated with marks of progression.

**Conclusions:** Apoptotic index can be considered as the prognostic marker in primary gastric lymphoma. The estimation of proliferative index as prognostic marker and the role of plasma cell-line differentiation require further investigation.

## P-206

INTRINSIC DENERVATION OF THE COLON LEADS TO HYPERPLASIA OF EPITHELIAL CELLS

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**Aims:** The colonic epithelium consists of colonocytes, endocrine cells and goblet cells, which differentiate from pluripotent stem cells located at the crypt bases. Intrinsic intestinal denervation by benzalkonium chloride (BAC) leads to enlargement of the colon. Our aim was to study the effects of myenteric denervation by BAC on changes in the populations of three different epithelial cell types of the colonic mucosa in rats.

**Methods:** The descending colons of rats were treated by serosal application of 2mM BAC, for 30 min. Control animals were treated with saline (0.9% NaCl). The animals were killed 3 months after the surgery and the colons were excised, fixed in formalin, paraffin embedded, cut and stained with H&S and periodic acid-Schiff for identification of goblet and columnar cells. Argyrophil reactivity using the Churukian-Schenk method was used to estimate the total endocrine population of the intestine in other sections. The myenteric neurons were estimated per unit length of mucosa. The mucosal endocrine, goblet and columnar cells were counted in 100 crypts/animal. Data were analysed by Mann-Whitney U-test, with the level of significance set at 1%.

**Results:** A significant reduction in neurone number was observed in the myenteric plexus of animals treated with BAC in comparison with normal animals (377.1/mm X 821.5/mm). The numbers of columnar, goblet and endocrine cells were significantly increased in BAC-treated animals when compared with controls, but with different proportions. These values, respectively, were as follows: columnar cells, 107.4 versus 77.1 cells per crypt; goblet cells, 87.9 versus 63.8 and endocrine cells, 13.36 versus 3.34.

**Conclusions:** These results confirm the existence of hyperplasia of the mucosal epithelial cells in experimental megacolon. They also show that intestinal cell differentiation in this experimental model is not a homogeneous process for all cell types but, instead, differences occur among them. This experimental model provides a simple method for further studies on the mechanisms of regulation of cell proliferation and differentiation in the colon.

## P-207

**EXPRESSION OF P53 AND NM23 IN ADVANCED STAGES OF STOMACH CANCER**

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**Aims.** Studies have been performed on the expression of p53 as a prognostic factor in different types of tumour. However, in gastric tumours, its use as an indicator of poor prognosis is debatable. Regarding nm23 the loss of expression seems to be associated with invasion and nodal metastasis.

Here we studied p53 and nm23 expression, alone and together, in gastric cancer and the association of their expression with clinical stages

**Methods.** We analyzed 90 gastric carcinomas using immunohistochemical techniques for p53 and nm23. Regarding clinical stages, the tumours were divided into two groups: localised (Ca. in situ. T1, T2, NO) and advanced (T3, T4, N1, N2, N3, M1). A Chi-squared study was performed to contrast independence among the variables, followed by implementation of a descending segmentation algorithm based on criteria of entropy.

**Results.** Of the 90 gastric carcinomas studied, 38 were localised and 52 advanced. 40 cases were positive for p53 and of these 28 (70%) were in advanced stage ($p=0.03$). Regarding nm23, positivity was found in 36 tumours and low expression in 54. Of the latter, 87% of the cases were in advanced stages ($p<0.0001$). Of the p53-positive cases, 26 were nm23-negative (p53+ and nm23-); of these 92% (24 cases) were in advanced stages while 8% corresponded to localised cases.

**Conclusions.** The expression of p53 and the loss of nm23 expression seem to be strongly linked to the clinical stage in stomach cancer since 92% of the tumours in advanced stages were p53+ and nm23-. The association of both variables affords more information than each of them independently.

## P-208

**DNA PLOIDY STATUS AND S-PHASE FRACTION AS MARKERS OF MALIGNANT POTENTIAL IN BARRETT'S ESOPHAGUS. A FLOW CYTOMETRIC STUDY USING ROUTINELY PARAFFIN-EMBEDDED TISSUE.**

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**Aims:** To investigate the usefulness of DNA flow cytometry in Barrett's esophagus in order to define subgroups of patients showing similar histological findings but with a different malignancy potential.

**Methods:** Routinely formalin-fixed and paraffin-embedded tissue of 49 patients with this disease were processed for flow cytometry measurements (ploidy, S-phase fraction) and the results were compared with the histological evolution observed in these patients.

**Results:** The presence in the flow cytometric analysis of a DNA aneuploid cell line is closely related to the presence of severe histological alterations (i.e., high-grade dysplasia: $p<0.001$, Fisher exact test). Only in the group of patients with "indefinite dysplasia" did we find statistically significant differences between the samples from patients with and without progression to more severe lesions (mean S-phase fraction of stable patients: 4.1% *vs.* 8.4% in patients with progression -$p<0.001$, "Student's" t test-). **Conclusions:** Our results suggest that this procedure are at least capable of distinguishing between a real, although incipient, neoplastic process and morphological changes of a reactive or reparative type. In Barrett's esophagus, genetic perturbations (manifested by the presence of DNA aneuploidy) may be generated in relation to a high proliferation rate.

## P-209

GASTROINTESTINAL STROMAL TUMOUR - A CORRELATIVE IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY

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**Introduction:** Gastrointestinal stromal tumour (GIST) is a recently, although not unequivocally defined entity, separated from the non epithelial tumours of the gastrointestinal tract. In the present study six gastrointestinal stromal tumours occurring in a two year interval were examined.

**Material and methods:** Eight GISTs of seven patients - one of them had a duplex tumour - were analysed by histological, immunohistochemical and electron microscopical methods. The main morphological features, the immunoreacitvity for vimentine, smooth muscle actin, S-100 protein, desmin, neuron specific enolase, chromogranin, neurofilament and CD 34 were evaluated together with ultrastructural characteristics.

**Results:** Two of the eight tumours were diagnosed as malignant, they metastatized within two years after removal. These tumours were cellular with high mitotic activity. Immunohistochemically all of them were positive for CD 34. Fore of them showed coexpression of smooth muscle actin and S-100 protein, the other fore were negative for both S100 and S actin. Vimentine was positive in each of them. Ultrastructurally indented nuclei, cell processes, skeinoid collagen fibres, occasional dense core granules and some incomplete cell junctions were observed.

**Conclusion:** The correct histological diagnosis of GIST and its histological subtypes is essential, because phenotype, especially immunophenotype is of crucial importance in predicting their biological behaviour.

## P-210

**HELICOBACTER PYLORI ERADICATION IN CHRONIC GASTRITIS**

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**Aims:** Helicobacter pylori ( HP ) infection is associated with an active chronic gastritis ( CG ). HP eradication improve CG activity. The aim of this study was to investigate the outcome of the CG parameters after HP eradication.

**Methods:** Sixty-five HP associated chronic gastritis patients ( mean age 28 years old ) with HP eradication ( lansoprazole 30 mg qd, amoxicillin 1.0 gm bid, and clarithromycin 250 mg bid for two weeks ). All the patients underwent endoscopy with antral biopsies before and 3 months after the end of the treatment. CG parameters have been evaluated according to the Whitehead classification. Gastric mucosa was considered as improved when the total parameters score $\leq$ 3 and as normal when the total score was 0. Patients with dysplasia were excluded.

**Results:** The mean CG score before and after HP eradication were respectively 5.7 and 4.2 for total score, 2.7 and 1.5 for inflammation, 1.1 and 1.2 for atrophy, 1.1 and 0.9 for intestinal metaplasia, and 0.8 and 0.6 for lymphoid follicles. The CG parameters improved in all the patients after HP eradication. Gastric mucosa was normalized in 48 % ( 31 / 65 ) out of patients.

**Conclusions:** 1. HP eradication was associated with a significant improvement in the CG parameters. 2. Gastric mucosa normalizes in 48 % of patients, 3 months after anti-HP treatment.

## P-211

EXPRESSION OF MICROSATELLITE INSTABILITY AND hMSH2 IMMUNOREACTIVITY IN 25 PATIENTS WITH EARLY ONSET OF COLORECTAL CANCER.

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**Aims:** To investigate the correlation between colorectal cancer with RER phenotype and immunohistochemical pattern of hMSH2.

**Methods:** Non neoplastic mucosa and tumor tissues of 25 patients with colorectal carcinoma under 40 years of age were retrieved from the files of the Department of Pathology of Medical School of "Federico II" University of Naples - Italy. Morphological and immunohistochemical analysis of p53, bcl2 and p27 expression was performed and correlated with proliferative index by Mib1, with hMSH2 expression and with microsatellite instability by molecular studies. The expression of immunohistochemical markers was tested using specific monoclonal antibodies and the results evaluated by a semiquantitative analysis.

**Results:** p53 was expressed in about 40% of poorly differentiated carcinomas, in which high proliferative index was seen. Bcl2 and p27 were expressed in well differentiated carcinomas.

We examined the rate of replication errors (RER) at microsatellite loci by determining the microsatellite markers BAT26, BAT40, and D2S123. We also screened the BAT-RII microsatellite sequences of the TGFβ type II receptor gene, which may be one of the target genes of the defective DNA repair in HNPCC tumors. Furthemore, we have examined the protein expression pattern of hMSH2 by immunohistochemistry in paraffin-embedded tumors with RER phenotype.

**Conclusions:** Our results suggest that the immunoistochemical examination of protein expression may be a rapid method for prescreening tumors for mutations in the MMR genes. However the utility of immunohistochemistry, as an adjunct to genetic diagnosis, has to be determined on a larger number of patients

## P-212

**CHARACTERIZATION OF KERATINOCYTE GROWTH FACTOR AND ITS RECEPTOR IN HUMAN COLON CANCER**

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**Aims:** Keratinocyte growth factor (KGF) is an angiogenic and mitogenic polypeptide synthesized mainly by mesenchymal cells. Its actions are dependent on its binding to a specific cell-surface KGF receptor (KGFR). It is not known whether cells of colonic adenocarcinoma within the tumor mass express KGF or KGFR. Therefore, in the present study, we estimated the expression of KGF and KGFR on human colonic cancer cell lines and human colonic cancer tissues.

**Methods:** KGF and KGFR mRNA expression in COLO 205, DLD-1, HCT-15, SW 480 and WiDr colonic cancer cell lines were examined by RT-PCR. The expression levels of these mRNAs in human colonic tissues were estimated using competitive RT-PCR. Cells overexpressing KGF and KGFR mRNA in cancer tissues were identified by in situ hybridization using a cRNA probe.

**Results:** KGFR mRNA was expressed in all five cancer cell lines. In contrast, KGF mRNA was not detected in any of them. Competitive RT-PCR revealed that KGF and KGFR mRNAs were expressed in colonic cancer and normal colonic tissues, and that both of these mRNAs were overexpressed to a greater extent in cancer tissues than in normal tissues. In situ hybridization showed that KGFR mRNA was expressed in colonic cancer cells.

**Conclusions:** These findings indicate that KGF and KGFR are overexpressed in cancer tissues, suggest KGF might contribute to the aggressiveness of human colonic cancer.

## P-213

**Papillary-cystic tumors of the pancreas: a report of three cases**
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**Aims:** Three new cases of papillary-cystic tumor (PCT) of the pancreas (the so called solid-pseudopapillary tumor) are reported.

**Methods:** Tissues were fixed in 10% neutral buffered formalin and routinely processed for light microscopy. Sections were stained with hematoxylin and eosin and periodic acid-Schiff (PAS) with and without diastase digestion. Immunohistochemical staining for Vimentin, chromogranin, CD-45, alpha-1-antitrypsin, enolase, CAM 5.2 and Progesterone receptors were performed. Cytometric determination of tumor cells was done.

**Results:** Two cases were well demarcated masses, surrounded by a fibrous capsule. The cut sections showed brown nodular areas in the periphery with hemorragic and necrotic zones. The third case was a solid mass similar to normal pancreas.
Histologically all tumors were similar. The patterns ranged from solid and trabecular to seudopapillary. Tumor cells have a monotonous clear eosinophilic cytoplasm with regular round-to-oval nuclei. Immunohistochemically, the tumor cells were positive for Vimentin, alpha-1-antitrypsin, enolase, CAM 5.2 and Progesterone receptors. Cytometric determination showed a DNA-diploid pattern.

**Conclusions:** PCTs are unusual tumors, diagnosis is either found incidentally on routine image examinations (ultrasound or CT). The prognosis is excellent after complete tumor removal. Agressive behavior cannot be excluded even in the absence of malignant histological appearance.

## P-214

THE MICROVESSEL NETWORK IN COLORECTAL NEOPLASMS
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**Aim:** The objective of this study was to evaluate multiple morphometric microvascular characteristics in addition to microvessel density (MVD) in colorectal carcinomas to provide a better approach to examining the relation between angiogenesis and clinicopathologic factors and prognosis.
**Methods:** Histologic sections from 106 colorectal adenocarcinomas and 17 adenomas, immunostained for factor VIII, were evaluated by image analysis for the quantification of MVD, total vascular area (TVA), and microvascular branching, as well as several morphometric parameters related to the vessel size or shape.
**Results:** MVD gradually decreased with progressing Dukes stage. The vascular branching counts were significantly higher in carcinomas than in adenomas, and remained unaffected through progressing Dukes stages. Shape-related parameters showed significant differences between carcinomas and adenomas and between different grades of differentiation. Branching counts and TVA were the only factors found to be of prognostic significance.
**Conclusions:** This study provides evidence that neovascularization is an early critical event in colorectal tumorigenesis, reaching a maximum level early in the malignant process. Its prognostic significance is better assessed by quantification of TVA and the branching pattern of microvessels, whereas MVD does not provide significant prognostic information for colorectal carcinoma patients.

## P-215

**EXPRESSION OF STROMAL DIAGNOSTIC MARKERS IN ACUTE AND CHRONIC GASTRIC DISORDERS.**
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The aim of this study was to evaluate the amount and qualitative characteristics of stromal cells in gastric mucosa and to determine their possible role in the destructive and repair processes.
31 children and 27 adult patients with different types of gastritis, erosions and chronic peptic ulcer were examined. Immunocytochemical analysis were performed on formalin fixed, paraffin-embedded tissue sections with antibodies against synaptophysin,vimentin, CD 68 , CD8, chromogranin A, NSE, serotonin. Immunolabelled cells were counted in 1 $mm^2$.
CD68 + macrophagal-monocytic cells were present in the areas of dense inflammatory exudation during activation of chronic gastritis till 2O,4 cells per 1 $mm^2$ and were located under multilayered proliferation of surface epithelium. Concentration of vimentin containing cells was more dense in the bottom of the healing erosions, in the edges of chronic peptic ulcer and in the microvessels of its granulation tissues.
The amount of cytotoxic lymphocytes in control group, in acute gastritis of children and in adults with chronic peptic ulcer were accordingly o,6 : 2,6 : 7,2 cells per 1 $mm^2$ .CD8 positive cells have increased during lingering the pathology of stomach. They were found in subepithelial areas and lymphoid follicles . Comparative topographic investigation of synaptophyzin, NSE , serotonin, chromogranin proved the location of these active substances in EC, ECL and mast cells of gastric mucosa and submucosal layer. They become exhausted in patients with permanent gastric ulcer and their amount and functional activity correlated with gastric acidity.
The results proved that the repair of mucosa is realised with vimentin and CD 68 + cells, but CD8 + lymphocytes are one of the mediators of its injury.

## P-216

**HELICOBACTER PYLORI ERACICATION FOR THE TREATMENT OF LOW-GRADE GASTRIC MALT LYMPHOMA: FOLLOW-UP TOGETHER WITH SEQUENTILAL MOLECULAR STUDIES**
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**Aims:** Helicobacter pylory infection is associated with low-grade gastric lymphoma, and available data support that the eradication of the H. pylori can cause histological regression of the lymphoma.
**Patients and Methods:** Eight patients with low-grade gastric MALT lymphoma were treated with amoxicillin, metronidazole, and omeprazole for 14 days in a prospective study. Patients were followed up with sequential endoscopy, mapping gastric biopsies, and molecular studies with PCR amplification of the IgH gene in order to asses the response to H.pylori eradication and the evolution of the histological molecular responses.
**Results:** H.pylori was eradicated in all patients and reinfection was demonstrated only in one patient (at 18 months). After H.pylori eradication treatment, the lymphoma regressed both endoscopically and histologically in all patients. In four patients, no clonal band was detected by PCR, and in the remaining patients, PCR identified a clonal band, which disappeared in all patients after a mean of 12 +/- months. In the last year, seven patients have a persistent clinical, histological resimision, and no clonal band was detected by the PCR analysis; but in the patient with reinfection, a clonal band was detected in spite of clinical and histological regression.
**Conclusions:** 1) Low -grade gastric lymphoma can be histologically cured with eradication therapy for H. pilory. 2) After histololgical regression, PCR amplification of the IgH gene can identify an eventually persisting clonal population .3) Sequential histological and molecular studies are essential for the assesment of the evolution of the lymphoma. 4) The clonal population trends to disappear, but its disappearance may be delayed for months.5) Patients with histological regression but with a persistent clonal band should not be treated unless the lymphoma can be histologically demonstrated.

## P-217

INTRATUMOR HETEROGENEITY AND RELATIONSHIP OF P53 PROTEINS, APOPTOSIS AND DNA ANEUPLOIDY IN HUMAN COLORECTAL ADENOCARCINOMAS.

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Aims: Intratumor heterogeneity of p53 protein, apoptosis, and DNA aneuploidy in human colorectal adenocarcinomas within the context of a morphology analysis still needs to be further investigated. To these aims we have examined multiple tumor sectors (from 4 to 6 per tumor) for 56 adenocarcinomas. Methods: Detection of p53 proteins was done by immunohistochemistry using Mab DO7. P53 mutations were also detected in a subgroup of cases. DNA aneuploidy was evaluated by multiparameter flow cytometry of nuclei suspensions for measuring Dapi fluorescence emission, forward and side scattering. Apoptotic index was calculated counting TdT positive neoplastic cells x 1000. Results: DNA aneuploidy was detected in 43 (77%) carcinomas, while presence of more than one DNA aneuploid subclone was found in 21 (49%) tumours. Immunohistochemical detection of p53 proteins done in at least 5-6 tumor areas indicated that aneuploid sectors were more frequently p53 positive. A proportion of 20% of the cases examined with p53 mutations did not show high p53 expression. A statistically significative correlation was found between p53 overexpression and DNA aneuploidy ($p<0.01$) as well as with DNA multiclonality ($p=0.02$). Contingency table analysis showed also a statistically significant correlation between p53 alterations and increased intratumor distribution of apoptotic cells ($p<0.005$). Conclusions: Intratumor heterogeneity of p53 protein, apoptosis, and DNA aneuploidy in colorectal carcinomas indicated that assessment of any one of these parameters is critical without a simultaneous morphological investigation. The inactivation of the p53 tumorsuppressor gene appears as one of the important selective causes of increased incidence of aneuploidy and multiclonality and decreased apoptosis in these tumors but clearly not the only genetic cause.

## P-218

FINE NEEDLE ASPIRATION PERFORMED UNDER ENDOSCOPIC ULTRASONOGRAPHY: A USEFUL MEANS OF DIAGNOSING SOLID PANCREATIC LESIONS. A SERIES OF 203 CASES.

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**The aim of this study** was to test the efficiency of fine-needle aspiration (FNA) performed under endoscopic ultrasonography (EUS) as a means of diagnosing solid pancreatic lesions.

**Material and Methods:** Between January 1994 and January 1999, 203 consecutive patients (110 men, 93 women) with a mean age of 66 years underwent EUS FNA on a solid pancreatic mass with a median diameter of 30.2 mm (range 8 to 40 mm). A conventional smear (CS) was obtained in each case, and in 83 % of all the cases, microbiopsies were also obtained. The ThinPrep (TP) method, a recently introduced fluid-based technique for collecting aspirates, was also performed in the last 28 cases.

**Results:** We diagnosed 167 malignant lesions: 126 adenocarcinomas, 32 endocrine tumors, 8 pancreatic metastases, 1 lymphoma and 11 non malignant lesions: 7 chronic pancreatitis and 4 post acute pancreatitis abscess. The diagnosis was confirmed by either surgery or follow-up. In this series, we observed one false positive case: an endocrine tumor FNA diagnosis corresponding in surgical specimen to an islet hyperplasia and 24 false negative cases : 22 adenocarcinomas, 1 endocrine tumor, and 1 sarcoma. In the majority of the latter cases, a poor cellularity and a large amount of fibrous stroma was observed in the surgical biopsy or specimen. The sensitivity and specificity of the FNA diagnosis were 87.6 % and 91.6 %. The positive predictive value of the FNA method was 99.4 % and the negative predictive value 31.4 %.

**Comments:** TP offers the additional advantage in comparison with CS that it reduces the screening time per case and like microbiopsy, can be used with other techniques, such as immunohistochemistry. With the method used here to obtain the TP material, it was not possible, however, to assess the cellularity, an important criterion in CS. The value of the TP method still remains to be confirmed, and microbiopsy is still the most efficient method available for diagnostic purposes.

**Conclusions:** FNA under EUS is a reliable technique for diagnosing pancreatic tumors and especially for screening adenocarcinomas and endocrine tumors. The limits of this technique are for the lesions showing poor cellularity and abundant stroma .

## P-219

PROTEIN p53 ACCUMULATION AND p53 GENE MUTATION IN COLORECTAL CANCER.

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**Aim**: 1. To investigate the relationship between p53 protein accumulation and p53 gene mutation in colorectal cancer. 2. To correlate these results with prognostic factors as sex, tumor localization, stage and grade.

**Materials**: A total 38 tumors were evaluated, 21 in male and 17 in female. Twenty seven cases were located in the rectum and 11 in the colon. Seventh were well differentiated, 28 were moderately and 3 poorly differentiated. According to Astler-Coller classification, 4 pts were B1, 16 pts were B2, 15 pts were C2 and 1 was D. The Jass classification was additionally used: I group was in 2 cases, II group in 10 cases, III group in 7 cases and IV group in 8 cases.

**Methods**: 1. Accumulation of p53 protein was analysed immunohistochemically (IHC) in formalin fixed and parrafin embeded material. Antibody p53 (DO-7, Dako) in dilution 1:100 was used. Control sections were used to replace the primary antibody with a nonrelated normal antiserum. A section with high p53 accumulation in colorectal cancer was used as a positive control. Staining cell numbers were scored as none (-), weak (+, 10-25%), moderate (++, 26-75%) and intense (+++, >75%). 2. For all cancer samples, exons 5 to 9 of p53 gene were amplified from isolated genomic DNA. PCR products were subjected to Single Stranded Conformational Polymorphism analysis. All products were also directly sequenced on ABI Prism 377 apparatus using fluorescent dideoxyterminators chemistry.

**Results**: p53 protein accumulation was detected in 19 among 38 (50%) colorectal cancers. The correlation between p53 expression and sex (Female 71%, Male 50%) and Jass classification was observed. Overall, 19 of 38 samples (50%) exhibited mutations in exons 5 to 9 of p53 gene. Mutational status correlated well with IHC results ($p<0.04$), there was, however, no correlation to any other clinico-pathological variables.

**Conclusions:** p53 protein accumulation corelated with gene mutations. p53 expression was higher in female and in advanced cases according to Jass classification.

## P-220

**p53 PROTEIN EXPRESSION IN BARRETT'S ESOPHAGUS.**

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**Aims:** There is a substantial interobserver disagreement in the diagnosis and grading of dysplastic lesions in Barrett's esophagus. The aim of this study is to evaluate the usefulness of tissue detection of p53 protein as a objective method to complement the conventional histological evaluation of dysplasia in this disease.

**Methods:** Tissue samples of 73 patients diagnosed of Barrett's esophagus were processed for p53 immunostaining. Cases were labelled as positive for p53 if they showed any obvious nuclear staining detected in at least 5% of the cells in the tissue area of interest.

**Results:** The positivity of the staining for p53 showed a statistically significant increase throughout the sequence: no dysplasia (0%) → indefinite for dysplasia (31%) → low-grade dysplasia (64.5%) → high-grade dysplasia (100%) → carcinoma (83.5%). (Linear trend: $p<0.0001$). **Conclusions:** The use of this procedure, technically simple, economical and quick, could play a role in the evaluation of patients with Barrett's esophagus.

## P-221

EXPRESSION OF GROUP II PHOSPHOLIPASE A2 IN TUMOURS OF THE LARGE INTESTINE

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**Aims:** Group II phospholipase A2 (PLA2) is a lipolytic enzyme that has been proposed to be involved in the genesis of colonic carcinomas. The PLA2 gene has been identified in mouse as the MOM-1 locus gene, a modifier of the pro-neoplastic effect of the APC gene. The purpose of the current study was to compare the expression of PLA2 in benign and malignant neoplasms of colonic mucosa.

**Methods:** Tissues from colorectal adenomas (16 patients), adenocarcinomas (9), juvenile polyps (5), hyperplastic polyps (15) and normal colonic mucosa (8) were studied by immunohistochemistry for the presence of PLA2 protein and by in situ hybridization for the mRNA of PLA2.

**Results:** There was mRNA of PLA2 in 3 out of 8 samples of normal colonic mucosa but no PLA2 protein. Colorectal adenomas contained PLA2 protein in 11/16 cases (6/9 cases of villous adenomas and 5/7 cases of tubulovillous adenomas). There was PLA2 protein in 4/5 cases of juvenile polyps but in none of hyperplastic polyps or neoplastic cells of adenocarcinomas. The mRNA of PLA2 was localised in the epithelial cells of adenomas and juvenile polyps.

**Conclusions:** The current results show that PLA2 is often expressed in the tumour cells of colorectal adenomas and juvenile polyps, whereas normal colonic mucosa, hyperplastic polyps and colorectal adenocarcinomas are practically devoid of the enzyme. The results support the notion that PLA2 is involved in the development of colonic tumours. The exact role of PLA2 in the evolution of colorectal adenomas and carcinomas remains to be investigated.

## P-222

**DO ANGIOGENESIS AND ANGIOGENETIC FACTOR VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) HAVE ANY ROLE IN ADENOMA-CARCINOMA SEQUENCE OF COLONIC NEOPLASTIC POLYPS?**

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**Aims:** To examine the difference between benign and malignant colonic neoplastic polyps in relation to angiogenesis and angiogenetic factor VEGF.

**Methods:** A total number of 64 colonic neoplastic polyps were classified histologically as benign (n=32), malignant (n=25), focally malignant (n=7) tubular, villous or tubulo-villous adenomas. Immunohistochemistry was performed for CD34 and VEGF expression. CD34 positive microvessels in the stroma of the polyps were counted in three different fields using light microscope on X200 magnification and mean microvessel count was considered as the MVD. Student-t test was performed for statistical analysis. The intensity of staining for VEGF was assessed on a 0-3+ scale.

**Results:** MVD of benign and malignant colonic neoplastic polyps showed no significant difference. Similarly, there was no difference between the malignant and benign areas of the focally malignant polyps. No significant difference was found between the MVD's of malignant or benign polyps of each histopathological type. VEGF expression was only observed in few of the cases, mainly in the epithelial cell cytoplasm or on the luminal surface, irrespective of the diagnosis.

**Conclusions:** Our results suggest that angiogenesis and angiogenetic factor VEGF do not seem to have significant role on adenoma-carcinoma sequence of colonic neoplastic polyps.

## P-223

GASTROINTESTINAL STROMAL TUMOR (GIST): ONE CASE WITH NEGATIVE INMUNOHISTOCHEMICAL MARKERS AND FATAL PROGNOSIS.

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The gastrointestinal mesenchymal tumors form a heterogenous group that consists of several different entities with distinctive clinico-pathological profiles. They can be divided into four categories on the basis of their phenotypical features.
1) Differentiation towards smooth muscle and neural elements (the less common). 2) Differentiation towards neural elements (Plexosarcomas). 3) Dual differentiation toward smooth muscle and neural elements (the less common). 4)Lacking differentiation towards either type. A high percentage of this category show immnureactivity for CD34 and some authors use the term GIST only for this group.

Our case is a 62 years old man with an extensive retroperitoneal tumor infiltring ureter (the inical symtom was a flank pain). The tumor was removed and the patient died two weeks after. Microscopically the tumor showed hypercellularity with epithelioid cells, necrosis and high mitotic activity. The inmunohistochemical study was positive only for vimentine.

CONCLUSIONS: The tumors refered to as "gastrointestinal stromal tumors" represent tumors of primitive mesenchymal cells. This terms reflects the incomplete understanding of their cells type lineage.and uncertain relationship with the differentiated smooth muscle and Schwann cell tumors. The CD34 antigen (a myeloid cells progenitor cell antigen also expressed in endothelial cells and some other mesenchymal cells) is generally expressed in this one. The no expression of CD34 is associated to a rather primitive phenotype and poor prognosis.

## P-224

**LACK OF MELANOSIS COLI IN COLONIC ADENOMAS SUGGESTS DIFFERENT PATHWAYS OF APOPTOTIC BODIES IN NORMAL AND NEOPLASTIC COLONIC MUCOSA**

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**Aim:** Melanosis coli is caused by increased epithelial apoptosis and pigment deposition in the lamina propria. Melanosis coli is lost in colonic neoplasia. The aim was to investigate this phenomenon in order to further contribute to the understanding of its pathogenesis.

**Methods:** We investigated and scored apoptotic fragments and their distribution and macrophages by CD-68 immunostaining in 7 human colonic adenomas associated with melanosis coli in nonneoplastic mucosa and in normal colonic mucosa.

**Results:** In adenomas we found 7 apoptotic bodies per 100 epithelial cells in the epithelial layer and only 0.2 apoptotic bodies per high power field (HPF) in the lamina propria, in normal mucosa, in contrast 2/100 in the epithelial layer and 5/HPF in the lamina propria. Our results show that apoptotic fragments remain in the neoplastic epithelium and do not reach the lamina propria and, therefore, melanosis production is inhibited in neoplastic colon lesions. Macrophages are reduced but not missing in adenomas. In adenomas arising in melanotic mucosa a mean value of 14 macrophages per HPF was found in the lamina propria. In colonic mucosa with melanosis coli a mean value of 34 CD68-positive macrophages per HPF was found in the lamina propria. In melanotic mucosa we made the following observations: (i) macrophages permeate blood vessels and reach the lamina propria; (ii) they approach the epithelium and accompany the epithelial cells during their migration to the top of the crypt, thereby taking up apoptotic material; (iii) pigment-laden macrophages migrate through the central space of the lamina propria between the crypts towards the lamina muscularis mucosae, reach the lymph vessels and are finally transported to the lymph nodes.

**Conclusion:** In colonic adenomas the uptake of epithelial apoptotic bodies and the pathway of macrophages seems to be disturbed as the basis of lack of melanosis in these lesions.

## P-225

**IMMUNOCYTOCHEMICAL DEMONSTRATION OF ALFA-FETOPROTEIN SYNTHESIS IN ADENOCARCINOMA OF ILEUM**

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**Aims:** Immunocytochemical (ICC) demonstration of alfa-fetoprotein synthesis in one small bowel adenocarcinoma, in a patient with very high serum levels of this substance, is reported. One only similar case, of ampulla of Vater location, has been reported in the bibliography.

**Methods:** The samples were obtained of an ileum neoplasia and of both ovaries of a 49-year-old woman, they were routinely processed and ICC study was carried out with the below mentioned antibodies.

**Results:** In the ileum there was a polypoid neoformation, of 3x2cm, infiltrating into the fat. The ovaries measured 5x4 and 4x3cm and they were solid, white and hard. Histologically, the ileum neoplasia was compound for a non-infiltrating component, represented by a well differentiated tubulovillous adenocarcinoma, and other infiltrating component with two growth patterns: (i) a moderately differentiated tubular adenocarcinoma, and (ii) solid masses of polygonal cells with plenty clear cytoplasm. Immunocytochemically, all neoplastic cells were positive for keratin and EMA, and negative for PLAP, HCG, vimentin and chromogranin. Non-infiltrating tubulovillous and infiltrating tubular components were positive for CEA and negative for alfa-fetoprotein. On the other hand, the clear cells component was positive for alfa-fetoprotein and negative for CEA. The ovaries showed bilateral metastasises of the ileum lesion with a similar ICC pattern to that of the non-infiltrating tubulovillous and infiltrating tubular components.

**Conclusions:** Alfa-fetoprotein is normally synthesized by the liver, yolk sack and gastrointestinal tract of the human embryo. At neoplastic level, this synthesis can be due to (i) liver cells metaplasia or yolk sack cells metaplasia, or (ii) imitation of the fetal gastrointestinal epithelium in early gestation by the neoplastic cells.

## P-226

HYPERPLASTIC POLYPS, ADENOMATOUS POLYPS, MIXED HYPERPLASTIC/ADENOMATOUS POLYPS AND SERRATED ADENOMAS OF THE COLON.

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AIMS: To define more clearly the relationship between certain polyps preliminary to assessing their biological nature and clinical significance.

METHODS: H.E. sections from 83 selected polyps were reviewed Polyps with high grade dysplasia were excluded. Formalin sections were stained with antibody Mib 1 for Ki 67 and anti body DO 7 for the p53 protein.

RESULTS: Mib 1 staining showed that hyperplastic polyps (HPs) had a basal profileration zone similar to that of normal epithelium, but more intense and expanded a variable distance towards the epithelial surface. In H.E. sections the columnar cells of this zone ferquently showed stratification, hyperchromasia and nuclear atypia thus mimicking mixed adenomatous/hyperplastic polyps. Mib 1 staining of tubular adenomas (TAs), was generally most intense at the surface and involved, with less intensity, the entire length of the glands. Only one true mixed hyperplatic/adenomatous polyp, distinct from HPs with expanded proliferation zones, and three serrated adenomas, defined as resembling a HP in architecture but with an expanded proliferation zone extending to the mucosal surface, were found. Staining for p53 was only rarely positive and then only in small groups of cells. In HPs p53 staining occurred at the base of the glands while in Tas staining was at the surface.

CONCLUSION: True mixed hyperplastic/adenomatous polyps and serrated adenomas are rare. HPs with expanded proliferation zones are quite common and may mimic mixed hyperplastic/adenomatous polyps. Criteria for differentiating HPs with expanded proliferation zones from mixed hyperplastic/adenomatous polyps and from serrated adenomas need to be improved.

## P-227

SUBEPITHELIAL MYOFIBROBLASTS IN MICROSCOPIC COLITIS

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**Aims:** The expression of α-smooth muscle actin (ASMA) is considered to be a marker of activated myofibroblasts. We intend to assess the pattern of subepithelial myofibroblasts in the two types of microscopic colitis , collagenous (CC) and lymphocytic (CL), by their ASMA expression.

**Methods:** Immunohistochemistry for ASMA in colorectal biopsies from patients with histological diagnosis of CL (n=10) and CC (n=10). As controls (CN) we included two groups of 5 patients with normal histology, either with or without symptomatology. In every case only well oriented biopsies (perpendicularly sectioned) were selected and stained. The morphology and number of immunoreactive cells in intercriptal spaces were assessed and scored from 0 to 4.

**Results:** As we expected, all colitis and control specimens contained ASMA positive myofibroblasts in intercriptal spaces. The two control groups did not show any difference and so they were considered together. In CN and CL cases, myofibroblasts showed mainly a spindle phenotype with polar slender prolongations; in contrast, the lamina propria myofibroblasts in CC were frequently stellate-shaped with more complex "dendritic" cytoplasmic extensions, that appeared embedded in the subepithelial collagen band. The mean score values of either group were: CN: 1.5 +/- 0.5, CL: 1.8+/- 0.4 and CC: 2.5 +/- 0.7. There were significant differences only between CC and the two other groups.

**Conclusions:** Subepithelial myofibroblasts are increased in number and more complex in shape in CC with respect to CL and CN. These differences would account for the increment of extracelular matrix production in CC. The pattern of myofibroblastic expression in CL does not differ from CN.

## P-228

**EVOLUTION OF GASTRIC MALT LYMPHOMA AFTER ERADICATION OF HELICOBACTER PYLORI INFECTION**

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**Aims :** 1) to investigate the effect of eradicating Helicobacter pylori (HPy) infection on associated low-grade gastric MALT lymphoma 2) to assess the significance of the detection of B-cell clonality in control gastric biopsies.

**Methods :** Nine patients with primary gastric low-grade MALT lymphoma associated with HPy gastritis were treated to eradicate HPy. Each patient had between 2 and 10 post-treatment biopsy controls, during a median follow-up period of 21 months (5 - 55 months).
In addition to histology in 36/46 biopsies, polymerase chain reaction (PCR) was used to analyse the IgH chain gene rearrangement.

**Results :** Following treatment, all patients became negative for H Py. Initially 8/9 patients showed partial or total regression of lymphoma. Four of them (50%) presented a relapse of MALT lymphoma at subsequent controls, respectively 5, 12, 18 and 36 months later.
By PCR a monoclonal B cell proliferation was found in 20/36 samples. Monoclonality was sometimes identified even in samples with an apparently complete regression of the lymphoid infiltrate. A relapse was diagnosed only when also morphological and not only PCR evidence were found. Two of the patients in total remission after respectively 3 and 5 years of follow-up, were positive at PCR examination in one or more of their control biopsies. Continued follow-up may clarify the significance of this observation.

**Conclusions :** 1) HPy eradication may result in complete or partial regression of lymphoma. 2) Prolonged follow-up is necessary in these cases as late relapse of lymphoma may occur. 3) Search for monoclonality by PCR is a useful tool in the follow-up of patients but results must be correlated with endoscopic and histological lesions.

## P-229

VALUE OF UPPER GASTROINTESTINAL ENDOSCOPIC BIOPSY IN DIAGNOSIS AND STAGING OF CROHN'S DISEASE.

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AIMS: To study the lesions that occur in the upper gastrointestinal tract (esophagus, stomach and duodenum) in patients with Crohn's disease (CD).

METHODS: a group of 45 patients were studied,40 of them with a demonstrated CD in small bowel and five with undetermined colitis. All of them were biopsied in esophagus( when macroscopical lesions were found),antrum, fundus, gastric body and duodenum.Routine stains for gastric biopsies were used in all of them, and in 14 cases we applied inmunoperoxidase techniques for CD3, CD45RO, CD20 and CD68 .

RESULTS:6 patients showed no lesions. 6 showed chronic superficial gastritis, with associated Helicobacter pylorii in 12 of them. 2 cases showed chronic atrophic gastritis with intestinal metaplasia in absence of HP. The lesions found in these 24 patients were not related, pathogenetically, with CD.

In the remanent group of 21 patients we found 7 cases with granulomas,11 with focal gastritis and 3 with acute duodenitis.

Nine of the 14 cases studied inmunohistochemically were focal gastritis. In them, we found a clear predominancy of T lymphocytes with abundant macrophages and only occasional B lymphocytes; this pattern is highly suggestive of CD and clearly different from the pattern observed in chronical superficial gastritis, in which there is a similar proportion between B and T lymphocytes and occasional macrophages.

CONCLUSIONS:the endoscopic biopsy , specially in stomach and esophagus,allow us to demonstrate specific lesions (granulomas and focal gastritis) of the upper intestinal tract in patients with Crohn's disease, in order to identify asimptomatic patients, classified undetermined forms and establish the real staging of the proccess.

## P-230

MICROCYSTIC ADENOMAS OF THE PANCREAS. A CLINICOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY

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**Aims:** Microcystic adenomas are benign tumors of the pancreas accounting for 1-2% of exocrine pancreatic tumors. It has been suggested that myoepithelial cells are present in these tumors. Since the tumor usually affects females, the authors also wanted to see if it has a hormonal background.

**Method:** 25 cases were retrieved from the archives of the 2nd Department of Pathology, Semmelweis University of Medicine. Blocks were available in 17 cases. HE slides were reviewed and a battery of immunohistochemical reactions [pan-cytokeratin, CK20, smooth muscle actin (SMA), S-100 protein, anti-estrogen (ER) and progesterone receptor (PR) proteins] were performed. A follow-up was available in 15 cases.

**Results:** The female:male ratio was 24:1. The average age of the patients was 61.5 years (ranging form 41 to 83). The tumors consisted of cysts of variable sizes, lined by cuboidal epithelium. Between the cysts there was a variable amount of stroma, usually showing hyalinization and myxoid degeneration. In 13 of the 17 cases the epithelial cells expressed CK20, which is not present in the normal pancreas. S-100 and SMA positivity was present on the basis of the epithelial cells or in scattered cells beneath the epithelium in 15 of the 17 cases. Nuclear positivity with ER was found in the epithelium in 12 cases and no reactivity was detected with PR. The stromal elements expressed ER and PR in three cases each.

After tumor resection 14/15 patients with follow-up are alive and well, one died of post-operative complications.

**Conclusions:** Based on the immunohistochemical study the presence of myoepithelial elements or the myoepithelial nature of the neoplastic epithelium is suggested. The expression of ER in the neoplastic epithelium might explain the fact that these tumors occur mostly in females.

## P-231

*HELICOBACTER PYLORI* INFECTION AND THE COURSE OF INFLAMMATORY BOWEL DISEASE

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**Aims:** Factors affecting the clinical course of inflammatory bowel disease (IBD) are largely unknown, although smoking may be associated with more severe Crohn's disease (CD). We have studied whether *H. pylori* infection has influence on the activity or extension of IBD.

**Patients and methods:** We studied 296 adult patients with IBD (females 144; mean age 43 years), including 185 with ulcerative colitis (UC), 94 with CD, and 17 with indeterminate colitis (IC). Smoking and follow-up data were collected from patient files and by interviews. Mean duration of follow-up was 10 years (range 0-35). All histopathological specimens were blindly re-evaluated. Serum samples were studied for *H. pylori* antibodies (Pyloriset-EIA-G, Orion Diagnostica).

**Results:** *H. pylori* seropositivity rate was 30% in UC, 29% in IC and 13% in CD. Rate was not related to medical treatment of IBD or current smoking habits. In UC no significant associations between the disease characateristics and seropositivity were observed. In CD, seronegativity was associated with higher cumulative endoscopic activity ($p=0.013$), trends for histological expansion ($p=0.155$) and increased cumulative histological activity ($p=0.148$), but no significant associations between activity and current smoking were observed.

**Conclusions:** Our results suggest that *H. pylori* positive CD patients have less severe intestinal inflammation. Smoking does not seem to have a significant role.

## P-232

CURRENT DIAGNOSIS OF BARRETT'S ESOPHAGUS: AN ANALYSIS OF 2605 HISTOLOGICALLY CONFIRMED CASES

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**Aims:** To evaluate the quality of diagnosis in case of Barrett's esophagus. It was examined wether: 1) there had been regular pretreatment investigations; 2) characteristic mucosal changes had been recognized by endoscopy; 3) a diagnosis of intraepithelial neoplasia had been made more often than of Barrett's carcinoma; 4) the frequence of early carcinoma.

**Methods:** Endoscopic and associated bioptic reports on 2605 consecutive patients with histologically confirmed Barrett's esophagus were analyzed (age: 63.6±14.6 years; m:f 3.8:1).

**Results:** Endoscopic diagnosis in cases without neoplasia was in 43.2% correct for actual or suspected Barrett's esophagus. At endoscopy dysplasia was suspected in 3.9%. The diagnosis or suspected diagnosis for Barrett's carcinoma was correct in 59.1%. The incidence of Barrett's carcinoma is 1 in 59 patients per year. The frequence of early carcinoma is 37%. Repeat endoscopy was performed in 17.4 % of patient's with Barrett esophagus without dysplasia, in 63.5% of patients with low grade dysplasia, 30.7% of patients with high grade dysplasia.

**Conclusion:** Neoplasia in Barrett's esophagus is found too late. But 37% of all carcinoma cases are early cancers. Only half of the histologically confirmed cases are found by endoscopy and follow-up is not sufficient.

## P-233

### STROMAL CHANGES ASSOCIATED WITH MALIGNANT CHANGE OF COLORECTAL POLYPOID ADENOMAS

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**Aims:** We analysed stromal changes in early polypoid colorectal adenocarcinomas (PCRC) in comparison with benign colorectal polyps.

**Methods:** A retrospective analysis was performed on biopsies of early PCRC (Dukes A, $T_1$=72%, $T_2$=28%). Adenomas with early malignant change (n 25), adenomas (n 11), juvenile polyps (n=11) and polyps connected with rectal prolapse (n=11) were included in the study.

Stromal changes were analysed by immunochemical methods using the following antigens: alpha-smooth muscle actin (α-SMA), von Willebrand factor (vWF), desmin, fibronectin, tenascein, collagen III and IV, laminin, Ki-67, CD68 and reaction with Ulex Europaeus Aglutinin-1 (UEA-1).

**Results:** There were some stromal changes characteristic for early PCRC: The number of myofibroblasts and pericytes was increased. They were arranged in multiple layers around tumor glands. In the myofibroblast cytoplasm, α-SMA but no desmin, was found. The orientation of myofibroblasts, pericytes and smooth muscle cells was irregular. Small vessels were increased in number and their morphology as well as architecture were altered in a characteristic way. The capillaries were arranged around carcinomatous glands in multiple layers and showed characteristic budding in all directions. There was also vWF and UEA-1 reactivity in the extracellular matrix around tumor glands, but the positivity was not found in the same regions. Fibronectin, tenascein and collagen III were increased in the stroma of the invasive tumor margin. Macrophages were increased in the number at the invasive tumor margin. The histological type, differentiation, inflammation and degenerative changes of tumor cells, influenced the intensity of stromal changes.

**Conclusions:** Our results have apparently demonstrated that the stromal changes in the early PCRC were specific. We revealed that the analysis of tumor stroma composition is an important diagnostic procedure in the differentiation of the polypoid colorectal lesions.

## P-234

### PROLIFERATION RATE AND LIGANDO-HISTOCHEMICAL PROPERTIES OF OESOPHAGEAL CARCINOMA

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**Aims:** To investigate the properties of intercellular recognition activities with potential prognostic significance.

**Methods:** Histological sections from tissue blocks of 43 cases with resected oesophageal squamous cell carcinoma were examined. Histological sections were incubated with panel of antibodies, biotinylated and carrier immobilised carbohydrates as well as with galactose-binding biotinylated endogenous lactins: KI-67, anti-galactin-1 and anti-galectin-3 antibodies, histoblood group trisaccharides A, B, H bound to biotinylated polyacrylamid, and biotinylated galectin-1 and galectin-3. The staining intensities of applied probes were qualitatively judged except MIb, which was measured with an image analysing system based upon commercially available basic software. DIAS in addition to numerical parameters such as percentage of positively and negatively stained nuclei structural features were analysed.

**Results:** Limited tumors stages (pT1/T2) comprised 10/43 (23%) and 65% of the carcinoma were excised without any notable lymph node involvement (pN0). The average percentage of tumours with detectable binding sites of histoblood group A, B and H antigens was 60%-65%. The percentage of detectable expression of binding capacities for galectin-1 and -3 as well as the presence of these lectins accounted 56%-84%. The proliferation activity of the tumour cells was high and amounted 75% at average. A statistically significant association of presence of galectin-1 and galectin-3 with the pN stage was seen and higher percentage of binding capacities of histoblood group A in pN0 stages compared to pN+ lymph nodes.

**Conclusion:** The data indicates that binding capacities of galectins are associated with the lymph node involvement of oesophageal carcinoma and therefore are probably of prognostic significance. These date are in agreement with results obtained from analogues examinations in lung carcinoma.

## P-235

### EARLY, INTRAMUCOSAL DIFFUSE CARCINOMAS OF THE STOMACH REPLICATE THE DIFFERENTIATION PROGRAMME OF ANTRAL GASTRIC GLANDS

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Diffuse carcinomas of the stomach are composed of mucin-filled 'signet ring' cells which infiltrate the gastric wall widely. Their diffuse growth pattern and wide dissemination do not suggest any organisation or spatial differentiation pattern. We have analysed the pattern of differentiation in a series of 6 patients who showed multiple small intramucosal diffuse carcinomas of the stomach. Formalin-fixed, paraffin-embedded sections were immunostained for the trefoil peptides TFF1 (pS2) and TFF2 (hSP) using monoclonal antibodies, while TFF1, TFF2 and TFF3 (ITF) mRNA was localised using hybridisation *in situ*. Trefoil peptides showed a remarkable spatial distribution of expression in these small, diffuse tumours: TFF1 mRNA and protein was localised to the superficial cells, while TFF2 peptide was found in the deep part of the intramucosal tumour. However, TFF2 mRNA was found more superficially. Moreover while MUC6 was readily demonstrable in the deep areas, MUC5AC was difficult to demonstrate superficially. This pattern of peptide and, to a lesser extent, mucin gene expression is found in antral gastric glands with TFF1 in the foveolar-pit cells and TFF2 localised in the deeper parts of the gland. Moreover, proliferating cells were seen in the mid-area of the tumours, between the upper TFF1-positive and lower TFF2-positive cells. However, TFF3 mRNA, which is not usually expressed in the normal gastric antrum, was also focally demonstrable, as was MUC2. E-cadherin was not demonstrable. We conclude that early, intramucosal diffuse carcinomas of the stomach replicate the growth pattern of antral glands, with a defined central proliferative zone from which the malignant cells migrate, again in a bi-directional manner, and acquire the differentiation antigens, here the trefoil peptides TFF1, TFF2 and MUC6 in the same manner as the antral gastric gland. The finding of TFF3 mRNA and MUC6, usually expressed in intestinal goblet cells, in these early tumours suggests that 'intestinalisation' of diffuse gastric carcinoma, hitherto considered a late event in their evolution, occurs much earlier in their development.

## P-236

### NM23 H-1 PRODUCT IN COLONIC ADENOCARCINOMA

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**Aim:** As colonic adenocarcinoma represent the second cancer in Egypt, we looked for new prognostic parameters in our population.

**Methods:** The immunohistochemical (IHC) expression of nm23 H-1 was studied in 35 primary colonic adenocarcinoma, in their metastatic deposits and in the adjacent normal colonic mucosa. The expression was correlated with CD44 IHC expression, DNA ploidy and S phase fraction (SPF) evaluated by image cytometry (ICM), patients age and sex, Astler-Coller's staging, tumor type and grade, mitotic figure count (MFC), lympho-vascular (LV) and perineural (PN) invasions and presence or absence of schistosomiasis.

**Results:** Positivity for nm23 H-1 were observed in 23 tumors (65.7%) with 16 out of the 23 cases (69.6%) showing more than 75% positive cells. Metastatic deposits in the lymph nodes (9 cases), liver (3 cases), ovaries (1case) and bone (1case) were negative. 54.3 % of the tumors were aneuploid and had higher SPF compared to diploid ones ($p < 0.001$). Positive CD44 IHC expression was seen in 19 (54.2%). The increased nm23 H-1 expression correlated with the lack of CD44 expression ($p<0.001$), DNA diploid tumors ($p< 0.001$), early staging ($p= 0.02$) and MFC less than 20 ($p=0.05$). Spearman correlation analysis confirmed the above correlations. No correlation has been found between nm23 H-1 and the other variables.

**Conclusion:** Our data are in favor for the non-metastatic role of the nm23 H-1 gene in colonic adenocarcinoma in the Egyptian population. The reduced nm23 H-1 IHC expression associated with increased CD44 expression, advanced clinical staging and DNA aneuploidy status could serve as an additional prognostic markers to select patients for adjuvant treatment.

## P-237

SOLID-PSEUDOPAPILLARY PANCREATIC TUMOR: ITS ORIGIN REVISITED?

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**Aims:** solid-pseudopapillary tumor of the pancreas (SPT) has distinctive morphologic and biologic features but an unclear origin. It is classified among the pancreatic epithelial tumors, but many are reported to be negative for cytokeratin. Also unclear is its neuroendocrine differentiation, its capability to express alpha-1-antitrypsin (AAT) and, in view of the tumor's striking prevalence in women, its relationship with the female genital tract.

**Methods:** to clarify these issues the immunoprofiles of 55 solid-pseudopapillary tumors were defined by applying a battery of antibodies against cytokeratin, vimentin, smooth-muscle actin, CD34, KiM1P, AAT, LeuM1, neuron-specific enolase (NSE), synaptophysin, chromogranin A, tyrosine hydroxylase (TH), alpha-inhibin, calretinin and placental alkaline phosphatase.

**Results:** the most consistent markers with the strongest immunoreactivity were vimentin, AAT and NSE, which were each found in more than 90% of the tumors. Using antigen retrieving immunocytochemical methods cytokeratin was demonstrated in almost 70% of the cases. Synaptophysin was found in 25% of the tumors, while chromogranin was absent and TH was only present in a few tumors. None of the other tested markers were expressed by SPTs.

**Conclusions:** this staining pattern fails to reveal a phenotypical relationship with any of the defined cell lineages of the pancreas. Therefore the hypothesis of an extrapancreatic origin is discussed, which relates the SPTs to primitive genital ridge cells that have come into contact with pancreatic cells during embryogenesis.

## P-238

INFLAMMATORY PSEUDOTUMOR OF MAXILLARY SINUS

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Inflammatory pseudotumor ( IPT ) is a rare benign lesion. It is most often located in the lung and orbit, however its occurrence in the maxillary sinus is unusual. The purpose of this work is to communicate a new case of IPT describing its clinical, microscopic and immunohistochemical characteristics.

A 34 year-old woman showed left hemifacial pain. A coronal CT scan of paranasal sinuses revealed a soft tissue opacity of the left maxillary sinus. Surgically it was removed a semisolid white mass filling the left maxillary sinus.

Microscopically there were tissular fragments of respiratory mucosa massively infiltrated by mesenchimal proliferation of spindle cells with inflammatory reaction. The former were fibroblast-like and myofibroblast, that could be arranged in a characteristic storiform pattern, admixed with collagen , lymphocytes, and plasma cells.

Immunohistochemically muscle-specific actin and vimentin were demonstrated in the cytoplasm of spindle cells, while the immunophenotype of lympfoid population was B cells ( CD-20+ ) and T cells ( CD-43+ ).

Primary IPT of the maxillary sinus is a extremely rare pathological condition of unknow etiology, which usually mimics malignancy both clinially and radiologically. Pathologic study of the case support a possible myofibroblast origin of the lesion.

## P-239

**Immunohistochemical expression of cathepsin D in laryngeal epithelial lesions. Correlation with CD44, p53 and pRb expression and proliferation indices**

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**Introduction:** Clinical studies in several tumour types have shown a strong correlation of cathepsin D (CD) expression and tumour progression. In order to elucidate the role of cathepsin D in laryngeal epithelial lesions we studied the immunohistochemical expression of CD in correlation with the expression of CD44 , p53, pRb and proliferation indices.

**Methods:** Immunohistochemical staining for cathepsin D (clone D13A) was performed in paraffin embedded tissues from 39 invasive squamous cell carcinomas, 13 in situ carcinomas, 35 cases of dysplasia, 10 papillomas and 17 cases of keratosis.

**Results:** Cathepsin D was highly positive in the groups of carcinomas compared to other lesions ($p<0.0001$). A statistically significant correlation of cathepsin D expression with CD44 expression was observed in invasive cancers ($p=0.037$). The relationship of cathepsin D immunoreactivity with p53, Rb and proliferation indices was insignificant.

**Conclusion:** The results show that cathepsin D is expressed in a higher proportion of cancerous lesions of the larynx than in non cancerous or premalignant lesions, fact which suggests that cathepsin D may be involved in laryngeal tumour cell growth process.

## P-240

**MALIGNANT TUMOURS OF THE SINONASAL TRACT**

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**Aims**: Most of the sinonasal malignant tumours are carcinomas, nevertheless there is a misconception about the prevalence of the squamous cell type. To assess the incidence of the different histological types of sinonasal carcinomas we perform this study.

**Methods**: We reviewed the histological and clinicopathological data of 229 sinonasal malignant tumours from the files of Hospital Clínic between 1976 and 1998.

**Results**: Seventy-three percent of malignant sinonasal tumors are carcinomas. However only 45% of them are of squamous cell type. The main group of carcinomas, are of non squamous cell type (55%): 20% are undifferentiated carcinoma, 14% cylindrical cell carcinoma, 12% high grade adenocarcinoma, 8% adenoid cystic carcinoma, 6% low grade adenocarcinoma, 4% adenosquamous carcinoma and 1% myoepithelial carcinoma. Other types are seen with lesser relative frequency.

Nasal cavity is the most common location of these tumours (37,3%) followed by maxillary sinus (34,7%) and ethmoid sinus (24%). Frontal and sphenoidal sinuses are rarely involved.

**Conclusions**: Among de malignant tumours involving the sinonasal tract, carcinomas are the most prevalent. Although squamous cell carcinoma is the most frequent histological type, the high prevalence of the other histological types requires a careful differential diagnosis before therapy is started.

## P-241

THYROID CANCER REGISTRY IN "LJUDEVIT JURAK" CLINICAL DEPARTMENT OF PATHOLOGY AND IT'S CONNECTIONS TO THE NATIONAL REGISTRY OF CANCER

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**Aims:** To connect Hospital Thyroid Cancer registry with National Registry of Cancer and therefore to gain access to follow-up data, that combined with our registry will make possible better patient control and various research studies.

**Methods:** Thyroid Cancer registry was filled with data from biopsy findings after surgical treatment in Univ.Hosp."Sestre Milosrdnice", accompanied with other clinical findings and relevant data from National Registry of Cancer.

**Results:** Department of clinical pathology created Thyroid Diseases registry, including all thyroid patients from 1980 to 1999. There is total of 3944 patients. Of those tumors were found in 1404 cases (35,6 %), benign tumors in 979 cases (24,8 %), and non-tumor changes in 2540 cases (64,4 %) with goiter as most common in 2021 cases (51,2 % of all changes). Thyroid cancer in our registry comes with 425 cases. Of those carcinoma papillare in 257 cases (60,5 %) with female to male ratio of 3,2 to 1. Ca. folliculare in 71 cases (16,7 %) with F:M=2,1:1. Ca.medulare comes with 35 cases (8,2 %) and F:M=1,5:1. Ca. anaplasticum was found in 32 cases (7,5 %) with F:M=1,9:1. Other carcinomas were found in 30 cases. Folowing our intention to make follow-up of our patients, we've connected to National Registry of Cancer and found that 5 year survival for Ca. papilare is 97 %, Ca. folliculare 93 % and for the Ca. medullare 78 %. Long term (15 year) survival as follows: Ca. papillare 91 %, Ca.folliculare 87 % and for Ca. medullare 55 % (both types).

**Conclusion:** Our results are in with correlation with those qouted in literature, analyzing large series and long time peroid studies.

## P-242

**EXPRESSION OF Ep-CAM AND E48 IN LARYNGEAL SQUAMOUS CELL CARCINOMA AND NORMAL TISSUES**

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**Background:** Adhesion molecules participate in cell-to-cell recognition and signaling during proliferation and differentiation processes. The exact roles and patterns of expression of each one are different in different tissues.

**Design:** E48 and Ep-CAM expression was ascertained by immunohistochemistry with two specific monoclonal antibodies upon frozen tissue samples of 13 primary tumors and 7 metastatic laryngeal squamous cell carcinomas, as well as of 13 normal squamous epitelium samples.

**Results:** E48 was detected in all normal squamous epithelium samples, in 12 out of 13 primary and 6 out of 7 metastatic tumors. E48 staining of normal epithelium was diffusse in 7 cases and located in the lower layers of the epithelium in the remaining 6 cases. Glandular epithelium was always negative. Half of the tumors showed a peripheral staining pattern, although 4 were diffusely positive and 2 showed a inverted staining pattern, with negative cells at the periphery of the tumor nests. Ep-CAM was detected in all normal squamous epithelium samples, 12 out of 13 primary tumors and all metastatic tumors. Normal squamous epithelium was positive in the lower cell layers and 9 tumors showed a peripheral staining pattern. Glandular cells showed strong Ep-CAM expression. Metastatic tumors retained the expression pattern of primary tumor in most of the cases.

**Conclusion:** E48 and Ep-CAM expression are frequent phenomenons in squamous cell carcinomas of the larynx, but their possible roles in transformation and progression remain undetermined.

## P-243

A CASE OF PERIPHERAL GRANULAR CELL AMELOBLASTOMA IN SOFT TISSUES.

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Ameloblastoma is the most common type of the epithelial odontogenic tumours, 80% with mandible location. There are also cases of peripheral (extraosseous) setting (2-5%).They arise most commonly in the third to fifth decades, 70% involving the molar-ramus area. While most of them shows a slow growing there are rare exceptions of locally aggressive tumours and ganglionar and lung metastasis are described. Some authors find that the histological subtype may affect the risk of recurrence (like granular cell variant).Extraosseous ameloblastomas appear to affect an older group of patients and also show a greater male preponderance than the intraosseous type.

In this setting we report a case of a 70 years old female with a parotid location tumour with clinical diagnosis of pleomorphic adenoma.

A tumour mass of 8 cm. of diameter was excised. Histological study resemble the more common follicular type but the epithelium, particularly in the centre of the tumour islands, forms sheets of granular cells resembling those of other granular cell tumours, been the diagnosis of peripheral granular cell ameloblastoma.

The electronic and immunohistochemistry study shows the epithelial histogenesis of the tumour and the lisosomic nature of intracitoplasmic granules.

## P-244

**CERVICAL ECTOPIC MENINGIOMA. FNA**

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**Aims:** We describe a case of a 30-year-old woman with a tumor located in the left laterocervical region.

**Methods:** An FNA was performed, that showed a monotonous population of epithelial and spindle cells with nuclear inclussions and grooves and psammoma bodies. The diagnosis of papillary carcinoma of the thyroid was suggested. Following the cytologic diagnosis, isolation of the cervical mass and total thyroidectomy was performed.

**Results**: Histologically, the cervical tumor was composed of lobulated nests of cells with pale and eosinophilic cytoplasm and regular nuclei with pseudoinclusions, arranged in concentric whorls, with occasional psammoma bodies. Special stains showed reactivity for EMA and Vimentin. There was a lack of reactivity for thyroglobulin.

**Conclusions:** Extracranial meningiomas are rare tumors. W hen they arise in ectopic places other differential diagnosis should be consider. Papillary carcinoma of the thyroid can show many of the cytologic features found in meningioma. Our case shows that extracranial meningiomas should also be taken in consideration when psamoma bodies are observed in FNA from the cervical region.

## P-245

AgNOR AND DNA INDEX IN LARYNGEAL CANCER

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Nucleolar organiser regions (AgNOR) and DNA content are considered useful prognostic markers in different tumors. So far only few studies including relatively small number of patients have been undertaken to investigate the relationship of AgNORs and DNA content of as well as their relation to other parameters (such as grade, stage or tumor size) in laryngeal squamous cell cancers (SCC). The aim of this study was to investigate the relation of AgNORs and DNA index of laryngeal SCC to different clinico-pathologic and nuclear morphometric parameters. **Methods.** Samples of tumor tissue from fifty consecutive patients undergoing laryngectomy for SCC were included in the study. After standard procedure consecutive sections were stained with HE, impregnated with silver for AgNOR demonstration and analysed by flow citometry. At the end a control HE slide was produced. Nuclear area as well as AgNOR area and number/nucleus were assessed morphometrically, using interactive semiautomatic image analysis system (VAMS, Zagreb). Obtained results were correlated to tumor grade and TNM stage as well as patient survival. Statistical analysis was performed using Statistica® for Windows 5.0. Kaplan-Mayer and Sperman log-Rank test were performed. Values were considered statistically significant at $p<0.05$. **Results.** Significant positive correlation was obtained for TNM and nuclear area ($p<0.0008$), and number of AgNOR/nucleus ($p<0.006$). Negative correlation was obtained for TNM and AgNOR area/nucleus ($p<0.05$). DNA status showed no correlation with analysed parameters. **Conclusion.** From our results we can conclude that AgNOR seems to be a useful prognostic parameters in laryngeal SCC.

## P-246

**EXPRESSION OF PROLIFERATIVE ANTIGENS (PCNA AND Ki-67), p-53 AND bcl-2 IN THYROID PAPILLARY CARCINOMAS WITH AND WITHOUT METASTASES.**

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**Aims:** The aim of this study was to analyse the expression of proliferative antigens (PCNA and Ki-67), p53 tumour suppressor protein and bcl-2 oncoprotein in localised papillary carcinomas and papillary carcinomas of the thyroid with metastatic involvement of regional lymph nodes.

**Methods:** The study was carried out by immunohistochemistry on paraffin embedded archival material using primary antibodies purchased from DAKO (Glostrup, Denmark). Tissue sections of 10 localised papillary carcinomas of the thyroid and 10 papillary carcinomas with regional lymph node metastases were analysed. The alkaline phosphatase/antialkaline phosphatase (APAAP) method was used. Microwave pre-treatment was performed to improve immunostaining.

**Results:** Positive immunostaining for PCNA and Ki-67 was observed in 6 and 3 cases of papillary carcinoma without metastasis, respectively, p53 in 4 cases and bcl-2 in 3 cases. Six cases of papillary carcinoma and their lymph node metastasis showed positive imunostaining for PCNA. Positive reaction for Ki-67 was observed in one primary tumour and lymph node metastasis in the same patient. Papillary carcinomas with lymph node metastasis showed positive staining for p53 in 4 primary tumours and 6 lymph node metastasis. bcl-2 immunopositivity was found in 7 primary papillary carcinomas and their metastases.

**Conclusions:** We did not observe a significant difference in the expression of PCNA, Ki-67 and p53 between papillary carcinomas without and with metastasis. However, we have found the expression of bcl-2 in the majority of papillary carcinomas with metastasis. Our results suggest that bcl-2 expression in papillary carcinomas might be associated with more aggressive behaviour of these tumours.

## P-247

BASALOID-SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK: FINE NEEDLE ASPIRATION FEATURES.

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**Aims**: Basaloid-squamous cell carcinoma (BSCC), originally described in 1986, is a rare distinctive variant of squamous cell carcinoma (SCC) ocurring mainly in the head and neck areas. Whereas his histologic features have been well defined, his cytologic picture has been only recently and scarcely characterized. Our purpose was to found distinctive features of this kind of tumors in Fine needle aspiration (FNA) specimens.

**Methods**: Five tumors primarily diagnosed as BSCC are reported. Chirurgical specimens and biopsies material were fixed in 10% buffered formalin and paraffin embedded. FNA was performed in primary or metastatic locations. The slides were air dried or 96% ethanol fixed and stained with "Diff-Quick"and Papanicolaou stain respectively.

**Results**: The smears showed a rich background composed by necrotic and diskeratotic cells, fibrina and naked nuclei. A stromal component, with a very tipical appearance, consisting in hyaline cores of magenta colour with a polymorphic and branched appearance. And a poorly conserved cellular component. The malignant cells, isolated, in small three-dimensional clusters or forming acinar structures had small or medium size, scanty and indistinct cytoplasm and moderately pleomorphic nuclei.

**Conclusions**: FNA slides of BSCC may be mistaken with other neoplasms that arise in the upper aerodigestive tract, being very important the differential diagnosis because of prognostic and therapeutical decisions. Then the recognition of his cytologic features can avoid delay in diagnosis and treatment, mainly in head and neck tumors that are readily accesible to either visual inspection or palpation.

## P-248

**ANALYSIS OF NONINVASIVE FUNGAL PARANASAL SINUSITIS**

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**Aim:** To define the pathologic features of noninvasive paranasal fungus infection.

**Methods:** We reviewed all cases of inflammtory sinus managed in our Center from 1994-1998. Of the 127 patients, 6 had fungus ball and 6 had allergic sinusitis (total 12, 0.94%), without invasion by fungal hyphae in the tissue specimen. The patients presented with proptosis, and extensive involvement of the sinuses was found on CT scan. Paranasal tumor was suspected. Cultures were performed in all cases.

**Results**: Fungus ball (mycetoma) occurred in 4 females and 2 males aged 42 to 68 years (mean 60). The sinuses affected were the maxillary (4) and the sphenoid (2). Microscopically, there were dense tangles of hyphae consistent with Aspergillus within necrotic material, with small groups of neutrophils and foci of calcification. Fragments of edematous sinus mucosa contained mixed chronic inflammatory infiltrate, lymphatic follicles with germinal centers, foci of ulceration and metaplastic epithelium. Granulomas were not seen. Allergic sinusitis occurred in 3 females and 3 males aged 15-64 years (mean 35). The sinuses affected were the maxillary (5) and the sphenoid (1). Microscopically, there were abundant basophilic mucin with a laminated appearance that consisted of densely packed bands of eosinophils. Scattered fungal hyphae consistent with Escherohilum were identified within the mucin in 4 cases. Fragments of edematous sinus mucosa contained marked eosinophilic infiltrate.

**Conclusion**: Noninvasive fungal paranasal sinusitis presents with marked symptoms. Biopsy of the sinus mucosa is necessary to rule out fungal infection or tumor. The type of fungus infection (mycetoma, allergic sinusitis) and, in most cases, to identify the etiologic factor, can be identified microscopically.

## P-249

ADENOSQUAMOUS CARCINOMA OF THE UPPER AERODIGESTIVE TRACT. A CLINICOPATHOLOGIC REVIEW OF 6 CASES

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**Aims**: To evaluate the clinico - pathologic features of a series of adenosquamous carcinomas located in the upper aerodigestive tract.

**Methods**: During a 18-year period (1982 - 1998) six adenosquamous carcinomas arising in the upper aerodigestive tract have been diagnosed in our Institution following the current histologic criteria: fairly malignant carcinomas presenting a biphasic pattern of growth with squamous areas on surface and glandular differentiation in depth. The clinical history was evaluated in all the cases. Representative areas of the tumors were stained with Alcian blue, PAS, CAM 5.2, 34 β E12, AE1-AE3, CEA, chromogranin, γ-enolase, and calcitonin.

**Results**: All the cases were males aged between 47 and 71 (mean 59,7 years). Three of them arose in the larynx (2 supraglottic and 1 in the right piriform sinus), and the others in the left maxillary sinus, oral cavity, and esophagus. At diagnosis, lymph node metastases were detected in 2 cases (1 supraglottic and 1 piriform sinus). Long-term clinical follow-up revealed that 2 out of 6 died of disease 19 and 58 months after the diagnosis. The rest remains alive and free of tumor at present (mean: 22 months). The glandular component (tubular/cribiform) showed mucins production. Glanz' score revealed all of them were high grade tumors. Basal-type keratins immunostained the squamous areas and CEA and low-molecular weight keratin the glandular ones. Neuroendocrine markers were consistently negative in all cases.

**Conclusions**: The typical arrangement of both histologic components of this rare neoplasm, together with its characteristic immunostaining pattern, allows its distinction from other neoplasms with lesser malignant potential, e. g., mucoepidermoid carcinoma.

## P-250

THE PROGNOSTIC VALUE OF INDIVIDUAL HISTOLOGIC PARAMETERS IN THE SURVIVAL OF T1 SQUAMOUS CELL CARCINOMAS OF THE LARYNX

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**Aims**: The present paper intends to define the predictive value of the Glanz'score and mitotic index in the survival of T1 squamous cell laryngeal carcinoma.

**Methods**: In the period 1974 - 1992, an amount of 73 laryngeal carcinomas with less than 2 cm in diameter (T1) were diagnosed and surgically treated in our Institution. The prognostic value of everyone of the Glanz' index parameters ( tumor pleomorphism and differentiation, pattern of invasion, intravascular and perineural invasion, and host cellular response ) was evaluated. The mitotic count was done in 10 consecutive high power fields (HPF = x40). Kaplan - Meier survival curves and Cox' regression model were performed.

**Results**: Five years overall survival was 80,4 %. Multivariate analysis demostrated that the pattern of deep invasion (expansive vs. infiltranting) ($p<.017$) and the mitotic index (<20 mitoses/10HPF) ($p<.0008$) obtained independent predictive values of survival. The tumor differentiation (keratinizing vs. non-keratizing) ($p<.052$) nearly reached significant values. Conversely, both vascular invasion and host cellular response were not significant parameters.

**Conclusions**: The present study demonstrates that the pattern of tumor invasion and mitotic count are useful histologic criteria predictive of survival in T1 squamous cell carcinomas of the larynx.

## P-251

BENING MYOEPITHELIOMA OF SALIVARY GLAND: REPORT OF A CASE.

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AIMS: We want to present a case of bening myoepitelioma of parotid gland in a young male.

METHODS: 18 year old man with tumor in parotid gland. The surgeon performed a FNCA to diagnose and then the patient was treated with surgical excision for his tumor.

RESULTS: The cytology showed a monomorphic proliferation of fusocelular cells with excentric nuclei, without pleomorfism and mitotic activity. There was no background substance. The diagnosis was: LOW GRADE FUSOCELULAR NEOPLASM. The histological study of the tumor showed a well delimitated nodule in the salivary gland. It was constituted by fusiform cells (previosly described) within a myxoid stroma, without evidence of chondroid differentation and tubular srtructures. There were no signs of malignancy (mitosis, atypia...).

Inmunohistochemistry results: CAM5.2, PS100 and Vimentin positives; Desmin, SMA, MCA, GFAP negatives.

Final diagnosis:BENING MYOEPITELIOMA.

CONCLUSIONS: Bening myoepitelioma of the salivary gland is a rare tumor (1,5% of oll salivary glands tumors) and it probably represents one end of the spectrum of mixed tumors.

It must be differentiated from malignant tumors (mesenchimal and other primary malignant salivary tumors), and the IHQ helps to make an accurate diagnosis.

Citology can be a good diagnostic method.

Many of these tumors occur in young patients without sex differences. Its biological behauviour is similar to the standard mixed tumors, therefore the treatment should be the same.

## P-252

ELECTRON MICROSCOPIC STUDY OF EXTRAOCULAR MUSCLE ALTERATIONS IN THE PARANEOPLASTIC PHENOMENON ASSOCIATED WITH RETINOBLASTOMA

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**Aims:** A study about the effects of retinoblastoma in non-invaded extraocular muscles and their microvasculature was performed in order to expand the knowledge on the muscle paraneoplastic phenomenon.

**Methods:** Extraocular muscle biopsies were obtained during surgery for retinoblastoma extraction in 4 patients. Samples were processed with routine techniques for transmission electron microscopy and observed in Hitachi H-500 and H-7100 electron microscopes.

**Results:** Muscle fibre atrophy which varied from slight to severe was observed exhibiting loss of myofilaments, disorganization of sarcomeric structure, and presence of nemaline and filamentous bodies. Irregular and hyperchromatic nuclei were seen. Hypercontracted fibres with segmental necrosis were observed. Microvascular changes included endothelial infolding invasion of capillary lumen, occlusion, and endothelial cell degeneration. Pericytes appeared vacuolated in some cases. Capillary basement membrane was usually widened. A mononuclear cell infiltration formed by macrophages located next to and around capillaries and muscle fibres was observed. Cancer cells invading muscle tissue were not seen.

**Conclusions:** This study shows that non-invaded extraocular muscles located around retinoblastomas were severely altered. The histopathological picture was essentially similar to that observed in the paraneoplastic phenomenon in muscles located far from the malignant tumour. Microvascular changes resemble those present in several autoimmune disorders. Our results suggest that circulating tumour factors and immune processes may play roles in the ethiopathogenesis of these muscle alterations.

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## P-253

**IMMUNOHISTOCHEMICAL ANALYSIS OF STROMAL CELLS IN EPITHELIAL HYPERPLASTIC LESIONS AND SQUAMOUS CARCINOMA OF THE LARYNX**

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**Aims:** The aim of our study was to analyze the distribution and immunohistochemical phenotype of stromal cells in epithelial hyperplastic lesions (EHL) and squamous carcinoma (SC) of the larynx and to reveal their possible diagnostic significance.

**Methods:** Samples of surgical specimens of 20 laryngectomies containing transition from EHL to SC were included. Control group consisted of 5 autopsy samples of normal laryngeal mucosa. EHL were classified according to the criteria of the Ljubljana classification (Histopathology 34: 226-34, 1999). Immunohistochemistry was performed using antibodies against vimentin, α-smooth muscle actin and desmin.

**Results:** Stromal cells, if present, were vimentin and α-smooth muscle actin positive, and desmin negative (phenotype VA). Stromal cells were absent in normal mucosa and in EHL. In SC, they were strongly increased in 18/20 cases; the 2 negative cases had little desmoplastic stroma.

**Conclusions:** The stromal cells were detected almost exclusively in the invasive SC indicating that invasion beyond the basement membrane is necessary to evoke a myofibroblastic stromal reaction. The stromal cells were consistently of VA phenotype (vimentin +, actin +, desmin -). Antibodies against α-smooth muscle actin also stained small blood vessels in the stroma demonstrating a gradual increase of the microvessel density as the grade of EHL increased. We conclude that immunohistochemical staining for α-smooth muscle actin enables evaluation of stromal cells and microvessel density providing additional information on phenotypic characteristics of the stroma in EHL and SC of the larynx.

## P-254

EXPRESSION OF Ki67, PCNA, P53 AND cerbB-2 AS DIFFERENTIAL DIAGNOSTIC MARKERS IN BASAL CELL TUMORS OF SALIVARY GLAND.

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**Aims:** Basal cell adenocarcinoma (BCC) and adenoma (BCA) can only be differentiated by the histologically infiltrative growth pattern of the malignant tumour. When working with small pre-operatory biopsies and cytology, this can make it difficult to establish the firm diagnosis. The purpose of this study was to determine if differences between these tumours existed by means of analysis of proliferative activity and c-erbB-2 oncoprotein expression.

**Method:** Immunohistochemical analysis of Ki67, PCNA, P53 and c-erbB-2 on 7 BCC and 6 BCA was performed..

**Results:** PCNA positive nuclei ranged between 14.5% and 52% with a mean of 32.8 %. The staining intensity was strong. In BCA the positivity ranged between 0.5% and 8.7%. The intensity was from moderate to weak In cases of BCC the Ki67 ranged from 9.7% to 45% with a mean of 24.2%. The staining intensity was strong. For BCA Ki67 was negative in 2 cases, reaching 1.5% with a mean of 0.5%. The intensity was weak in all cases. P53 revealed positive staining in all cases of BCC and only in 1 case of BCA. Nuclear immunoreactivity in carcinomas ranged from 1.06% to 63% (mean of 32.7%). The intensity was moderate to weak. The case of BCA showed positivity in 0.25% of tumour cells. Expression of c-erbB-2 was not observed. A significant statistical differences between Ki67 (p=0.003), PCNA (p=0.001) and P53 (p=0.001) for benign and malignant basal cell tumors was observed.

**Conclusions:** The results show that the presence of positive cells for Ki67, PCNA and P53 is higher in malignant than benign basal cell tumors.

## P-255

EPITHELIAL-MYOEPITHELIAL CARCINOMA OF THE LACRIMAL GLAND

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**Aims:** We report the case of a 53-year-old woman who presented a primary epithelial-myoepithelial carcinoma (EMC) of the lacrimal gland with two recurrences over a 15 years period. EMC is a rare salivary gland neoplasm of intercalated duct origin. It is now recognized to be a low grade malignant tumor, prone to local recurrence and may sometimes metastasize. It most commonly occurs in the parotid gland but can also arise in minor salivary glands.

**Methods:** Immunohistochemistry was performed with an automaton (Techmate 500, Dako) using the Dako ChemMate detection Kit.

**Results:** Histologically, the tumor was composed of small ducts with a double cell lining. The inner layer of cells was cuboidal, contained eosinophilic cytoplasm, and small uniform nuclei without conspicuous nucleoli or mitotic activity. These were surrounded by a continuous peripheral layer of spindle cells. Immunohistochemistry: the inner cells showed a strong positivity with anti-cytokeratin 7,18,19,20, anti-CD10, and anti-EMA monoclonal antibodies. The outer layer stained with anti-cytokeratin 7, 20, anti-CD10, anti-vimentine and anti-α smooth muscle actin monoclonal antibodies. Cells were GFAP and desmine negative. The basement membranes stained with anti-IV collagen monoclonal antibodie. Two % of tumoral cells nuclei, mostly in the myoepithelial component were Ki67 positive. Correlations have been shown between solid pattern, nuclear atypia, DNA aneuploidy, high proliferative activity and unfavorable outcome. Our case presented a tubular pattern, a low proliferation index and lacked nuclear atypia.

**Conclusion:** EMC is a neoplasm characterized by a biphasic proliferation of epithelial and myoepithelial cells. The lacrimal gland is a very rare location, with to our knowledge, only one case described before in association whith a pleomorphic adenoma. Our observation confirms the low grade course of EMC in this particular site.

## P-256

**ASSOCIATION OF A VARIETY OF NUMERICAL CHROMOSOMAL ABERRATIONS TO DIFFERENT SITES AND STAGES OF HEAD AND NECK CARCINOMA AS DETECTED BY INTERPHASE CYTOGENETICS**

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**Aims**: Identification of a possible correlation between numerical chromosomal aberrations and different stages or head and neck sites in squamous cell carcinomas.

**Methods**: Isolated interphase cells from paraffin sections of 53 squamous cell carcinomas of the head and neck region (13 oropharyngeal, 7 hypopharyngeal, 33 laryngeal) were investigated by fluorescence in situ hybridization techniques (FISH) with centromeric DNA probes for chromosomes 1, 3, 4, 6, 7, 9, 10, 11, 12, 15, 17, 18, X and Y.

**Results**: An underrepresentation of chromosomes 3 (26%), 6 (17%), 9 (26%), 10 (23%) and 18 (32%) could be revealed as main numerical chromosomal abnormalities. The Y chromosome was lost in 53% of male tumors. The investigated tumor sites and stages of the head and neck squamous cell carcinomas showed obvious differences in the patterns of chromosomal aberration. A loss of chromosomes 3 and 10 was predominantly seen in laryngeal squamous cell carcinomas (39% and 30% of tumors, respectively), the underrepresentation of chromosomes 9 was a frequent event in oropharyngeal squamous cell carcinomas (54%) and a copy number decrease of chromosomes 18 was detected in 31% of oropharyngeal and 57% of hypopharyngeal tumors.

**Conclusions**: Although only a low number of samples were investigated, our results offer evidence for characteristic chromosomal changes in head and neck tumors with regard to different sites and stages.

## P-257

**NODULAR FASCIITIS OF THE TONGUE**

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**Aims :** Presentation of an histological and immunohistochemical study of a rare lesion of the tongue, and a review of the literature.

**Case report :** a 26 year-old healthy man complained of a painless submucosal nodule of the tongue, 1cm in diameter, slightly raised, covered by a depapillated mucosa with a small ulcer. First noticed 2 weeks ago, growing rapidly, the lesion was considered aggressive. A total excision was performed. After 3 years the lesion did not recur.

**Pathology :** The specimen contained a 1.5 x 1 x 1 cm nodule, firm, greyish-white, well-demarcated from the surrounding muscle, bulging under the overlying mucosa. Histology showed that the non encapsulated lesion formed of bundles of spindle cells arranged in a plexiform pattern encroached on the muscular tissue. There was a slight polymorphism. Mitoses were numerous but normal. Degenerated rhabdomyocytes, and inflammatory cells, mostly lymphocytes, were mixed with the spindle cells. Collagen fibres were present and merged in a central hyalinized and almost acellular sclerosis. Spindle cells expressed strongly *vimentin* and *smooth muscle actin*, but not *desmin* which was positive only in the degenerated residual muscle cells. The reaction was positive with *CD68* in nearly 10% of the cells, negative with *MAC'387* and *S100* protein.

**Comment :** these results plead for the oral location of *Nodular Fasciitis* (NF). Head and neck NF is rather common (13 - 20% in literature), but in most cases the lesions develop in subcutaneous tissue. Only 26 cases of oral NF have been reported, of which only 2 cases occured in the tongue. Other locations were the cheek (9), alveolar mucosa (6), alveolar ridge (3), upper lip (3), lower lip (2), and palate (1). In some of these cases the diagnosis had to be discussed with fibrosarcoma. In 2 cases the oral NF presented features of the intravascular type. In every case these lesions of probably reactive nature did not recur after surgical treatment.

**Conclusion :** Pathologists should be aware of the possible oral location of Nodular Fasciitis, to avoid unnecessary overtreatment in certain cases.

## P-258

**HYALINE RING GRANULOMA (PULSE GRANULOMA)**

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**Aims :** Hyaline Ring Granuloma (HRG) is an unusual chronic inflammatory lesion of the jaws (intraosseous) or of the gingiva (extraosseous) characterised by granulation tissue with pale-staining hyaline rings or horseshoe-shaped formations, surrounded and penetrated by multinucleated giant cells, inflammatory cells and connective tissue elements. Its origin is unclear, attributed to inclusion of alimentary particles (Pulse Granuloma), or to a hyaline degeneration of vessel walls (Giant Cell Hyaline Angiopathy). We have investigated the nature of the involved structures by immunohistochemistry.

**Methods :** we have selected in our files 3 recent cases of HRG :

*Case 1* : HRG in the wall of a suppurated radicular cyst developed on a traumatised right central incisor (root canal left open during treatment) in a 15-year-old male.

*Case 2* : HRG in a suppurated periapical granuloma around the roots of the 2nd left upper molar in a 32-year-old man.

*Case 3* : HRG in a painful and suppurated mass of the palatal papilla of the gingiva between the left canine and 1st premolar in a 30-year-old male.

In each case we have stained the paraffin-embedded sections with hematoxylin-eosin (HE) and PAS, examined the slides in polarised light, and applied the vimentin, CD 68 and CD 34 antibodies.

**Results :** The hyaline rings stained like collagen in HE, and were weekly PAS-positive. Within some of them polarised light showed a few birefringent debris. Around and inside the rings, cells were strongly positive with Vimentin and CD 68, and negative with CD 34.

**Conclusion :** These results favour an extrinsic foreign body origin (altered food), at least for some of the cases of HRG.

## P-259

**PROGNOSTIC IMPLICATIONS OF NUMB IMMUNOREACTIVITY IN SALIVARY GLAND CARCINOMAS.**

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**Aims**: to evaluate the expression of the human Numb protein in normal and neoplastic salivary glands.

**Methods**: formalin-fixed, paraffin-embedded samples of normal (6 parotid and 4 submandibular) and neoplastic (28 adenoid cystic and 34 mucoepidermoid carcinomas) salivary glands were immunostained with a polyclonal antibody to Numb, using the EnVision detection system (Dako, Glostrup, Denmark). The results were semiquantitatively evaluated and compared with the clinico-pathological parameters by uni- and multivariate analyses. In addition, one sample each of cryopreserved parotid gland and adenoid cystic carcinoma were subjected to Numb mRNA Northern hybridisation.

**Results**: Numb immunoreactivity was detected in normal salivary (lobar, striated and intercalated) ducts and in a subset of submandibular acinic cells. Diffuse Numb immunoreactivity in more than 50% neopalstic cells was documented in 5 adenoid cystic and 8 mucoepidermoid carcinomas, moderate immunoreactivity (10-50% neoplastic cells) in 14 and 5 cases, and focal positivity in 9 and 21 cases, respectively. Numb immunorectivity was inversely correlated with tumour grade and positively correlated with prolonged disease-free survival by statistical analysis. Also, a coordinated expression of Numb mRNA was documented in the normal parotid gland and adenoid cystic carcinoma.

**Conclusions**: Numb protein may influence cell differentiation in both normal and neoplastic salivary glands and could serve as a prognostic marker in patients with adenoid cystic and mucoepidermoid carcinomas.

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## P-260

**ALTERATIONS IN $p16^{INK4a}$, CYCLIN D1, AND CDK4 MAY COLLABORATE IN LARYNGEAL CARCINOMA PROGRESSION**

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**Aims**: Alterations in the elements that participate in control of $G_1$-S phase transition are frequent among human neoplasms. However, most of these alterations, particularly those belonging to the pRb/CDK4/cyclin D1/$p16^{INK4a}$ pathway, have been described as mutually exclusive in different tumor models.

**Methods**: A series of 34 carcinomas of the larynx was analyzed for CDK4 and cyclin D1 mRNA expression by Northern blot. $p16^{INK4a}$ was analyzed for protein expression (by western blot with anti $p16^{INK4a}$ G175-405, Pharmingen), mutations (by non-isotopic SSCP and sequencing), promoter hypermethylation (by Southern blot with SacII/EcoRI restriction), and homozygous deletions (by multiplex PCR co-amplification of $p16^{INK4a}$ exon 2 along with β-actin exon 4 of DNA obtained from microdissected material).

**Results**: Cyclin D1 overexpression (10/15 vs 2/19) was associated with advanced stage (stage 4), but neither CDK4 overxpression (14/15 vs 12/19) nor $p16^{INK4a}$ alterations (12/15 vs 9/19) were. Overexpression of cyclin D1 and CDK4 were associated. Simultaneous CDK4 overexpression and $p16^{INK4a}$ alterations were associated with advanced stage (12/15 vs 4/19), even among cases without cyclin D1 overexpression (4/5 vs 3/17).

**Conclusions**: Association of simultaneous alterations in members of the CDK4/cyclin D1/$p16^{INK4a}$ pRb regulatory pathway with advanced stage of disease suggests that a proliferative advantage can be obtained from the disregulation of more than a single element of this pathway.

## P-261

PROGNOSTIC FEATURES IN SALIVARY GLAND CARCINOMAS

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**Aims**: A retrospective study in salivary gland carcinomas(SGCs) was carried out to try on proliferation indices and expression of both metallothionein (MT) and Fas-receptor (APO-1, CD 95), as prognosticators.

**Methods**: Formalin fixed paraffin-embedded tissue sections from 32 archival SGCs of different histological types and grades were classified according to the 1991 WHO criteria. Mitotic index (MI) was calculated there and MIB1-labelling index (MIB1-LI) was also assayed by standard immunocytochemistry, comparing them to the incidence of MT and Fas-positive tumor cells.

**Results**: Low, intermediate and high-grade SGCs included 19 mucoepidermoid (ME), 6 adenoid cystic (ADC), 4 acinic cell (AC), 1 papillary-cystic (PCA), 1 in pleomorphic adenoma (inPA), 1 undifferentiated (UD), 11 of which were node positive (high-grade 6:19 MECs; 1:4 ACCs; 1:5 ADCCs, 1:1 CinPA; 1:1 UDC; and intermediate-grade 1:4 ACCs). MI and MIB1-LI were calculated to be on average 0.8 and 2.2, in low-grade SGCs; 7.5 and 11.9, in intermediate-grade SGCs; 38.8 and 56.9, in high-grade SGCs, respectively.
Immunohistochemically detected MT+ cells mostly occurred in the invasive SGCs, showing a strong granular positivity (+++), with cytoplasmic, cell membrane and/or nuclear patterns. This finding was lacking in the low-grade SGCs, while intermediate-grade SGCs contained occasional MT+ cells, with a moderate(++) or weak (+) cytoplasmic immunostaining.
On the other hand, cell-membrane Fas-positive intense reactions were often revealed in both the high- and intermediate-grade SGCs and their nodal recurrences. Statistical analysis by Cox multiple linear regression demonstrated that MI, MIB1-LI, and MT+ cells were positively correlated with one another, and also with the tumor grading, at a highly significant rate ($P < 0.001$). Differently, no reciprocal coherence was found between Fas+ cells and the other individual features.

**Conclusions**: These results show proliferation indices and MT expression to be coherent to pathological grades of SGCs, as independent prognosticators in evaluating the tumor cell dedifferentiation and invasiveness.

## P-262

EXPRESSION OF MASPIN IN SALIVARY GLAND TUMOURS

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Most salivary neoplasms likely develop via a series of consecutive, partly independent, molecular events, one of which may be loss of tumour suppressor (TS) genes. Maspin (mammary serpin) is a newly discovered human TS gene, whose protein product of 42 kDa contains sequence homology with the serine protease inhibitor superfamily (serpins). Maspin has been shown to inhibit tumour motility, invasion, and metastasis in breast carcinoma. It is strongly immunodetectable in the myoepithelial cells surrounding normal breast ducts and acini, and loss of Maspin expression correlates with the progression of breast carcinoma.

The expression of Maspin protein was investigated in normal salivary gland myoepithelial cells, and in a series of 33 salivary gland tumours. Routinely processed sections were incubated with a polyclonal antibody against Maspin (Pharmingen, CA, USA). The myoepithelial cells around ducts and acini in normal salivary gland tissue showed a strong cytoplasmic immunoreactivity, and all 10 pleomorphic adenomas showed equally strong positivity for Maspin. A similar strong positivity for Maspin was observed in all carcinomas (10 adenoid cystic and three mucoepidermoid carcinomas, five polymorphous low grade adenocarcinomas, and two adenocarcinomas. Three cases of acinic cell carcinoma were negative.

Hence, this study did not show any decrease of the expression of Maspin in malignant salivary tumours with myoepithelial participation, nor any deletion of the gene. Although recombinant Maspin has been shown to induce changes in cell surface levels of different integrins on tumour cells, thereby contributing to inhibit the invasive process, Maspin apparantly does not play a major role in the malignant progression of salivary gland tumours.

## P-263

CLEAR CELL ODONTOGENIC CARCINOMA. REPORT OF THREE CASES

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**Aims:** clear cell odontogenic carcinomas (CCOC) and clear cell odontogenic tumors are rare neoplasms of the jaws that histologically may be confused with metastatic carcinoma and salivary gland tumours. Cases of ameloblastoma with clear cell differentiation have been reported. Although these three neoplasms have been independently described, most of pathologists consider these tumours to be the same lesion.
We search for odontogenic tumours with clear cells in our institution since 1968 to 1998 and review the clinical, radiological, histopathological, and inmunohistochemical studies.

**Methods:** we found three CCOC among 222 odontogenic tumours: 50, 62, and 82 year-old females, all of them with lesions confined to the body region of the mandible. Radiological, histopathological and inmunohistochemical studies were achieved

**Results:** bone lesions appear as diffuse multilocular radiolucencies. Microscopically, two patterns are identified: islands of clear cells with surrounding stromal elements, many of them encircled by a narrow zone of hyalinized connective tissue, that suggests an inductive capacity of this tumour, and a pattern of typical ameloblastoma. Inmunohistochemically, both types of tumour cells showed positive expression for various cytokeratins and for epithelial membrane antigen. A radical hemimandibulectomy was performed in all of cases. One patient suffered from multiple recurrences and died 18 months later. The other two are alive, free of disease.

**Conclusions:** we conclude that all of these tumours are malignant and should be referred as clear cell odontogenic carcinoma. They require an aggressive surgical approach.

## P-264

CATHEPSIN B EXPRESSION IN MYOEPITHELIAL CELLS OF SALIVARY GLAND TUMORS

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Cathepsin B (cat B) is a lysosomal cysteine proteinase involved in extracellular matrix degradation which has been associated with tumor progression and prognosis in some human malignancies.

**Aim:** To study the expression of cat B in myoepithelial cells in salivary gland tumors and normal salivary gland tissue.

**Methods:** Expression of cat B was assessed by immunohistochemistry with the polyclonal antibody Cathepsin B, Oncogene (1: 3500) in 23 cases of salivary gland tumors which contained normal tissue (8 adenoid cystic carcinomas, 6 pleomorphic adenomas, 4 epimyoepithelial carcinomas, 3 myoepitheliomas, 1 myoepithelial carcinoma and 1 carcinoma ex-pleomorphic adenoma). Cat B expression in normal and neoplastic myoepithelial cells was semiquantitatively analysed (scored 1-3. 1: less than 10% of positive cells; 2: 10-50% of positive cells and 3: more than 50% of positive cells). The intensity was also evaluated and graded 1 to 3 according to the expression in macrophages.

**Results:** In normal salivary tissue, myoepithelial cells were mainly negative whereas ductal cells expressed Cat B in the apical pole with a granular pattern. Four pleomorphic adenomas showed positivity in less than 10% with a mild to moderate intensity. Two myoepitheliomas were scored as 1 with a mild to moderate intensity. Six adenoid cystic carcinomas were scored as 3 with a moderate intensity, predominantly in the basal pole. All epimyoepithelial carcinomas showed positivity higher than 10% and 2 of them were scored as 3 with a mild to moderate intensity. The only myoepithelial carcinoma showed positivity in less than 5% of cells with a moderate intensity whereas the carcinoma ex-pleomorphic adenoma was scored 3.

**Conclusions:** Myoepithelial cells in normal salivary gland tissues are mainly negative for cat B. Benign salivary gland neoplasms show weak and scarce Cat B expression, whereas myoepitelial cells of malignant salivary gland tumors are more frequently cat B positive with higher intensity. Yet, exception like in one case of myoepithelial carcinoma might indicate a late downregulation of this proteinase during malignant progression. Therefore, further studies with larger series are needed to confirm our results.

## P-265

MIOEPITHELIAL COMPONENT, PROLIFERATIVE ACTIVITY AND P53 EXPRESSION IN MALIGNANT SALIVARY GLAND NEOPLASMS

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**Aims:** Myoepithelial cells (MEC) are found in benign and malignant salivary gland tumours (MSGT). It is not clear if their presence has any prognostic value in clinical outcome of the affected patients. The aim of this study was to investigate the myoepithelial component and to correlate it with proliferative activity of the neoplasm .

**Methods:** To detect the myoepithelial component, 97 MSGT were studied immuhistochemically using monoclonal antibody anti-vimentin, S-100 protein, smooth muscle actine, pan-cytocheratin. The proliferative activity was investigated by immunohistochemical analysis of Ki67 and PCNA proliferation markers. p53 expression was immunohistochemically assessed, too.

**Results:** Immunoreactivity for vimentin was expressed in 70% of the tumours studied, S-100 and actin immunostaining were showed respectively in 60% and 32% of the cases. The patients, whose cancers had positivy staining for at least two myoepithelial markers, had lower desease free and overall actuarial survival rates than those with no myoepithelial markers. Moreover our results show that there is a correlation between the histological grade (low, intermediate, high) and the overexpression of the proliferation markers, although there are some low grade neoplasms showing moderate and high PCNA and Ki67 expression. p53 positivity (from focal to diffuse) was found in 85% of all cases.

**Conclusions:** Our findings suggest that: a) myoepithelial component is very frequently immunophenotipically detectable in MSGT; b) the presence of more differentiated MECs in MSGT appears to be related with a poor prognosis; c) PCNA and Ki67 can be useful adjunctive markers of prognosis sometime independent from histological grade, tumour size and evolvement of regional lymphonodes; d) p53 can play a role in the oncogenesis of salivary gland tumours and its presence appears to be related to a more aggressive behaviour of the neoplasm.

## P-266

UNDIFFERENTIETED CARCINOMA ASSOCIATED WITH ADENOID CYSTIC CARCINOMA OF THE TONGUE.

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Intoduction: Adenoid cystic carcinoma (ACC) is the fifth most common malignant epithelial tumor of the salivary glands and constitutes about 7.5 percent of all epithelial malignancies. The parotid gland, submandibular gland, and palate, in this order, are the sites of most frequent occurrence. We describe a tumor of the tongue composite of a usual adenoid cystic carcinoma and an undifferentiated carcinoma large cell type.

Case report: A 61-year-old man presented with a rapidly growing mass, 3 cm in diameter, located on the ventral tongue, covered by non-ulcerated mucosa. Histopathological examination of the tissue showed the presence of a classic ACC with a predominant cribriform pattern and areas with a tubular pattern. Some tumor lobules were seen in close proximity to areas of an undifferentiated carcinoma of large cell type. The relative proportion of the two components was roughly equal. At the present time, after 5 years of follow-up, the patient is alive without sign of recurrent disease.

Discussion: To our knowledge, no similar cases have been reported in the literature thus far, but a specifically differentiated salivary gland carcinoma in association with undifferentiated carcinoma, has been describe in other tumors of the salivary glands and in other anatomic sites, specially the parotid gland. Treatment of these tumors must be dictated by the undifferentiated carcinoma component and radical resection and eletive neck dissection should be performed. In summary, pathologist should be aware of the potential for ACC like for others salivary glands tumors, to harbor areas of high-grade carcinoma.

## P-267

PATTERNS OF CHROMOSOMAL ALTERATIONS IN LARYNX AND PHARYNX CARCINOMAS BY COMPARATIVE GENOMIC HYBRIDIZATION (CGH)

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Although considerable knowledge exists on clinical-pathologic, cytometric and genetic characteristics of larynx-pharynx carcinomas (LPC), still little is known about the relationship with tumor progression.

DNA was extracted from frozen tissue of 16 LPC, and analysed by comparative genetic hybridization (CGH). Results were related to DNA ploidy, tumor localization and pTNM data.

A high number of gains and losses was found: on average 8.3 and 5.4 per case, respectively. The highest number of gains were observed at 3q (13 cases), 8q (10 cases), 7q and 1q (7 cases), 17q and 2q (6 cases). Recurrent losses occurred at 3p (8 cases), 4q, 18q and 21q (5 cases). High level amplifications were found at 3q26-27 (5 cases), 11q13 (3 cases), 11q22 and 18q11 (2 cases), 7p22, 8q24, 13q34 and 22q11 (1 case).

We found no differences in the average number or location of chromosomal changes in relation to DNA ploidy, tumor localization and pTNM stage. However, nearly all high level amplifications were displayed in larynx cases.

We can conclude that chromosome 3 (3q gain and 3p loss) could have a high importance in the LPC progression. More cases are being investigated and the genetic alterations will be studied in relation to clinical follow-up data.

## P-268

LARYNGEAL SARCOMATOID CARCINOMA: CLINICAL AND PATHOLOGICAL REPORT

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**Aims**: To asses our achievements in the treatment of this rare biphasic variety of squamous carcinoma to which, in general, a better prognosis is attributed than to the conventional form and establish relation between the clinical evolution and morphological findings.

**Methods**: Clinical revision (treatment, evolution) and pathological study (proportion of components, immunohistochemical study) of all the cases in the period 1976-98.

**Results**: Ten cases. M/F 10/0. Mean age 64 years (43-74) Localization: Glottis (G) seven, Supraglottis (SG) three. Clinical stage: T1 two G; T2 six, G three and SG three; T3 two G.

Morphology: 8 of polypoid shape. Size: max. 7 cm, min. 2.2 cm. Squamous carcinoma component in all, low grade in 8. Sarcomatoid component in all; predominant (75% or more of the tumor) in 7; High grade in 8. Vimentin positive in all, keratin w.s. positive in 4. Mucosa adjacent to the tumor: dysplastic in 4, squamous carcinoma in 4.

Therapy: Radical surgery (laryngectomy) in 3 cases: two T3 G, one T2 SG. Good evolution in all. Conservative surgery in 4 cases: two T2 G, two T2 SG . Evolution: good in 2, regrowth and death (because of the tumor) in 2. Radiotherapy in 3 cases: two T1 G, one T2 G. Incomplete response in all, second treatment: radical surgery in two, conservative surgery in one, good outcome in all three.

**Conclusions**: 1- Neither the proportion of the components (squamous/sarcomatoid) nor their grade had any relation to the response to the treatment. 2- Local control of the tumor was not achieved with radiotherapy. The results of conservative surgery were inferior to those obtained in conventional squamous carcinoma. 3- The best prognosis seems to depend on the polypoid growth which allows diagnosis and treatment at an early stage.

## P-269

**EXPRESSION OF P-53 AND RETINOBLASTOMA SUPRESSOR GENES IN LARYNGEAL CARCINOMAS**

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Tumour suppressor genes control cell proliferation by means of the suppression of specific mechanisms which stimulate the proliferative response, being Rb and P-53, both nuclear antigens, the most studied.

We have evaluated the individual immunoexpression of both markers in 72 carcinomas of the larynx, also making a correlation with classical prognostic parameters.

P-53 was positive in 56% of cases with a mean staining of 40%. There was no significant correlation with the prognostic parameters, but this marker was frequently detected in dysplastic and incipient neoplastic lesions.

Rb gene was positive in 87% of the cases with a mean staining of 43%. There were significant differences concerning the histological grade (p=0,0001), as well as the existence of tumoural adenopathies (p=0, 0113).

P-53 and Rb suppressor genes expression is a frequent event in laryngeal carcinomas.

The relationship of Rb expression with tumour grade and the existence of lymph node metastases could reflect a failed attempt to control the mechanisms of proliferation, given its cell cycle regulatory function.

## P-270

**TUMOR ANGIOGENESIS EVALUATION AS A NEW PROGNOSTIC FACTOR IN NASOPHARYNGEAL CARCINOMA PATIENTS**

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**Aims:**Several previous studies have demonstrated that tumor angiogenesis (TA) is essential for the growth of solid tumors, including head and neck cancer. Morover, several observations have proposed that intratumoral microvessel density (IMD) is associated with overall survival. The aim of this study was to determine the possible prognostic significance of tumor angiogenesis in nasopharyngeal carcinoma (NPC) patients.

**Methods:** 54 patients with NPC morphologically diagnosed at the University Hospital La Fe were evaluated for TA in relation to survival. Endothelial cells were immunohistochemically stained with anti-von Willebrand factor (F-VIII) and microvessels counted in the most active areas of neovascularization or *hot spots*. Microvessels were manually counted using a ligth microscope in a 200x field (0.7386 $mm^2$), and later automatically using an image analyzer in a 250x field (0.5028 $mm^2$). Additionally, we analyzed other angiogenic parameters such as perimeter, roundness, and relative area of the microvessels.

**Results:** The mean values for the IMD were 44 and 37 for manual and automatic counting, respectively. We ordered all samples with respect to their vessel numbers, from the lowest to the highest. We, then assigned as a cut off point the number of microvessels include in the sample, from which 75% of sample values were belof thereof. We chose this method to differentiate cases with a low and high angiogenesis. Both methods were correlated with a statistical significance between (IMD) and overall survival, either using manual (p= 0.0141) or automatic counting (p=0.0117). Additionally, cases over *cut off* point demonstrated a prognostic significance in relation with the relative area (p=0.0072).

**Conclusions:** TA measure may be another prognostic indicator that can be used in junction with other factors in order to achieve a more reliable evaluation in NPC patients.

## P-271

**Apoptosis in nasopharyngeal carcinoma as related to histopathological characteristics and clinical stage**

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***Aim:*** We investigated the significance of apoptosis, using the terminal deoxynucleotidyl transferase mediated dUTP-digoxigenin nick end labelling method, in nasopharyngeal carcinoma biopsy samples.

***Methods:*** The apoptotic index (AI) in 50 nasopharyngeal carcinomas was compared with various histopathological features and clinical stage. Also, the AI was correlated with p53, bcl-2 and Ki-67 expression by immunohistochemistry.

***Result:*** In histopathological studies, the AI was significantly higher in mixed cellular type (MC) than in keratizing squamous cell type (KS) and spindle cell type (SC) ($P<0.001$) which worsens prognosis. In tumor stage analyses, AI was higher in early stage (stage 2 and 3) than in high stage (stage 4). In addition, there was a significant correlation between the AI and p53 expression ($P<0.001$) but not with proliferative activity ($P=0.15$). in NPC containing p53 protein positive tumour cells, there was a significantly higher apoptotic rate.

***Conclusions:*** these findings indicate that apoptosis is related to type and stage of nasopharyngeal carcinoma. They also confirm the role of p53 in regulating tumour apoptosis.

## P-272

**Mandibular leiomyosarcomas**

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Leiomyosarcomas are uncommon in the head and neck region. The rarity of smooth muscle cells in the oral cavity makes leiomyosarcoma as a tumor occurring in the mandible extremely rare. All the knowledge obtained with regard to the treatment of this malignant tumor in the jaws is derived from case reports in the literature. Since the first description of Carmody in 1944 less than 20 primary mandibular leiomyosarcomas have been published in the medical literature.

We report a case of a 10 year old boy with a leiomyosarcoma of the left mandible.

The primary tumor involved the mandibular bone and gingiva. There were no indications for lymph node metastasis. The patient had a slowly enlarging, ulcerated painless mass in the left mandible. Biopsy showed spindled-shaped cells with eosinophilic cytoplasm and elongated, blunt ended nuclei arranged in fascicles. Immunohistochemical examination in combination with histological characteristics led to the diagnosis of mandibular leiomyosarcoma.

A case in which the dilemmas encountered in therapy is presented with a review of all reported mandibular leiomyosarcomas.

## P-273

AN UNUSUAL EXTRAMEDULLARY LEUKEMIA OF THE THYROID GLAND

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Extramedullary leukemia (EML) also known as granulocytic sarcoma (GS) or chloroma, is an uncommon tumor composed of myeloblasts and myeloid precursors, that may occur most frequently in acute and chronic myelogenous leukemia, and rarely in myelodysplastic disorders. Most sites of occurrence reported are bone, periosteum, soft tissue, lymph node, skin and cervix.

We report the case of a 78-year old female who presented a left subdigastric node and a thyroid isthmic nodule. The patient had a history of refractory anemia with excess of blasts (RAEB) treated by Danazol for 7 months. The diagnosis of EML was first suggested by a fine needle aspiration of thyroid nodule on the existence of some few immature myeloid cells. This diagnostic was confirmed on histological sections of excised isthmic nodule and lymph node, and by immunohistochemical stains. The cells in both lymph node and thyroid nodule were stained by CD43, CD68, CD15, CD45 and CD34, but negative with CD20 and CD3.

The patient remains stable with normal peripheral blood, without any therapy seven months after the initial diagnosis.

This is the first case of extramedullary leukemia of thyroid gland associated with RAEB. The cytologic features of EML are indicative of the correct diagnosis.

## P-274

THE MI15 MONOCLONAL ANTIBODY (ANTI - SYNDECAN - 1) IS A RELIABLE MARKER FOR QUANTIFYING PLASMA CELLS IN PARAFFIN-EMBEDDED BONE MARROW BIOPSIES.

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**Aims:** Plasmocyte selective mAbs recognizing syndecan-1 have recently been described (CD138). Using the mAb MI15, we investigated the expression of syndecan-1 in routinely paraffin-embedded tissues.

**Methods:** Immunohistochemistry was performed with an automaton (Techmate 500, Dako) using the Dako ChemMate detection kit.The MI15 mAb was used at 5mg/ml concentration.

**Results:** Non-tumoral lymph nodes (25) and bone marrow (BM) biopsies (63) showed strong membrane staining of plasma cells only, allowing accurate analysis of the nuclear structure. The percentages of plasma cells calculated in BM biopsies after MI15 staining were respectively 2.1% (range 1-4%) in normal BMs, 8.5%, (range 5-17) in reactive plasmocytosis and 4.6% in MGUS patients (range 1-13), slightly higher than those obtained on smears or on HE stained sections. In multiple myeloma (40), all plasma cell types were marked and MI15 mAb gave additional information in 8/40 (20%) patients. In lymph nodes, MI15 mAb reacted with Reed Sternberg cells of classical Hodgkin's disease in 23/31 cases (74%) with variable intensity. In contrast, nodular lymphocyte predominance Hodgkin's disease (10), most B cell lymphomas (88/107) and all T cell lymphomas (30) were negative. In B cell lymphomas, plasmocytomas (8), plasmocytic lymphomas (2) and 5/13 cases of immunoblastic lymphoma with plasmocytoid differentiation were stained. In lymphoplasmocytoid lymphomas (4 lymph nodes and 20 BM biopsies), only mature plasma cells were positive. Moreover, a wide distribution of syndecan-1 was observed in normal and tumoral epithelial tissues.

**Conclusions:** MI15 mAb appears to be a reliable marker for identifying and quantifying normal and tumoral plasma cells in paraffin-embedded BM and lymph node samples.

## P-275

**LOW GRADE NON-HODGKING LYMPHOMAS: PROGNOSIS FACTORS AND BEHAVIOR IN 118 CASES.**

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**Aims:** To class 118 patients (1987-1997) of low grade non-Hodgkin lymphomas (NHL) according to REAL classification. Immunohistochemical (IH) and clinical prognostic factors were analysed.

**Methods:** Examining H-E stained slides we chose the most representative paraffin blocks to IH semiquantitative study using p53, Ki 67, bcl-2 and cicline D1. These dates and biological characteristics of International Prognostic Index (IPI) and related clinical factors were correlated with overall survival (OS) and relapse-free survival (RFS).

**Resuts:** Cases were classified in CLL/ small lymphocitye lymphoma (32), lymphoplasmocitoid lymphoma (4), mantle cell lymphoma (16), follicle center lymphoma (44), marginal zone B cell lymphoma MALT type (26: 23 in gastrointestinal tract, 1 in parotid gland and 2 in eyelids) and splenic marginal zone lymphoma (2). Two more cases were unclassifiable. bcl-2 was positive in 92.3 % cases with independence of the histologycal type. Cicline D1 was positive in 65% of mantle cell lymphoma. p53 was observed in 22.5 % cases and Ki67 was observed in 57.6% cases. OS was 120 months and RFS was 45 months. Only age> 65 year-old, systemic symptoms, β2 microglobulin >2.5 mg/l, creatinine > 1.2 mg/dl, VSG>25mm/h, and p53 and Ki67 positivity >50% of neoplastic cells were associated with a shorter OS, while creatinine >1.2mg/dl and splenomegaly > 3 cm were associated with smaller RFS.

**Conclusions:** Similar to others series expression of p53 and Ki67 in > 50% of the neoplastic cells were associated with a shorter OS. Expression of cicline D1 was observed in mantle cell lymphoma and it has no prognostic incidence. All follicle center lymphomas were positive to bcl-2 protein and also in 87.8 % of the other histological types. There was correlation between OS/RFS and some IPI and clinical factors.

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## P-276

NUCLEOLAR ORGANIZER REGIONS QUANTIFICATION IN MEGAKARIOCYTES OF BONE MARROW BIOPSIES FROM PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISEASES

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**Aims**: estimate the number of NORs in the megakaryocytic nuclei in bone marrow biopsies from patients with CMPD.

**Methods**: we have studied bone marrow biopsies from 10 patients with CML-common type, 10 patients with CML-megakariocytic increase, 10 patients with CML-megakaryocytic predominance, 6 patients with PV, 5 patients with PTH and 5 patients with CMGM; three micrometer thick paraffin sections were mounted on silani slides, immersed in sodium citrate and microwave-treated for 3x5 minutes; after silver stainine, we have counted the AgNORs in 50 megakaryocytic nuclei for each case.

**Results**: at the microscopic level we have identified three dimensions for AgNOR: big, medium, small; the median number per nucleus was:

| | | | |
|---|---|---|---|
| CML-CT: | 0,712(big) | 4,268(medium) | 9,028(small) |
| CML-MI: | 0,723(big) | 4,822(medium) | 9,623(small) |
| CML-MP: | 0,822(big) | 5, 232(medium) | 10,646(small) |
| PV: | 1,250(big) | 10,437(medium) | 18,687(small) |
| PTH: | 1,432(big) | 12,416(medium) | 19,820(small) |
| MGMC: | 1,040(big) | 6,928(medium) | 9.948(small) |

**Conclusions**: our microwave technic permit a good visualization of AgNORs: the number of AgNORs may be related to the proliferative activity of megakariocytes.

## P-277

BONE MARROW FIBRIN-RING ("DOUGHNUT") GRANULOMAS (FRG) AND PERIPHERAL T CELL LYMPHOMA : A CASE REPORT.

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Fibrin-ring ("doughnut") granulomas (FRG), with characteristic polymorphic histiocyte reaction, centered by a lipidic vacuole, and peripheral fibrinoid material, were first described in the liver of Q-fever patients. FRG granulomas have been occasionally observed in the bone marrow of patients suffering Q-fever, typhoid fever, infectious mononucleosis, mycobacterium avium intracellulare infection and Hodgkin disease. We herein report a case of non-Hodgkin lymphoma (peripheral T cell type) with bone marrow FRG, an association not previously published, to our knowledge.

A 69 year-old male patient had B symptoms, multiple lymphadenopathies and splenomegalia. A peripheral T cell lymphoma diagnosis, with epithelioid component, was made on an axillary lymph node biopsy. Paratrabecular and central lymphoid nodules were observed in the bone marrow biopsy, with a predominant T immunophenotype (CD3+ and CD43+). In addition, scattered FRG were observed. After treatment, a follow-up biopsy performed 15 months after the initial diagnosis revealed FRG lesions, without remaining lymphoid nodes.

The clinicopathological features observed in this case support the non-specific nature of FRG lesions.

## P-278

USEFULNESS OF THE LYMPH NODE IMPRINT AT THE INITIAL DIAGNOSIS OF LYMPHADENOPATHIC SYNDROMES. CONCORDANCE DEGREE WITH DEFINITIVE HISTOLOGICAL DIAGNOSIS.

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**Introduction**: The aim of this study is to compare the approxiinate diagnosis of lymphadenopathic syndromes by lyinph node imprint (LNI) with the definitivo diagnosis obtained by histological examination of excised lytnph node. We analyzed the concordance of results between both procederes and the usefulness of lymph node imprints.

**Method**: Approximate diagnosis by LNI in sixty-four lymph node biopsies perforined in our institution from 1991 to 1996, were retrospectively analyzed. The study comprised 33 males and 31 females, mean age 49 years and range 380 years. May-Grünwald/Giemsa stain was used in all imprints.

**Results:** Overall concordance between both diagnostic procederes was 7 1 % (45/64). According to different final diagnosis, concordance was: 1) Absolute in Hodgkin's disease (515, 100%) and high-grade malignancy non-Hodgkin's lymphomas (Working Forinulation classification) (8/8); 2) High in low-grade malignancy non-Hodgkin's lymphomas (9/11, 82%), intermediate-grade malignancy non-Hodgkin's lymphomas (8/10, 80%) and infiltration of nodes by metastatic malignant cells (3/4, 75%); 3) Acceptable in non-specific chronic lymphadenitis (1 1/ 1 6, 69%).; 4) Low in tuberculous lyinphadenitis (1 /3, 3 3 %) and null in rare, low-incidence lymphadenopathic processes.

**Discussion:** In our experience, LNI are very useful in Hodgkin's disease, non-Hodgkin's lymphomas (mainly high-grade malignancy) and node metastases, and acceptable in chronic non-specific lymphadenitis. However, LNI gives very little information in tuberculous lymphadenopaty and proves to be null in rare, low-incidence lymphoproliferative syndromes.

## P-279

PROGNOSTIC IMPACT OF EPSTEIN - BARR VIRUS EXPRESSION IN REED - STERNBERG CELLS OF HODGKIN'S DISEASE.

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**Aims:** The expression of Epstein - Barr virus genome and LMP1 EBV in Reed - Sternberg cells of Hodgkin's disease in adults and their prognostic impact on survival were studied.

**Methods:** 100 cases of Hodgkin's disease from two Croatian regions were examined using EBER PNA in situ hybridisation technique and LMP1 EBV immunohistochemistry. Clinical data of patients' complete remission rate, disease free survival and overal survival rate in ten years period were retrieved from the hospital list.

**Results**: According to Rye convention, 8% patients were of lymphocyte predominant (LP), 48% of nodular sclerosis (NS), 37% of mixed cellularity (MC) and 7% of lymphocyte depletion (LP) subtype. Overal survival was 73%. Positive EBER expression was found in 28/100 (28%) and positive LMP1 EBV in 26/100 (26%) cases, respectively. Negative expression of EBER in NS subtype was higher than in other subtypes ($p=0.04$). Subtype MC was not significantly connected with EBV ($p=0.22$). Complete remission rate were lower among EBER negative patients who had subtype NS ($p=0.01$) and LD ($p=0.056$). Disease free survival was connected with positive expression of LMP1 EBV ($p= 0.023$) and complete remission rate ($p<0.0001$).

**Conclusions**: The expression of EBV in Reed - Sternberg cells of immunocompetent patients with Hodgkin's disease has positive impact on disease free survival and complete remission rate.

## P-280

GAIN OF CHROMOSOME 7 THAT MARKS THE PROGRESSION FROM INDOLENT TO AGGRESSIVE FOLLICLE CENTRE LYMPHOMAS IS RESTRICTED TO THE B-LYMPHOID CELL LINEAGE.

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**Aims:** In a previous study using fluorescence in situ (FISH) analysis of interphase nuclei, we found that gain of chromosome 7 occured in almost all cases of high grade follicle cell lymphomas (FCLs grade III) and diffuse large B-cell lymphomas (DLBCLs) while this chromosomal abnormality was uncommon in low grade FCLs (grade I-II). Interestingly, we often found gain of chromosome 7 in bone marrow (BM) cell nuclei even though malignant tumor cells could not be morphologically identified in the corresponding cell smears. This raised the question wether the gain of chromosome 7 was really confined to the B-lymphoid tumor cells or if other cell lineages were also clonally involved.

**Methods:** In the present study, we thus applied FISH in combination with immunomarkers and morphology on BM smears and lymph node imprints from seven patients with high grade FCLs and DLBCLs.

**Results:** Three of the seven BM samples were found to contain clonal CD 20 positive B-lymphoid cells (range; 0.4-96% of the cells) and in none of the cases did we detect extra copies of chromosome 7 in the myelomonocytoid or erythroid precursor cells nor in the CD 3 positive T-lymphocytes. All seven patients demonstrated gain of chromosome 7 in the lymph nodes and this cytogenetic abnormality was also restricted to the CD 20 positive cells (range; 0.7-80% of the cells).

**Conclusion:** The present findings thus confirm that the gain of chromosome 7 in these high grade lymphomas is confined to the B-lymphoid tumor cells. Our results suggest that lymphotoxic agents either alone or in combination with CD 20 antibodies should be used in the treatment of high grade B-cell lymphomas irrespective of BM engagement.

## P-281

IMMUNOPHENOTYPIC MODULATION OF HODGKIN AND REED-STERNBERG CELLS BY EPSTEIN-BARR VIRUS

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**Aims:** To investigate the role of Epstein-Barr Virus (EBV) in pathogenesis of Hodgkin's disease (HD) we analysed immunophenotypic modulation of Hodgkin and Reed-Sternberg (H&R-S) cells made by this virus.

**Material:** Lymph nodes from 66 cases of HD and 10 reactive, non-neoplastic lymph nodes.

**Methods:** The expression of antigenes and/or direct products of EBV genetic material transcription in H&R-S cell was detected by immunohistochemical (IHC) method (with use of monoclonal antibodies to LMP-1) and by in situ hybridisation (ISH) method (with use of florescein-marked oligonucleotides complementary to EBERs). The immunophenotypic modulation of H&R-S cells was assessed by expression of CD30, CD15, CD74, CD45, CD 20, CD45RA, CD3, CD45RO, EMA and PCNA. We compared expression of these antigenes between groups of EBV-positive and EBV-negative cases of HD.

**Results:** Expression of CD30 was significanty higher in EBV-positive (assessed both by IHC and ISH) H&R-S cells. In the group of LMP-1-positive cases of HD, expression of CD45 in H&R-S cells was significantly lower. In the group of ISH-positive cases of HD, we found higher expression of CD20. In the group of EBV-positive (assessed both by IHC and ISH) cases of NS type HD, expression of CD15 and CD20 in H&R-S cells was significantly higher, and expression of EMA was lower In the MC-type of HD we found lower expression of CD45 in LMP-positive cases and CD45RO in ISH-positive cases. We didn't find any differences in expression of CD74, CD3, CD45RA and PCNA.

**Conclusions:** Though the precise mechanism of immunophenotypic modulation of H&R-S cells in not known yet, the results of our studies suggest the active role of virus itself in this process. The probable aim of the modulation is to prevent immunologic selection and/or to increase proliferation rate of infected cells.

## P-282

**NK/T-CELL LYMPHOMAS – THE LINEAGE AND COMPARATIVE GENOMIC HYBRIDIZATION STUDY-**

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**Aims:** The purpose of this study is to investigate the lineage and genetic changes of NK/T-cell lymphomas.

**Methods:** Forty-two cases of NK/T-cell lymphomas including twenty-six nasal(and nasopharyngeal) NK/T-cell lymphomas, fourteen nasal type NK/T-cell lymphomas, and two blastic NK-cell lymphomas were studied. Immunohistochemical staining using antibodies for pCD3, CD20, CD56, TIA-1, TdT, and CD30 was done on paraffin-embedded tissue. PCR assay for TCR-γ gene rearrangement and EBV in situ hybridization study were performed. In five cases of which fresh frozen tissue was available, comparative genomic hybridization study was done. The LOH analysis of chromosome arm 6q was performed by PCR-amplification of microsatellite sequence using informative five markers.

**Results:** Clonal TCR-γ gene rearrangement was detected in 3 cases out of 29 nasal and nasal type NK/T-cell lymphomas(10.4%). Twenty-eight of thirty-seven nasal and nasal type NK/T-cell lymphomas (76%) was positive for EBV , in which nasal and nasopharyngeal tumor showed higher positive rate(91%) than tumors arising in the oral cavity and oropharynx(56%). Two cases of blastic NK-cell lymphomas were negative for EBV. Chromosomal imbalance was noted in all 5 cases. High level amplifications were detected in 22q12-13 of blastic NK-cell lymphoma. Common loss of 1p31-ter and 12q24-24.3 and common gain of 21q were detected. No 6q abnormality reported in NK/T-cell lymphoma was seen by LOH analysis .

**Conclusion:** Based on these results, we concluded that about 90% of NK/T-cell lymphomas in Korea belong to NK-lineage and share common immunophenotypic and viral profile with those of other Asian countries. Common chromosomal imbalances are noted irrespective of clinicopathologic subgroups of NK/T-cell lymphoma and these changes might be associated with tumor progression.

## P-283

**PROGNOSTIC SIGNIFICANCE OF RETINOBLASTOMA GENE AND P21 (WAF1/CIP1) EXPRESSION IN NON-HODGKIN'S LYMPHOMAS**

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**Aims:** To investigate whether the expression of two negative regulators of the cell cycle, namely retinoblastoma gene product (pRb) and WAF1/Cip1 gene product (p21), is related to clinicopathological parameters, proliferative fraction, p53 expression and survival in non-Hodgkin's lymphomas (NHL).

**Methods:** Paraffin sections from 93 patients with NHL (mean follow-up 48 months) were stained immunohistochemically with antibodies to pRb (PMG 3-245), p21 (4D10), PCNA (PC10), Ki-67 (MIB1)and p53 (DO1).

**Results:** Rb labelling index (LI) increased with malignancy grade and proliferative activity but was unrelated to other clinicopahtological parameters. In 33% of cases, especially those of the high grade category, we observed diminished pRb expression (i.e. low pRb/Ki-67 ratio). P21 expression on the other hand did not correlate with any parameter except with histological grade, Rb LI and p53 LI. In multivariate analysis, Rb LI was a negative predictor of disease-free survival but was linked to a higher probability of complete response. However, diminished pRb expression as well as p21 expression were not statistically significant prognostic indicators.

**Conclusions:** Our results suggest that pRb as a cell cycle related molecule may play an important role in determining prognosis and therapeutic response in NHL patients.

## P-284

DETECTION OF EPSTEIN-BARR VIRUS IN HODGKIN'S DISEASE (PATIENTS IN CZECH REPUBLIC)

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**Aims**: The frequency with which EBV is demonstrated in patients with Hodgkin's disease (HD) shows geographical variability. In the present study, we investigated the frequency of EBV in HD in the Czech Republic.

**Methods**: The presence of EBV was determined by immunohistochemistry (IHC) with anti LMP-1 antibody (DAKO) and by in situ hybridization (ISH) method for EBERs.

**Results**: We studied 142 cases of HD. The age of patients ranged from 4 to 82 years. The ratio males to females was 1.2 (males 55.6%). In a series of 142 patients 47 (33%) positive cases were found. The incidence of EBV positive results was significantly higher in males - 70.2%, than in females - 29.7%; $p = 0.023$. Five patients were found in the group below 10 years. They were all positive with LMP-1 antibody (IHC) and for EBERs in ISH method. The same results were discovered in two patients above the age of eighty. The most frequent histologic types of HD were nodular sclerosis (64 cases) and mixed cellularity (62 cases), respectively. The former mentioned type contained 16 cases - 25 % EBV positive. There were 24 cases - 38% in the latter. Positivity in other histologic types: lymphocyte predominance 12 cases - 42%; lymphocyte depletion 3 cases - 67%. ISH method determined EBERs in the nuclei not only in Reed-Sternberg (R.S.) cells but also in some intermediate-size lymphoid cells and in some small lymphocytes. LMP-1 was indentified in the cell membrane and paranuclear region of R.S. cells and in some Hodgkin's cells.

**Conclusions**: The results of the study indicate that EBV infection in latent form is present in one third (33%) of HD patients in the Czech Republic. In most frequent histologic types - nodular sclerosis and mixed cellularity, positive results clearly prevail in the latter mentioned type of HD (38%). In a small group of patients with lymphocyte predominance and lymphocyte depletion types, the frequency of positive cases was 42% and 67%, respectively.

## P-285

EBSTEIN BARR VIRUS ANTIGEN EXPRESSION IN HODGKIN'S DISEASE IN KUWAIT

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**Aims:** Based on much evidence in the literature of a significant association of Epstein Barr Virus (EBV) expression in Hodgkin's disease (HD), our aim was to study EBV in HD in Kuwait and compare it to similar studies in other countries.

**Methods:** Archival paraffin blocks of cases previously diagnosed as HD were retrieved and used for the study. The diagnosis of HD was confirmed by reviewing routine H&E as well as immunohistochemical stains for CD15, CD30, CD45, & CD20. Immunohistochemical stains for EBV-LMP was performed using avidin-biotin complex method. Cases were labeled as positive for EBV expression if the neoplastic cells were reactive with a membrane or Golgi staining.

**Results:** 59 confirmed cases were studied, 28 mixed cellularity (MC), 20 nodular sclerosis (NS), 2 lymphocyte predominance (LP), 2 lymphocyte depletion (LD) and 7 unclassifiable. Demographic data was available for 51 patients. There were 39 males (76%) and 12 females (23%). EBV antigen was positive in 22/28 MC (79%) and 7/20 NS (35%), a statistically significant difference (p=.0012). EBV was also positive in 50% LP, 100% LD, and 28% unclassifiable cases. EBV positivity did not vary with age. However, the percentage of EBV positive NS cases was slightly higher in the over 35 year age group.

**Conclusion:** The incidence of EBV in Kuwait is one of the highest reported in the literature for MC HD compared to other reports. The expression of EBV in MC does not correlate with age but is significantly higher than in NS.

## P-286

**PROGNOSTIC VALUE OF HISTOPATHOLOGY IN CASTLEMAN'S DISEASE. CLINICO - PATHOLOGIC AND IMMUNOHISTOCHEMIC STUDY OF THREE CASES.**

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**Aims.** To describe clinico-pathologic and immunohistochemical features in three patients with Castleman's disease (CD), two with generalised lymphadenopathy and one with abdominal mass.

**Material and Methods.** Two lymph nodes and a tumor-like mass from abdominal wall. Histological sections from formalin-fixed paraffin embedded tissue and stained by current techniques. Immunohistochemical study using antibodies against human immunoglobulin subclasses (Kappa, Lambda), L26, UCHL-1, Ki-67 and factor VIII-related antigen, using an avidin-biotin-peroxidase complex (ABC) method.

**Results. Patient one:** woman, aged 25 yr, with abdominal mass. The internal organisation of the mass showed a certain resemblance to a lymph node (numerous follicle-like structures and a richly vascular interfollicular tissue). The follicle-like structures, smaller than reactive follicle, had a centre composed by a blood vessel with swollen, often proliferated, endothelial cells in concentric arrangement and interposed hyaline material. Immunohistochemically, polyclonal B-lymphocyte proliferation expressing surface IgM and IgD. In the middle of the hyaline-vascular follicles the endothelial cells were positive for factor VIII -related antigen. We concluded a histologically localized hyaline-vascular (HV) type. **Patient two:** man, aged 32 yr, with generalised adenopathy and splenomegaly; the left axillary lymph node biopsy reveled a histologic aspect characteristic for the hyaline-vascular type but "multicentric" CD, which showed mixed or "intermediate" position between the HV and plasma cell (PC) types. **Patient three:** man, aged 69 yr, with generalized lymphadenopathies and hepatomegaly; supraclavicullar and axillary lymph node biopsies showed numerous concentric hyaline-vascular "follicular" structures and abundant plasma cells in interfollicular zones. Immunohistochemical studies: hyaline-vascular structures are proliferated endothelial cells and polyclonal lymphocytes. Interfollicular plasma cells showed a predominance of lambda chain.

**Conclusions.** This study pointed out that ● CD is a hyperplastic, non-neoplastic B lymphoproliferative disorder ● there is an intermediate form of CD with few plasma cells representing the "inactive" variant ● plasma cells of "multicentric" type are clearly associated with a generalised disturbance of immunoregulation lymphokine-mediated (as Il-6) ● possibly, a hyperplastic phase precedes and creates the environment in which malignant cells (neoplastic plasma cell clones) proliferate.

## P-287

PRIMARY LYMPHOMA OF PERIPHERAL NERVE

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**Aims:** Malignant lymphoma presenting as a solitary tumor of peripheral nerve is exceedingly rare. We describe four cases of primary lymphoma of peripheral nerve involving the sciatic nerve (two cases), the radial nerve, and the sympathetic chain.

**Methods:** All cases of primary lymphoma of peripheral nerve at MGH were identified. All cases were stained with antibodies directed against CD45, Ig M, kappa and lambda light chains, CD20, CD3, CD56, MIB-1, S-100, and neurofilament. Clinical details of the cases were obtained by reviewing pertinent records. DNA was extracted from formalin-fixed, paraffin embedded tissues. To evaluate the possibility of homozygous deletions of the *CDKN2A/p16* gene, we performed a comparative multiplex assay. To evaluate expression of the p16 protein, encoded by the *CDKN2A/p16* gene, we employed immunohistochemistry with the monoclonal anti-p16 antibody, JC8.

**Results:** The patients were two males and two females with an average age of 55.5 years. All lymphomas were high grade B-cell lymphomas. Two of the three patients with follow-up experienced relapse of disease with involvement of other nervous system sites and died of lymphoma. The third is alive with stable local disease at five years. We found homozygous deletion of the *CDKN2A/p16* gene in one case, confirmed immunohistochemically by absent staining for p16. An additional case showed absent staining for p16, suggesting inactivation of the gene in this case as well.

**Conclusion:** Primary lymphoma of peripheral nerve occurs rarely, usually is large B-cell type, has a poor prognosis, and appears to have a pathogenesis different from primary CNS lymphoma.

## P-288

**MANTLE CELL LYMPHOMA: A CLINICOPATHOLOGIC STUDY OF 9 CASES**

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Introduction: Mantle cell lymphoma (MCL) is a relatively uncommon type of malignant lymphoma (ML) with unique morphological, immunophenotypic, cytogenetic and molecular genetic features. It accounts for aproximately 2,5-4% of ML in North America and 7-9% in Europe.

Method: We reviewed clinicopathologic characteristics of 9 cases of MCL diagnosed in our hospital between 1993 and 1998. Clinically, valued: stage at the moment of diagnosis, peripheral blood involvement and clinical situation of patients (Estern Cooperation Oncologic Group's valoration). Morphologically, valued: site of diagnosis, histologic pattern, blastic features and bone marrow biopsy findings.

Results: The MCL constituted 16,6% of ML in our environment and accounts with a male-to-female of 4/5. Most patients presented with advanced-stage disease. Extranodal involvement at presentation occurred in one case (Waldeyer's ring). This patient also presented a erythematosus lupus.

Conclusions: Our results were similar from those present in literature. However, in our collection of ML(48 cases) MCL accounts for 16,6%, an incidence higher than other series; and the male-to-female ratio (4/5) is uncommon. We found a case with MCL and erythematosus lupus associated, an extraordinarily rare event.

## P-289

ANAPLASTIC LARGE CELL LYMPHOMA CD 30+/KI-1+ WITH HIGH CONTENT OF REACTIVE NEUTROPHILS. "NEUTROPHIL RICH". REPORT OF TWO PEDIATRIC PATIENTS.

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*Aims*: We describe two pediatric patients with anaplastic large cell lymphoma (ALCL) with high content of reactive neutrophils.

*Methods*: Forty three cases of ALCL were retrieved from the files of the department of pathology of the ABC Medical Center. Two cases of ALCL were encountered. #1, a 5-years-old male with cervical adenopathies. #2, 8-years-old female with tumor in soft tissues of chest. Immunohistochemical staining was performed using MoAbs CD45, CD20, CD3, CD30, CD15, p53 and EMA. In the two cases there were numerous neutrophils intermixed with the CD30+/CD15- tumor cells. Case #1 showed a B cell phenotype and case #2 did not stain for CD20 and CD3.

*Conclusions*: "Neutrophil rich" was described as a variant of ALCL and only has been reported in adults. We describe two cases of ALCL-neutrophil rich, in children. In this variant the neutrophil infiltration without necrosis is a hallmark of the disease. All cases described so far appear to be T and null phenotype. Citokines probably are important in mechanism to cell recruitment associated with ALCL. The prognosis of this subtype in children is unknown. Our first case is alive and receiving chemotherapy; the second one had disseminated disease and died 6 months after diagnosis.

## P-290

STATUS OF EPSTEIN-BARR VIRUS IN SEQUENTIAL BIOPSY MATERIAL OF MALAYSIAN HODGKIN'S DISEASE

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**Aims:** With the exception of a recent case report, all cases of Hodgkin's disease with reported associated Epstein-Barr virus (EBV) infection in the tumour cells remain EBV positive in all involved sites and during the course of the disease. This study aims to investigate the EBV status in sequential biopsies of Malaysian HD patients.

**Methods:** A total of 25 biopsies from 10 patients diagnosed with HD were retrieved from the files in the Department of Pathology, University of Malaya. The tissue sections from all biopsy material were reviewed. Serial sections from all the tumour involved tissue were selected for the detection of EBV by in situ hybridization technique, with peptide nucleic acid (PNA) probe for EBER

**Results:** The ages of patients ranged from 13 to 49 years at the time of initial tissue diagnosis. 5 patients had initial tumour showing presence of EBV in the tumour cells and the other 5 without. Three patients had 2 biopsies within 2 weeks interval, one patient 3 months apart, the remaining 6 had subsequent biopsies which were minimally 1½ years from the time of initial diagnostic material. The longest follow-up was one EBV associated case with a total of 5 biopsies in 9 years, followed by a non-EBV associated case with 3 biopsies in 5 years. Irrespective of the sites and interval of subsequent biopsies, all the 5 EBV associated cases showed persistence of virus in all the biopsy material and the 5 non-EBV associated tumours remained virus free.

**Conclusion:** The persistent presence of EBV in all the sequential biopsies suggests that the presence of virus is probably vital for the survival of the tumour. The consistent absence of the virus in the non-EBV associated cases refutes the notion that cases with presence of EBV in the tumour cells was probably the result of secondary infection of the susceptible tumour cells by the virus.

## P-291

**EXPRESSION OF hPTTG ONCOPROTEIN IN MALIGNANT LYMPHOMAS**

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**Introduction:** *hpttg*, a human homologue of pituitary tumor transforming gene, has been recently isolated and characterized as a novel proto-oncogene. In normal adult tissues, its expression is restricted to thymus, testis and placenta. Overexpression of *hpttg* has been documented in pituitary adenomas, tumor cell lines and peripheral blood cells from several hematopoietic disorders. Our aim is to study the occurrence of *hpttg* overexpression in malignant lymphomas and its distribution among the different histological types.

**Methods:** A series of 93 malignant lymphomas were selected and classified according to the Revised European-American Lymphoma (REAL) classification. Immunohistochemical detection of PTTG was carried out on routinely formalin-fixed, paraffin-embedded tissue sections by using standard ABC method. Polyclonal anti-hPTTG antibody was raised against a GST-hPTTG fusion protein containing the amino terminal hPTTG sequence.

**Results:** T cell lymphomas (TCL) were the most intensely immunostained with anti-hPTTG. Up to 70% of TCL were positive. On the other hand, only 26% of B cell lymphomas were immunoreactive for hPTTG, being the staining fairly weak. h-PTTG protein was detected in most cases (79%) of Hodgkin's di-sease ( HD ). Hodgkin/Reed-Sternberg cells were highlighted by a se-lective and intense hPTTG immunostaining.

**Conclusions:** *hpttg* overexpression in lymphoproliferative disorders has been previously reported. We have analyzed the expression of *hpttg* in malignant lymphomas. PTTG protein is frequently detected in TCL and HD. *hpttg* overexpression might have an important role in lymphoma tumorigenesis but it remains to be determined if this is a primary or secondary event.

## P-292

SIGNIFICANCE OF THE ASSESSMENT OF DIPEPTIDYL PEPTIDASE IV IN DIFFERENTIATING ANAPLASTIC LARGE CELL LYMPHOMA FROM HODGKIN' S DISEASE

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**Aims:** Anaplastic large cell lymphoma (ALCL) and Hodgkin's disease (HD) show morphologic and immunophenotypic overlap that may be cause of diagnostic difficulties. In this study the activity of dipeptidyl peptidase IV (DPP – IV) was determined in order to examine the defining characteristics of tumor cells of ALCL and HD and to assess the possible value of the DPP – IV activity in differentiating of these two entities.

**Methods:** The study included 5 cases of CD30 – positive ALCL with T – and null cell phenotype and 13 cases of all subtypes HD. The diagnosis of ALCL and HD was based on histologic and immunohistochemical criteria. Enzyme histochemistry was performed as described by Lojda et al. ( 1979) , using glycyl – L-proline – 4 – methoxy-β- naphthylamide as substrate and fast blue B as coupler.

**Results:** All malignant ALCL cells were strong positive for DPP – IV. By contrast, in HD, irrespective of the subtypes, Reed – Sternberg cells and mononuclear Hodgkin's cells were consistently negative. In all cases of ALCL and HD nonmalignant small T lymphocytes with dot – like cytoplasmic DPP – IV activity can be found.

**Conclusion:** This study demonstrate that the difference of the DPP - IV – activity between malignant cells of ALCL and HD may be diagnostic important and be useful addition to distinction of these diseases.

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## P-293

MICROSCOPIC STUDY OF 500 SPLENECTOMIES :

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**Aims** : we wanted to precise principal indications of splénectomies

**Methods:** we overviewed microscopic analysis of splénectomies performed during the ten last years in your hospital ,an universitary surgical and médical center with a department of hematology. Splenectomy cases were found by ADICAP code (SR)

**Results** : 500 cases listed with by order of frequency :

-187 splenectomies after traumatic injury (37,4%)
- 96 for malignant hemopathies (19,2%) :
79 NHL :76 B (63 small c ,11 large c.,2 myeloma) and 3 NHL T
4 HK diseases
13 myeloid disorders (5 LAM, 7 MPS )
- 62 immune cytopenia with 51 ITP (12,4%)
- 46 splenectomies during visceral surgical of proximity (9,2%)
- 19 necrosis ,infarctus (3,8%)
- 16 hereditary hemolytic anemia ( 3,2%)
- 14 cystic lesions : 6 trues cysts ,8 pseudo-cysts (2,8%)
- 13 infectious lesions :2 MNI with rupture (2,6%)
- 11 vasculars tumors with 1 angiosarcoma (2,2%)
- 10 fibro-congestive spleens with 4 liver transplant (2%)
- 9 normal spleens :bilan HK , NHL ,unspecified etiology (1,8%)
- 6 follicular hyperplasia : 1 tropical splenomegaly (1,2%)
- 5 storage diseases (1 %)
- 3 metastasis : 2 renal and 1 ovarian adenocarcinomas (0,6 %)
- 3 malformatives (0,6 %)

Traumatic injury stay the first indication of splénectomy followed by hematologic indications : small B cells NHL and ITP
Primary splenic tumors were uncommun except splénic NHL

## P-294

CRYSTAL-STORING HISTIOCYTOSIS IN LYMPHO-PLASMACYTIC DISORDERS

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Aims: Crystal-storing histiocytosis (CSH) is a rare disorder described in patients with Ig-secreting lymphoplasmacytic disorders that can involve many organs.

Methods: We report two new cases associated with plasmacytoma and low grade MALT type lymphoma (MALT) involving lymph node and lung respectively. The first patient, a 72 year-old woman presented with pancytopenia and poliadenopathies. The second patient was a 59 year-old woman with a pulmonary mass. Samples were embedded in paraffin and stained by immunohistochemical (IHC) techniques using a panel of antibodies. An ultrastructural study (US) was performed in case 1.

Results: Patient 1: The lymph node showed neoplastic plasma cell infiltrate masked by sheets of large benign histiocytes containing crystals. Immunoelectrophoresis of serum demonstrated and IgGκ monoclonal protein. Bone marrow aspirate showed 13% of plasma cells. A diagnosis of plasmacytoma with CSH was done. In the second patient, the tumour in the right lower pulmonary lobe was composed of small lympho-plasmacytic cells and crystal laden cells. The diagnosis was MALT with CSH. Bone marrow biopsy was negative. IHC in both patients demonstrated monoclonal staining of crystals and plasma cells with antibody to κ light chain.. The crystal-containing cells were also positive for CD68. US demonstrated cells containing electron-dense inclusions surrounded by a thin membrane that appeared to be a lysosomal structure.

Conclusions: CSH can be associated with plasmacytoma and other lymphoplasmacytic disorders such as MALT. Multiple organs can be involved by CSH but lung is very infrequent. IHC confirmed that these cells are histiocytes. CD68 is essential to confirm the histiocytic nature of the cells and monoclonal positivity staining is very helpful in identifying the crystals as monoclonal κ light immunoglobuline deposits.

## P-295

BONE-MARROW LYMPHOCYTOSES IN CHRONIC MYELOID LEUKEMIA (CML) WITH CYTOGENETIC REMISSION AFTER INTERFERON-α (IFN-α) THERAPY

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**AIMS**: Histological and immunohistochemical study of bone-marrow in six patients with complete remission of CML after IFN-α therapy.

**METHODS**: Six patients with CML (mean age 57.5 years) were admitted to IFN-α therapy (6-9 MIU daily, from 1 to 5 years). Bone-marrow biopsies, performed before and after therapy, were fixed in B5 and immunohistochemically studied with a lymphocyte antibody panel CD20, CD79a, CD3, CD4, CD8, CD57, CD45RO.

**RESULTS**: All patients manifested their desease with marked leucocytosis ($50\text{-}100 \times 10^9$/L), anemia, splenomegaly and clonal disorder with t (9;22) chromosomal translocation (Ph+). Bone-marrow biopsies showed the modifications of CML. At the end of the therapy a normal peripheral range of leucocytes, red cells and platelets with complete cytogenetic response (100% Ph-) was observed. Bone marrow biopsies showed normal hematopoietic cell component; lymphocytes were increased (20-25%), sometimes assembled in small clusters. Immunohistochemistry revealed polymorphyc B and T cells with a moderate increase in CD8+ cells.

**CONCLUSIONS**: The availability of IFN-α has had a particularly promising impact on CML therapy because this drug can produce a complete hematologic and cytogenetic remisson. Many factors may be involved such as: IFNs could enhance spontaneous NK activity; IFN-α could restore LAK cytotoxicity against CML cells; autologous IL-2 activated NK cells could suppress malignant hemopoiesis in CML; IFN-α could enhance the terminal differentiation of dendritic cells, capable of inducing a T-cell mediated cytotoxicity against the leukemic cells. These data may be in accordance with the bone-marrow lymphocytoses, found in the 6 patients with complete histomorphologic and cytogenetic remission of CML after IFN-α therapy.

## P-296

LARGE CELL ANAPLASTIC LYMPHOMA OF THE MEDIASTINUM SIMULATING MALIGNANT HISTIOCYTOSIS

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**Aims:** Malignant histiocytosis, i.e. true neoplastic proliferations of differentiated tissue histiocytes/macrophages, is an extremely rare hematologic neoplasm.

**Methods:** Formalin-fixed, paraffin-embedded tissue samples of liver, bone marrow and mediastinal tumor tissue of a 58-year-old woman with a four-week history of fever of unknown origin were investigated using immunohistochemical and molecular biological methods.

**Results:** Liver and bone marrow both exhibited a diffuse increase in CD68R-expressing macrophages with signs of an active hemophagocytosis but no lymphomatous infiltrates. Initial diagnosis of a malignant histiocytosis was suggested. However, the mediastinal tumor (tissue removed 2 weeks after liver and bone marrow biopsies had been performed) could be classified as large cell anaplastic lymphoma of T-cell origin with monoclonal rearrangement of the chain of the T-cell antigen receptor and tumor cells expressing the antigens CD3, CD30 and CD45RO, but lacking CD15, CD20, CD68R and CD79a.

**Conclusion:** This case report demonstrates a typical but rare example of a malignant lymphoma simulating malignant histiocytosis clinically and morphologically indicating extreme caution for diagnosis of true histiocytic neoplasms.

## P-297

PROGNOSTIC SIGNIFICANCE OF IMMUNOSTAINING EXPRESSION OF Ki67, BLC-2 AND P53 PROTEINS IN FOLLICULAR AND DIFFUSE LARGE B CELL LYMPHOMAS

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**Introduction:** Follicular and diffuse large B-cell lymphomas were studied to correlate their clinical with immunocytochemical parameters of accepted prognostic value.

**Methods:** 104 non-Hodgkin lymphomas were reviewed. Clinical aspects relevant to the prognosis were correlated with light microscopic and immunocytochemical (CD-3, CD-20, CD-43, Bcl-2, Ki67 and p53) features.

**Results:** Among the 49 follicular lymphomas studied, the high grade (III) ones showed higher p53 expression (P=0.073). Follicular lymphomas I and II showed higher levels of Bcl-2 expression than the grade III ones (P =0.056). Low p53 expression in follicular lymphomas was associated with either none or just one extraganglionar area affected (P= 0.019). Among the diffuse large B-cell lymphomas high Ki67 expression (>50% nuclei) was also associated to extraganglionar involvement of more than one area (P= 0.03). No significant differences were observed in 5 and 10 year survival between diffuse lymphomas with percentages of Ki67 nuclei staining either below or above 50% (P= 0.8585).

**Conclusions:** P53, Ki67 and Bcl-2 immunostaining did not seem to correlate with patient survival in our series of follicular and diffuse large B-cell lymphomas. However, significant associations were found with certain features of prognostic interest.

## P-298

**DETECTION OF EBV IN GASTRIC MALT LYMPHOMA AND NASOPHARYNGEAL LYMPHOMAS BY IN SITU HYBRIDISATION.**

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**Aims :** Our aim was to detect EBV in 23 lymphomas of the stomach (MALT) and 25 lymphomas of nasopharyngeal localisation.

**Methods :** We used in situ hybridisation with $EBER_1$ and BHLF PNA probes (Dako).

**Results :** We detected EBV in 3 cases (13%) of low grade gastric lymphoma in few scattered large or medium sized cells and 14 cases (6 low grade – 8 high grade) of nasopharyngeal lymphomas. The rate of positively was significant only in 3 cases (upper than 5%) with respectively 10, 20 and 80% of positive cells. Non tumorous gastric mucosa was always negative but EBV was detected in 5 from 11 cases of nasopharyngeal non tumorous mucosa with rates lesser or up to 5% positive cells.

**Conclusion :** The etiopathogenic role of EBV in the genesis of gastric MALT lymphoma remain hypothetic. But EBV may play on etiopathogenic role in the genesis of nasopharyngeal lymphomas.

## P-299

**IMMUNOHISTOCHEMICAL EXPRESSION OF CELL-CYCLE RELATED PROTEINS AND APOPTOTIC REGULATORS IN HODGKIN'S DISEASE (HD)**

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**Aims:** The aim of the study was to investigate the expression of the cell-cycle regulators. Rb and p-53 oncosuppressor proteins, the wt-p-53 induced waf-1/p-21 protein, in classical HD in correlation with the proliferative index ki-67. In parallel, the expression of the proapoptotic bax and antiapoptotic bcl-2 proteins was also studied.

**Methods:** The APAAP method was performed on paraffin sections of 59 cases HD (20 nodular sclerosis, 39 mixed cellularity) for the detection of Rb, p-53, waf-1/p-21, ki-67 (MIB-1) bcl-2, bax proteins with moAbs. The expression of Epstein-Barr virus (EBV) was investigated using the RISH (EBERs) and APAAP (LMP-1).

**Results:** p-53 protein was detected in 32% of the cases showing a nuclear expression in a limited number of neoplastic cells. Rb and Waf-1 proteins were detected in all cases Rb protein was expressed in a high percentage of neoplastic cells (>25%). In the majority of the cases, while Waf-1 demonstrated a variable expression. Ki-67 was detected in all cases showing a heterogenous expression in a limited number of RS cells. In 38 cases, an inverse correlation between Rb↑/ki-67↓ expression was observed with a parallel Waf-1/Rb expression in 22 of them (16 cases: Rb↑/ki-67↓/Waf-1↓) (22 cases: Rb↑/ki-67↓/Waf-1↑). Bcl-2 and bax proteins were detected in 56% (21/37) and 67% (25/37) of the examined cases, showing a parallel or inverse expression in 22 and 21 cases respectively. There was no correlation between EBV detection and the expression of the examined proteins.

**Conclusions:** According our results, dysregulation of cell cycle control and imbalance of apoptosis may contribute in the pathogenesis of HD.

## P-300

**IMMUNOHISTOCHEMICAL EXPRESSION OF Rb, p-53, Waf-1/p-21, ki-67 (MIB-1) IN MULTIPLE MYELOMA (MM)**

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**Aims:** The aim of this study was to investigate the expression of the oncosuppresor proteins Rb, p-53, the wt-p53 induced Waf-1 protein in MM, in correlation with the proliferative index ki-67 (MIB-1).

**Methods:** The APA-AP immunohistochemical method was performed on paraffin sections of 24 bone marrow biopsies with MM and of 4 extramedullary MM (classified into three grades) for the detection of Rb, p-53, Waf-1/p-21 and ki-67 (MIB-1) with monoclonal antibodies.

**Results:** Rb protein was detected in all cases, showing a high nuclear expression (>25%) in 61% (17/28). p-53 protein was detected in 46% (13/28) of the cases in a limited number of tumour nuclei (5-10%) and in only 3 cases (2 extramedullary) in more than 25% of the MM cells. Waf-1 protein was detected in 75% (21/28) of the cases showing a variable nuclear expression. ki-67 (MIB-1) protein was detected in all cases showing a limited expression (<25%) in 23/28 and an increased one in 5 cases (3 extramedullary). In the majority of the cases (18/28) an inverse Rb↑/ki-67↓ expression was observed and a parallel (Rb↑/ki-67↑) in 8. The combined immunoexpression of p-53, Waf-1 proteins suggests that Waf-1 expression may be p-53 independent in a number of cases (8/27).

**Conclusions:** ki-67 expression in MM correlates with tumour grade and reflects the proliferative compartment. The high Rb protein expression in MM probably corresponds to the phosphorylated form. Nevertheless, even if Rb proteins is expressed most of the time in MM cells, it remains possible that its function is abnormal.

## P-301

EXPRESSION OF CD99 AND bcl-2 IN SOLITARY FIBROUS TUMOR: A STUDY OF TEN EXTRAPLEURAL CASES

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Solitary fibrous tumor was initially described in pleura and considered like a variant of mesothelioma. At present time its different origin is known, and has been found in multitude of locations. Diagnosis can be done generally with conventional optical microscopy techniques. However, differential diagnosis problems can sometimes occur with others neoplasias of mesenchymal origin. Immunohistochemically expresses CD34 in practically all of the cases, being negative for most of other habitual markers, except for Vimentin.

We have studied ten cases of solitary fibrous tumor, all of them with an extrapleural origin. The age range oscillated between 31 and 69 years (mean 49). They were located in meninge, orbit, nasal cavity, mediastinum, intrapulmonary, liver, intraabdominal (2) and soft tissues of extremities (2). Immunohistochemical study of the cases revealed positivity in all of them, for CD34 (QBEND10) and Vimentin. Expression of CD99 (O13) was also found in all ten cases. Bcl-2 was positive in 9 of the ten cases (negative in the medistinal tumor). Positivity of these markers was equally intense in the most hypercellular areas of the neoplasias, than in areas of collagen rich pattern of low celularity.

Although CD99 and bcl-2 are two inespecific markers, their expression in a high percentage of solitary fibrous tumors has diagnostic interest in doubtful cases, since its most characteristic immunohistochemical marker, CD34, has been described also in others neoplasias of potentially similar characteristics.

## P-302

**PROGESTERONE RECEPTORS IN DUCTAL INFILTRATING CARCINOMA. IMMUNOHISTOCHEMICAL EVALUATION OF TWO ANTIBODIES.**

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**Aims.** To evaluate two antibodies for progesterone receptors (PgR) in formalin-fixed, paraffin-embedded tissues from breast carcinomas

**Methods.** 43 cases of ductal infiltrating carcinoma N.O.S. were included in the study. All tumors were histologically graded according to the Elston-Ellis method. For immunohistochemical determination of PgR two commercially available antibodies were used, PGR-1A6 (Dako, 1:50) and PgR-636 (Dako 1:50). One section of each routinely fixed specimen was immunostained with PgR-1A6 and with PgR-636 (Dako), using Dako Envision system. Nuclear staining was scored as positive, and the number of positive cells was determined semiquantitatively from 0 to 100%. A cut-off value of 5% was used. Intensity of staining also was scored, from 1 to 3. Results were correlated with age, tumor size and nodal status.

**Results.** 30/43 (70%) cases were positive for PgR-636 and 21/43 (49 %) were positive for PgR-1A6. 21 cases were positive with both antibodies. With PgR-646 a high level in the intensity of staining was observed (25 % score 3 and 28 % score 2, versus 5 % and 21 %, respectively). A strong correlation was found between the two antibodies and histological grade ($p<0.05$). No correlations were observed with patient's ages, tumor size and nodal status.

**Conclusion.** We believe that PgR-646 is more sensitive and a reasonable substitute for PgR-1A6 to be used on routinely fixed tissue from breast cancer.

## P-303

APOPTOSIS IN NEUROENDOCRINE TUMOUR OF THE LUNG

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**Aim**: The investigation of the morphological particularities of apoptosis in Neuroendocrine Tumours of the Lung (NETL) in correlation with the expression of p53, Ki-67, bcl-2.

**Methods**: 17 paraffin-fixed patterns of NETL were examined with hematoxylin-eosin staining, immunohistochemical assay of chromogranin (DAKO), pancytokeratin (Immunotech), mutated p53 (DAKO), bcl-2 (DAKO), Ki-67 (DIANOVA) and ApopDetek Cell Death Assay System (Enzo Diagnostics, US). Apoptotic index (AI) and expression of p53 and Ki-67 were calculated in percent among 3000 tumor cells.

**Results**: The morphological particularities of apoptosis in NETL include the specific localisation of the process and the absence of AB phagocytosis. The prominent apoptosis of tumour cells could be seen in the tumour borderline zone with surrounding tissue, in adjacent zones to intratumour "detritic" areas, in the walls of intratumour vessels. ApopDetek test brought out various types of apoptotic figures, which are probably responsible for different stages of apoptosis. Small cell carcinoma distinguished from carcinoids by the higher levels of AI (42% and 34%), p53 (62% and 20%), Ki-67 (43% and 7,5%) and bcl-2. Due to this was supposed that the high rate of proliferation activity respected to the high level of apoptosis.

**Conclusion:** Obtained data show that AI may be used as a prognostic factor for SCLC together with expression of Ki-67, bcl-2 and mutated p53. High levels of these markers correlate with high proliferation activity of tumour cells and may be considered as sings of high malignant potential in NETL.

## P-304

**IMMUNOHISTOCHEMICAL DIAGNOSIS OF EMERY-DREIFUSS MUSCULAR DYSTROPHY**

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**INTRODUCTION**: Emery-Dreifuss muscular dystrophy is a rare neuromuscular disease, linked to Xq28 and of adolescence onset, with characteristic muscular contractures, particularly in elbows and Achilles tendons, scapulo-humeral muscular atrophy and cardiac conduction defects. Mutations of this gene cause deficiency of a nuclear membrane protein called emerin.

**METHODS**: Two male patients from the same family, with a clinical picture suggestive of muscular dystrophy, were studied. Muscle biopsy was performed in both cases with immunohistochemical study for dystrophin, sarcoglycans, DRP, merosin and emerin. Exfoliative buccal cytology was then performed for emerin determination in both patients, two obligate female carriers and two other females from this family.

**RESULTS**: All nuclei of muscle biopsy and buccal epithelial cells proved to be negative for emerin in two patients; immunolabelling for dystrophin and associated proteins was normal in both. Cytology study of females showed a mosaic staining pattern for emerin combined with Feulgen in four carriers.

**CONCLUSIONS**: Emerin immunodetection in oral mucosa cells is a non-invasive, low-cost method, useful for the diagnosis of cases with suggestive clinical features of Emery-Dreifuss muscular dystrophy, as well as for carriers. Early identification of these patients implies better prognosis, since the onset of characteristic heart disease, which may also affect oligosymptomatic carriers, can be controlled.

## P-305

**UTILITY OF CYTOKERATINS 7 AND 20 IN THE DIFFERENTIAL DIAGNOSIS OF ADENOCARCINOMAS.**

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Aims: To assess the utility of combined use of cytokeratins 7 (CK7) and 20 (CK20) in the immunohistochemical study for the diagnosis of primary and metastatic adenocarcinomas (ADC). This study was limited initially to lung ADC and brain metastases.

**Methods:** A prospective study of 21 ADC of lung (16 likely primaries and 5 with known antecedent of primary colorectal ADC), and 7 brain metastasis of ADC of unknown origin. In every case, immunohistochemistry for CK7 and CK20 was done simultaneously with the conventional histological study for diagnosis.

**Results:** Lung ADC: 15 of primary cases expressed only CK7 (diffuse), 2 cases CK7 (diffuse) and CK20 (focal), 1 case showed equal diffuse expression of the two cytokeratins and the last one was negative for both antibodies. The 5 ADC of patients with previous colorectal cancer expressed only CK20 diffusely, so they were considered metastases. Brain metastatic ADC: 3 cases expressed only CK7. The possibility of lung primary was suggested in the final report. In all three patients lung ADC was confirmed later. The remaining 4 cases expressed both cytokeratins although CK20 positivity was focal and lesser intense in three cases. In one of these patients a gastric adenocarcinoma was found. We are unaware of the location of primary cancer in the three other patients.

**Conclusions:** The combined use of CK7 and CK20 is helpful in the differential diagnosis of primary and metastatic ADC. We consider that they would be incorporated in routine diagnosis.

## P-306

**COMPARISON OF p53 IMMUNOREACTIVITY IN FRESH-CUT VERSUS STORED SLIDES IN UROTHELIAL CELL CARCINOMA OF THE URINARY BLADDER.**

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**Aims**: Recent reports have indicated that storage of unstained parraffin slides may lead to false negative immunostaining of tumor markers. This is not a problem for daily practice but could be a real problem for retrospective works. A strong decrease of p53 immunoreactivity in these circumstances has been observed in carcinomas of several locations; however, overall status (negative or positive) seems to be consistent in individual cases. Successfully antigen retrieval using microwave heating has also been reported. The aim of this work is to investigate the effects of slide storage on immunohistochemical staining of p53 in urothelial cell carcinoma of urinary bladder. **Methods:** To do this, we performed immunohistochemical staining with use of the p53 monoclonal antibody DO7 (Dako) in 28 cases of urothelial cell carcinoma. Slides were stored for more than one year. Fresh cut (FCS) and stored slides (SS) of the same paraffin block were simultaneously stained by using a standard PAP method combined with a citrate buffer microwave heating antigen-retrieval technique. To quantify the positivity, an automatic image analysis system (CAS200) was utilized, and the percentage of positive cells and stained nuclear area was obtained. **Results:** Only 6 cases showed positive cells in the stored group while there was 24 reactive cases in the fresh group. 18 negative cases in the SS, showed positivity in the FC. There was coincidence in 4 negative and 6 positive cases. All positive SS showed less percentage of positive cells than the same cases in FCS. The mean percentage of stained cells was 3,9 (+/- 12,7) in all SS group, and 19,8 (+/-15,9) in all FCS group, with a statistically different signification between groups of $p<0,001$. Similar results were obtained for percentage of nuclear area.

**Conclusions:** Our study demonstrated a strong decrease in percentage of p53 positivity in stored tissue sections that affect overall status. Antigen retrieval by means of a citrate buffer and microwave heating did not improve reactivity. Therefore, we conclude that the use of stored unstained slides for p53 immunostaining is not recommended for urothelial cell carcinoma of urinary bladder.

## P-307

**USE OF PROSTATE SPECIFIC ANTIGEN IMMUNOHISTOCHEMISTRY IN DIFFERENTIAL DIAGNOSIS OF PROSTATİC CARCINOMA; IS IT RELIABLE?**

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**Aims:** Prostatic carcinoma may present as metastasis in some cases. It usually metastasizes to pelvic or cervical lymph nodes, bone or lung. Differential diagnosis may be troublesome on solely histopathologic base. Prostate Specific Antigen (PSA) is widely used immunohistochemically to identify prostatic tumours. Some of previous reports mentioned about faint staining in extraprostatic tissues and tumours. We tried to assess the reliability of this method in differential diagnosis.

**Methods:** Archival paraffine blocks of nine bone metastasis of malign tumours (5 lung carcinomas, 2 prostatic adenocarcinomas, 1 colon adenocarcinoma, 1 invasive ductal carcinoma), ten lymph node metastasis (7 colon adenocarcinomas, 3 lung adenocarcinomas), thirteen colon adenocarcinomas and seventeen lung adenocarcinomas stained with PSA immunohistochemically. All the patients were males. The presence and intensity of staining was evaluated.

**Results:** None of the primary colon and lung adenocarcinomas or lymph node metastasis stained. Three of the bone metastasis showed weak staining with PSA immunohistochemistry. The diagnosis of primary tumours of these cases were; moderately differantiated squamous cell carcinoma of lung, invasive ductal carcinoma and prostatic carcinoma.

**Conclusions:** Extraprostatic tissue originated malign tumours may stain with PSA immunohistochemically. This staining is weak, thus strong staining of metastatic tumour may be evaluated as specific for prostatic carcinoma.

## P-308

**OLD PARAFFIN BLOCKS: A PROBLEM IN IMMUNOHISTOCHEMISTRY?**

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**Aims:** To examine whether the age of paraffin blocks influences the outcome of immunohistochemistry.

**Methods:** The material consisted of 81 formalin-fixed, paraffin-embedded, left-sided, colorectal adenocarcinomas from 81 patients. The patients had participated in a randomized study on follow-up after radical surgery for colorectal carcinoma at Odense University Hospital in the period 1983-1994. 39 of the patients had surgery in the period 1984-86 (group I) and 42 in the period 1992-94 (group II). Fresh tumor sections were cut (1 section/tumor) and pretreated with either microwave irradiation or proteolytic enzymes, depending on the antibody. The sections were stained with antibodies against p53, Ki67, and components of the Urokinase Plasminogen Activating System (uPA, uPAR and PAI1) on an automated immunostainer. The intensity and the staining pattern were evaluated semiquantitatively.

**Results:** There were no significant differences between the two groups, neither regarding the intensity of the staining nor the staining pattern.

**Conclusion:** The immunohistochemical reactions for p53, Ki67, uPA, uPAR and PAI1 were independent of the age of the paraffin blocks in this study. This result does not rule out a loss of antigenicity due to processing of the tissue. On the other hand, comparison of immunohistochemical reactions in archival material of different age, for diagnostic or prognostic purposes, seems to be reasonable as long as the tissue processing an antigen retrieval procedures are standardized. The results are currently being tested in a sample of tumors from 1998, and evaluated in a standardized sampling design (random, systematic sampling).

## P-309

MORPHOLOGY OF THE VASCULAR STROMA AND ANGIOGENESIS IN GLIOBLASTOMA MULTIFORME.

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Glioblastoma multiforme is a grade IV astrocytic neoplasm histologically characterized by high cellularity, nuclear atypia, necroses and microvascular proliferation. Clinical prognosis is poor and the only favourable prognostic factor is age below 50 years.
The study was performed on 38 cases of glioblastoma, which were divided into two groups according to the patients' age: group I up to 49 and group II over 50 years of age. Tumor vascularization was characterized and types of vessels were specified: microvessels with normal morphology, teleangiectases, angioma- like forms, and steps of microvascular proliferation- simple and glomeruloid. In the older group glomeruloid proliferation was more frequent. Immunohistochemical staining with antibodies against vWf and CD31, α–SMActin and Collagen IV was made to visualize the vascular wall components. The immunomorphological analysis was performed with respect to the individual types of vessels.
Vascular density (specimens immunostained with anti vWf serum) and α–SMA and the vWf expression ratio (percent immunopositive area) were measured with computer image analyzer. Statistical analysis revealed a significantly higher vascular density in group I (170 vessels/mm$^2$). It was no difference in the vWf and α–SMA expression ratio between groups. However the α–SMA expression ratio was higher than the vWf ratio within both groups. This indicates the prevailing presence of smooth muscle cells and pericytes in the vascularization of glioblastoma as compared to endothelial cells. The second conclusion is that in glioblastoma not only vascular density indicates the intensity of angiogenesis but vascular morphology is of basic importance.

## P-310

**EXPRESSION OF CD44 IN COLORECTAL CARCINOMA**

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**Aim :** To study the expression of the cell adhesion molecule CD44, that belongs to the family of hyaluronan binding proteins, in carcino embryonic antigen (CEA) positive colorectal carcinoma using immunohistochemical methods.
**Methods :** Immunohistochemical analysis for CEA and CD44 were carried out in formalin fixed paraffin sections of normal and neoplastic colorectal tissues by the standard peroxidase-antiperoxidase method.
**Results:** In this study 36% of the cases of CEA positive colorectal carcinoma were moderately positive and the remaining were highly positive for CD44.
**Conclusion:** Tumor progression and metastasis is a complex process that involves constant interaction of tumor cells with one another and with the microenvironment in which they survive. Dysregulation in adhesion mechanism determines the metastatic potential of various tumors. As CD44 is one of the key molecules in cell adhesion, it is possible that the highly positive cases of colorectal carcinoma may have a higher metastatic potential as compared to the cases in which CD44 expression is moderate. Further studies on the expression of other cell adhesion molecules and cytokines that are involved in tumor progression and metastasis in colorectal carcinoma in correlation with CD44 expression are in progress in our laboratory.

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## P-311

MOLECULAR REGULATION OF THE KERATINIZATION IN SQUAMOUS CELL LUNG CANCER

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**Aim:** This study was undertaken to determine the role of p53, Ki-67, c-myc, c-fos, bcl-2 expression in the progression of squamous cell lung cancer (SCLC) and their relation to the process of the keratinization, as a variant of apoptosis.
**Methods:** 46 paraffin-imbedded samples of SCLC were examined with hematoxilin-eosin and alcian blue staining, immunohistochemical assay to pancytoceratin (DAKO), mutant p53 (DAKO), c-myc (Novocastro), c-fos (DIANOVA), bcl-2 (DAKO), Ki-67 (DIANOVA), IGF-II (Pepto tech inc.), IGF BP1,2,3,4,5,6 (Immun Diagnostic) and chromogranin (DAKO). Expression of wild-type p53 and Ki-67 was calculated in percents on 3.000 tumour cells.
**Results:** The obtained data show that the process of keratinization in SCLC is regulated by c-fos, c-myc oncogenes, IGF-II and IGFBP2,5. These oncogenes and the members of IGF system have the highest level of expression in tumours with deposition of extracellular keratin. Overexpression of mutant p53 (63% - 97% positive cells) was found more often in SCLC without keratinization. The level of Ki-67 expression was correlated with high proliferation activity in poor differentiated tumours. Bcl-2 was determined only in 39% of SCLC without any correlation with the process of keratinization.
**Conclusion:** C-fos, c-myc oncogenes and the members of IGF system may facilitate the process of the keratinization in SCLC. The high expression of these markers in SCLC can be considered as a good prognostic factor for this type of tumours.

## P-312

**IMMUNOHISTOCHEMICAL CORRELATION OF STEROID HORMONE RECEPTORS STATUS WITH BCL-2 EXPRESSION IN PROSTATE CANCER**

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**Aims**: For elucidation and better understanding of hormone independence development we have investigated the association of expression of Bcl-2 and related proteins with the expression of receptors for androgens (AR), estrogens (ER), progesterone (PR) and retinoid receptor X (RXR).
**Introduction**: Gene bcl-2 encodes a 26 kD protein Bcl-2 which, when over-expressed in prostate cells may prevent apoptosis and be implicated in the development of hormone refractory tumours which are more aggresive and their prognosis after relapse is poor. At present, intensive discussion on the regulatory role of receptors for various steroid hormones in the prostate cancerogenesis continues.
**Methods**: Immunohistochemical and Western blot analysis of Bcl-2, Bax, Bcl-$X_{S/L}$, AR, ER, PR and RXR proteins expression was performed in the formalin fixed and wax embedded samples of untreated primary prostate carcinomas obtained from 132 patients and also in androgen-sensitive prostate cancer cell line LNCaP.
**Results**: The significant indirect relationship exists between expression of Bcl-2 and expression of AR in the group of cancers with low/medium content of AR. In the group with the highest positivity of AR we have found a rather higher expression of Bcl-2. We did not find any significant relationship between expression of Bcl-2 and other investigated receptors. The tested cell line revealed the constitutive expression of Bcl-2 as well as of the others apoptosis regulating proteins Bax and Bcl-$X_{S/L}$ and very intensive expression of AR. The expressions of another receptors were not demonstrated by Western blot analysis.
**Conclusions**: Bcl-2 expression is arised in the lowest as well as in the highest grade tumours and it is indirectly related to AR status in AR low/medium positive cases of prostate cancer. The possible dual role of Bcl-2 in the regulation of prostate cancer progression is discussed.

**Acknowledgement**: The work was supported in part by grant IGAMZ CR and GACR 204/96/K047

## P-313

LOCALLY ADVANCED BREAST CANCER: PROGNOSTIC VALUE OF CLINICOPATHOLOGICAL PARAMETERS.

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**Aims:** p53, c-erb-B2, Ki 67, and EGFR are prognostic factors in node positive and negative breast cancer, but their significance in locally advanced breast cancer (LABC) has not been fully assessed.
**Methods:** Paraffin-embedded tissue from tumors of 168 patients with LABC were studied (median follow-up 8 yr). Conventional pathological parameters were evaluated and correlated with immunohistochemical overexpression of p53 (Oncogene Science), c-erb-B2 (Dako) and EGFR (Menarini), and Ki67 Index (Dako). Distant disease-free and overall survival were analyzed for each parameter.
**Results:** Mean age of patients was 61 yr (74% were postmenopausal). Immunohistochemical overexpression of p53 was found in 67% of tumors, c-erb-B2 in 52%, and EGFR in 45%. Ki67 Index were less than 5 in 33%, 5-10 in 19%, 11-19 in 18%, and >20 in 30%. No correlation was found between conventional and new parameters. Patients with c-erb-B2 overexpression had shorter survival (p=0.02).
**Conclusions:** Besides histological grade and hormonal receptor status, only c-erb-B2 overexpression offers additional prognostic information in LABC.

## P-314

APOPTOSIS IN DERMATOMYOSITIS

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Aims: exploration of the role of apoptotic pathways in the course of dermatomyositis (DM)
Methods: immunohistochemical study of participation of caspase system and bcl-2 in affected muscles using antibodies CPP32 to caspase 3 and BCL2 Oncoprotein (Dako) to bcl-2 protein.
Results: the expression of activated caspase 3 as well as of bcl-2 in normal muscles is negligible. In the stage of inflammamtory infiltration of the muscle with DM, the activated caspase 3 is expressed in the nuclei of dystrophic or atrophic fibres, less frequently in some myoblasts and regenerating fibres. A strong immunoreactivity for bcl-2 was recorded at the periphery (or in perinuclear areas) of a proportion of inflammatory cells, in the cells inside dystrophic (flocculent) muscle fibres and in the walls of some blood vessels. A similar distribution of bcl-2 positive cells was found in perivascular inflammatory infiltrates in the dermis, while activated caspase 3 was recorded in some cells of the stratum germinativum of affected skin.
Conclusions: expression of activated caspase 3 (which appears relatively early before DNA fragmentation) in some muscle fibres damaged by dermatomyositis gives evidence of the presence of apoptosis in the course of the disease. The presence of caspase system in regenerating fibres may be the cause of abortive regeneration of them. The occurence of bcl-2 in the inflammatory cells and in walls of blood vessels proves that apoptosis-related complex may control the inflammatory response and (to a certain extent) vascular compartment of both muscle and skin.

## P-315

**GLYOFIXX®, AN EXCELLENT FIXATIVE FOR IMMUNO-HISTOCHEMISTRY (IHQ)**

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**Background:** Antigen retrieval (AR) techniques have allowed the use of many policlonal and monoclonal antibodies (Abs) in formalin-fixed, paraffin embedded tissues. However, AR has some problems: it is not effective for some Abs, there are false positives due to the enhancing of the endogenous biotin activity, it impairs the morphology and it causes a loss of tissue attachment to the slides. Moreover, a standard protocol for all Abs is not available yet. Recently, some fixatives have been described that adequately preserve the morphology and allow to apply IHQ staining for different Abs without AR.
**Aim:** To test the applicability of GLYOFIXX® (GLX, Shandon, UK), a new commercially available fixative in surgical samples, and to determine the results obtained with IHQ.
**Methods:** Samples from tonsils, lymph nodes, spleen, liver, thyroid, pancreas, brain, kidney, and bowel were fixed 4-20 hours in GLX ,embedded in paraffin, and routinely processed. TechMate 500® and EnVision System ®(Dako) were used in IHQ stains.
**Results:** Excellent morphological and IHQ results without AR were obtained with the following Abs: CD3, 15, 20, 21, 23, 31, 34, 35, 43, CD45, 45RO, 68, 79a, KiM4p, CNA42, EMA, Keratin, TPA, 34E12, CEA, PSA, Actin, Desmin, Vimentin, Factor VIII, Rb and p53. Suboptimal results were obtained with CD1a, 4, 7,8, and 10, but they improved with the use of overnight (18 hours) low-temperature heating (80ºC) in 1mM EDTA at pH 8.
**Conclusions:** GLYOFIXX® adequately preserves the morphology of tissues and allows the use of IHQ without AR techniques for many Abs. GLX is a safe fixative, much less toxic than formalin.

## P-316

**EXPRESSION OF NM23 AND CATHEPSİN-D IN SQUAMOUS CELL CARCINOMAS OF LUNG AND LARYNX**

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**Aim:** The purpose of this retrospective study was to investigate if there was a difference in the expression of nm23, metastase suppressor gene, and Cathepsin-D, aspartic proteinase, in squamous cell carcinomas of two different regions.
**Methods:** Immunohistochemical localisation of nm23 and Cathepsin-D was performed on formalin-fixed, paraffin embedded tissues of 28 laringeal and 24 lung carcinomas. Tumors which expressed more than 5 % cytoplasmic staining were evaluated as positive. The results were correlated with the grade and the stage of the tumor in each group. Also the positivity of the two groups (lung and laryngeal tumors) for each marker were compared.
**Results:** There was no correlation with either the grade or the stage of the tumors. Yet there was a significant difference in the immunostaining percentage of nm23 and Cathepsin-D in these two different organs ($p<0.001$). For nm23, twenty-two (92.9 %) laringeal carcinomas were positive where only five (20.8 %) lung carcinomas were positive. Cathepsin-D expression was inversely positive: twenty-one lung carcinomas vs twelve laringeal carcinomas.
**Conclusion:** The difference in the expression of nm23 could be due to the advanced stage of all lung carcinomas (stage III ). However for Cathepsin-D, the high percentage of positive staining in lung carcinomas, and the low percentage in larynx tumors can be explained by the different stromal and matrix structure of these two organs.

## P-317

**EXTERNAL QUALITY ASSESSMENT FOR IMMUNOCYTOCHEMISTRY: A USEFUL EDUCATIONAL TOOL FOR THE ROUTINE LABORATORY**

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**Aims:** To provide a comprehensive and impartial external quality assessment (EQA) service for immunocytochemistry (ICC), with an emphasis on providing information on how to achieve the standards deemed to be acceptable.

**Methods:** Over 400 laboratories from 30 countries are registered with the UK National External Quality Assessment Scheme for Immunocytochemistry. Laboratories have the opportunity to participate in one of five different EQA modules for ICC; general pathology, breast pathology, neuropathology, lymphoid pathology and cytology. Participants were sent formalin fixed paraffin processed sections (alcohol fixed cytospins for cytology) and requested to demonstrate various antigens. A panel of assessors comprised of pathologists and technologists then assessed the slides. Feedback on individual performance was accompanied by a review of each assessment.

**Results:** The analysis conducted after each assessment was able to show that optimal demonstration of CD3, CD15, CD20, CD30 and CD45, occurred after the use of heat mediated antigen retrieval. For S-100, cytokeratin, polyclonal factor VIII, the use of proteolytic enzyme digestion allowed for optimal demonstration, whilst no pre-treatment allowed a large proportion of participants to achieve optimal demonstration of neuron specific enolase, chromogranin A and thyroglobulin. Choice of antibody was the other major variable influencing the quality or pattern of staining, particularly for CD15, IgM, kappa light chains, prolactin, growth hormone, neurofilament and CEA.

**Conclusions:** EQA is a useful educational tool promoting awareness of the standards possible for various antigens and identifying main parameters which assist in optimal demonstration.

## P-318

TUMOR NECROSIS FACTOR α AND ITS RECEPTORS IN NORMAL, HYPERPLASTIC AND CARCINOMATOUS HUMAN PROSTATE

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**AIMS:** Tumor necrosis factor α (TNF-α) is a 17 kDa polypeptide that induce tissue damage when overproduced. Two distinct receptors, TNFR1 and TNFR2, have been described in a variety of cell types. Activation of one of the receptors leads to either apoptosis or proliferation and activation of the NF-κB transcription factor.

**METHODS:** Prostatic biopsies from 25 men with benign prostatic hyperplasia (BPH), 25 diagnosed of prostatic cancer (PC), dominant Gleason grade 3, and 15 histologically normal prostates (NP) obtained at autopsy were immunohistochemically examined for TNF-α, TNFR1, TNFR2.

**RESULTS:** In NP, TNF-α expressed intensely in all epithelial cell types, and slightly in some stromal cells. In BPH, immunoreaction intensity was lower. In PC specimens, immunoreaction was similar to that of the NP. TNFR1 was found immunoreactive in both cell types of the NP epithelium, and in the smooth muscle cells of the stroma. In BPH, the same immunohistochemical pattern was observed. In PC, this intensity was even stronger. Immunoreactiveness to TNFR2 also appeared in all epithelial cell types and some stromal cell. In BPH the reaction was very increased. In the PC specimens, differences in the intensity were found in relation to the histological pattern, being similar to BPH in the papillar pattern but weaker in the microglandular pattern.

**CONCLUSIONS:** in BPH both receptor are increased respect to NP, but the levels of TNF-α are lower, leading to a compensation of the possible effects of TNF-α in these cells. In contrast, in PC, the increase of both receptors with maintenance of TNFα levels implies an increase of both proliferation-apoptosis ratio, leading to physiological waste and chance of mutations. In addition, the detection of the overproduction of these polypeptides could serve as an indicator of malignancy in human prostatic biopsies.

## P-319

FRACTIN IMMUNOSTAINING FOR THE DETECTION OF APOPTOTIC CELLS AND APOPTOTIC BODIES IN FORMALIN FIXED AND PARAFFIN EMBEDDED TISSUE

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**Aims:** To illustrate a new antibody specific for apoptotic cells and apoptotic bodies in formalin fixed and paraffin embedded tissue. That antibody named fractin recognizes an actin fragment generated by caspases during apoptosis (Yang et al. Am J Path 1998;152:379-389).

**Methods:** For fractin immunostaining, sections were treated in the pressure cooker with EDTA pH 8. The slides were incubated with the fractin antibody, diluted 1:1000 in 1% BSA. Visualization was achieved with a one step direct peroxidase detection system, followed by chromogen development with DAB and counterstaining with hematoxylin.

**Results:** Apoptotic cells and apoptotic bodies were visualized in different epithelial and mesenchymal cells. Weak and moderate fractin immunostaining was seen in cells with light microscopic features of apoptosis, such as nuclear shrinkage and chromatin condensation, whereas fragmented apoptotic bodies showed strong immunostaining . In human tumors of epithelial origin, apoptotic bodies were typically seen within the proliferative compartment of the tumor. In colonic adenocarcinoma many apoptotic bodies were present within luminal debris in glandular tumor formations and focal staining was also seen in the cytoplasm of carcinoma cells lining these formations. In soft tissue sarcomas, areas with apoptotic bodies also harboured some tumor cells with cytoplasmic fractin staining. In lymphoid tissue (appendix, tonsil and lymph node) apoptotic bodies were abundantly present in hyperplastic follicles, in the cytoplasm of starry sky macrophages, but also in between the follicle center cell population. In a liver allograft with chronic rejection many apoptotic bodies were seen ingested by Kupffer cells.

**Conclusion:** The preferential staining of fractin in apoptotic bodies supports the general concept that caspase cleavage of actin occurs in the late, irreversible phase of apoptosis. It still has to be established whether actin cleavage by caspases is a universal mechanism in apoptosis in different pathological conditions.

## P-320

**THE EXPRESSION OF p53, p21 AND INDUCTION OF APOTOSIS BY ULTRAVIOLET RADIATION IN HUMAN SKIN IS DOSE DEPENDANT**

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**Aims:** To determine the time course and dose response for p53, p21 expression and apoptosis in response to solar simulated radiation *in vivo* in human skin.

**Methods:** The minimal erythema dose (MED) was determined for six volunteers (V). Adjacent areas 4cm$^2$ were exposed to 0, 0.5, 1, 2, 3 MEDs. Biopsy specimens (4mm) were taken from these sites at 4.5 (Va + Vg), 9 (Vb), 24 (Vc), 33 (Ve) 48 (Vf) hours after UV irradiation. Ethical approval was obtained from Beaumont Hospital Ethical Committee. Induction of apoptosis was determined histologically using Haemotoxylin&Eosin staining and the *In Situ* Cell Death Detection Kit (Boehringer Mannheim). p53 and p21 positive cells were detected immunohistochemically.

**Results:** A dramatic increase in the numbers of apoptotic cells, p53 and p21 positive cells were seen in volunteers exposed to 3 MED in comparison to lower UV doses. The maximum number of p53 positive cells was seen at 9 to 24 hours whereas maximum expression of p21 and the highest number of apoptotic cells occurred at 33 hours post UV irradiation.

**Conclusions:** These data indicate that expression of p53 precedes induction of apoptosis while expression of p21 occurs simulanteously *in vivo* in human skin and and all three effects are dose dependent.

# Joint first author

# P-321

Correlation of MHC I expression and antigen processing machinery in malignant tumors and corresponding normal tissue.

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Background: Low molecular weight polypeptides (LMP2 + LMP7) and transporters associated with antigen processing (TAP1 + TAP2) play a crucial role in antigen processing and cell surface expression of HLA class I molecules. Recently it has been shown, that in malignant tumors reduced or defective MHC I expression is due to functional deficiendes of the AG-processing machinery, such escaping immunosurveillance.

Design: Formalin-fixed, paraffin embedded and fresh frozen tissue of 30 malignant tumors, tumor precursors and the corresponding normal tissue was immunostained with monoclonal antibodies to TAP1, TAP2, LMP2, LMP7 and MHC I, using Immunoperoxidase, ABC methods and computer aided double staining procedures in order to establish normal or defective MHC I expression or antigen processing.

Results: Most malignant tumors and malignant precursor lesions (PIN, AAH, Ca i.s.) showed highly reduced or completely lacking AG-processing peptides and lacking MHC I expression, although in some cases (PIN and PC) as well as in certain cells of the endocrine and lymphatic system MHC I was expressed without detectable AG-processing peptides. Furthermore, in non-neoplastic germ cells neither MHC nor TAP's or LMP's could be detected. Although in cells of the normal placental trophoblast the AG-processing peptides are synthesized, no MHC I-molecules at the cell surface could be detected.

Conclusions:
1. Most malignant tumors may escape immunosurveillance by functional defects of the MHC I and AG-processing machinery.
2. There are alternative ways of AG-processing and presentation.
3. Physiologically, cells or tissue of immunological risks may show a down regulation of the AG-processing peptides and the MHC I molecules.
4. The results are to be confirmed by molecular genetic methods.

# P-322

## HODGKIN'S DISEASE IN A HIV-PATIENT TREATED WITH A HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

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**Aim of the study:** Highly active anti-retroviral therapy (HAART) effectively reduces RNA levels of HIV in the plasma and lymph nodes. As a consequence immune function improves and progression of disease is delayed. However, classic Hodgkin's disease (HD) is increasing in HIV-1 positive individuals. These patients present with B-symptoms and advanced disease. There is an unusual prevalence of the mixed cellularity type of HD. We report on a 39-year old HIV-1 positive individual who is HIV-1 positive since ten years and under HAART since two years. The aim of our study was to investigate specimens infiltrated by a mixed cellularity HD including the spleen, perisplenic lymph nodes and bone marrow. The goal was to determine, whether there is an association of the Epstein-Barr virus (EBV) in this HD and which role plays the HIV-1 in the development of the malignant disease.

**Methods:** Formalin fixed, in paraffin embedded specimens from the spleen, from perisplenic lymph nodes and from bone marrow were routinely stained with HE and Giemsa. *Immunohistochemical analysis* using a AVBD complex method was carried out with antibodies directed against CD3, CD15, CD20, CD30, CD45, CD45RO, CD79a, EMA and LMP1 of EBV (DAKO, Denmark). *In situ Hybridisation*: Early mRNA of EBV (EBER) was carried out according to a routine protocol (DAKO, Denmark). For HIV-1-RNA detection in spleen an $^{35}$S-labeled, single-stranded, antisense RNA probe (Lofstrand Labs., Gaithersburg, MD) was used. As a positive control, cytospin preparations of H9 cells infected with HIV-1 were hybridised with the same probe. As a negative control, sections were hybridised with a radiolubeled sense-strand probe. *PCR for EBV*: The primers used were EBER, EBNA2 and EBNA3 for the viral subtypes.

**Results:** Histology showed a classical mixed cellularity type with malignant cells expressing CD30. The Hodgkin-Reed-Sternberg (HRS) cells strongly expressed LMP-1 and the viral RNA (EBER1). In situ hybridisation for HIV-1-RNA in spleen and lymph nodes showed signals only in areas not infiltrated by HD. The content of viral RNA was very variable among different germinal centres. The EBV PCR-results revealed positive signals for EBER, EBNA2 and EBNA3, indicating an EBV-infection of the latency-type II and the presence of the viral subtype A.

**Conclusion:** Despite a long-term HAART and a viremia below the detection level (100c/ml) in the peripheral blood, the spleen and perisplenic lymph nodes are a reservoir for HIV-1. HIV-1 in contrast to EBV seems not to be directly associated with HD.

# P-323

VISCERAL BOTRYOMYCOSIS (VB) OR BACTERIAL PSEUDOMYCOSIS A PROPOSAL OF TWO CASES. ONE PULMONARY AND OTHER HEPATIC.

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**Aims**: Our aim was to study an infectious bacterial disease (VB) which clinically resembles a malignant tumor and pathologicaly can be erroneously diagnosed as actinomycosis.

**Methods**: We studied two cases of VB, the first was pulmonary: 48 years old male with LSD solitary nodule suspicious of malignancy at Rx. The second was a 73 year old female with fever and jaundice, and a cystic mass that blurred the left lobe of the liver by ECO, Rx and TAC. The pulmonary case is studied from a surgery specimen and the liver case by PAAF.

**Results**: Both cases had the same findings, they were abscesses that contained one or more basophlics granules (organized aggregates of filaments) that are bordered by eosinophilic, club-like, refractil material (Splendore-Hoeppli). The granules can be mistaken for those of actynomicosis and actynomycotic mycetoma. The bacteria most commonly involved include: pseudomona aeruginosa, staphilococus aureus, escherichia coli and species of streptococus and proteus.

Botryomycotic granules and those of actynomycosis and mycetoma can be distinguished from each other if apropppiate bacterial and fungal stains are used. The differential diagnosis includes: actynomycosis (filamentous branched flora); nocardiosis (filamentous long delimited an weakly acid-fast) and VB (organized aggregates of non-filamentous gram+ and gram- bacterias).

**Conclusions**: 1.- The VB is an uncommon infectious human disease, known after the "sixties", but rarely diagnosed.

2.- It is important to make the correct diagnostic preoperatively, which is feasible by paaf, since this process is candiate for medical treatment.

# P-324

## GASTROINTESTINAL SOLITARY ULCER CAUSED BY CYTOMEGALOVIRUS: DIAGNOSTIC NEED OF IMMUNOHISTOCHEMISTRY

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**Aims.** To emphasize the need of immunohistochemistry for the detection of cytomegalovirus (CMV) in solitary gastrointestinal ulcers lacking typical intranuclear inclusions.

**Methods.** Five cases of CMV-associated large-sized gastrointestinal solitary ulcers (four in the stomach and one in the jejeunum) were retrieved from the files. Two of the cases had a previous history of immunosuppressive treatment for renal transplant. Biopsy samples had been fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin (H&E). Afterwards, immunohistochemistry had been performed with a monoclonal antibody against cytomegalovirus late antigen (clone QB1/06; Novocastra, Newcastle, UK).

**Results.** Three of the five cases showed characteristic intranuclear CMV inclusions on H&E examination. Multiple sections of the remaining two biopsies failed to show either intranuclear or cytoplasmic inclusions. In contrast, anti-CMV immunohistochemistry techniques provided positive results in all five cases.

**Conclusions:** Independently of the patient's immune status, CMV should always be suspected as the causative agent when confronted with large solitary ulcers of the gastrointestinal tract. Failure to find inclusions in multiple sections does not exempt from the use of immunohistochemistry, which is necessary for the diagnosis of CMV infection in almost half the cases.

## P-325

### A *SCID* MOUSE MODEL OF METHYCILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) PNEUMONIA

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**Aims:** *Staphylococcus aureus* is the leading cause of hospital acquired bacteremia in Europe and North America. A significant number of *S. aureus* isolates are resistant to multiple classes of antibiotics (MRSA) making the infections increasingly difficult to treat. It is hypothesized that the success and persistence of certain epidemic strains of MRSA to cause pneumonia is due to their ability to colonize the respiratory tract more efficiently than other strains of *S. aureus*. No clinically relevant and robust animal model of MRSA infection has been previously described in the literature.

**Methods:** Following administration of distinct epidemic strains of MRSA, colonization and infection was assessed at 24, 48, and 72 hours post-aerosolization. Histological sections of lung were examined using Hematoxylin and Eosin (H&E) and Brown & Brenn (B&B) special-stained sections. The bacterial *cfu* was also determined.

**Results:** Lungs of *scid* mice infected with Lab strain MRSARN6390 were characterized by severe acute multifocal suppurative bronchopneumonia (Figure 1). Special staining with B&B confirmed multiple clusters of gram-positive coccoid organisms within inflammatory foci (Figure 2). Lungs of *scid* mice infected with Punjab strain MRSA308C had less severe and progressive bronchopneumonia. Special staining in these animals also confirmed clusters of gram-positive coccoid organisms within inflammatory foci.


Figure 1


Figure 2

**Conclusions:** We have established a clinically relevant pneumonia model to study colonization and infection by epidemic strains of MRSA in *scid* mice. This model can be used in the development of alternative therapeutic approaches to the treatment or prevention of antibiotic-resistant *S. aureus* pneumonia.

## P-326

### RETROSPECTIVE STUDY OF NEPHROPATHIES. REVIEW OF OUR EXPERIENCE SINCE 1974

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PURPOSE: Renal biopsy is actually the main diagnostic method in a great number of pathologies, having an important prognostic value. The aim of this study was the analysis of the incidence and prevalence of the different nephropathies biopsied, and their evolution through these three decades.

METHODS: We reviewed the non transplant renal biopsies studied in our Department of Pathology since 1974 to 1998.

RESULTS: Primary glomerulonephritis ( PGN ) accounted for 628 cases ( 62 % ); there were 318 cases ( 31 % ) of secondary glomerulonephritis ( SGN ), 60 of tubulointerstitial nephritis ( 6 % ), and a miscelaneous group with 18 cases ( 1% ).

IgA nephropathy was the most common form of glomerulonephritis found in our series, with 150 cases, followed by minimal change disease ( 125 cases ) and crescentic glomerulonephritis and focal and segmentary sclerosis, both with 79 cases.

Between SGN, the most common pathologies were systemic lupus erythematous ( 104 cases ) and vasculitis ( 55 cases ).

Along these years, the number of extracapilar glomerulonephritis associated to vasculitis had a substantial increase.

CONCLUSIONS: During the last 25 years, it has been found an increase in IgA nephropathy, crescentic GN and vasculitis, and a decrease in the number of difuse mesangial proliferative GN, diabetic nephropathy and rheumatoid purpura.

## P-327

### bcl-2 EXPRESSION IN EOSINOPHYLIC RENAL CELL TUMORS

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**Aims**: Eosinophylic epithelial tumors of the kidney contain variable proportions of mitochondria. Distribution of mitochondria in the cytoplasm, as well as their association with other organelles, result in specific patterns for every tumour variety - granular renal cell carcinoma (RGC), eosinophylic variety of chromophil carcinoma (CP), eosinophylic variety of chromophobe carcinoma (CBC), and oncocytoma (OC), that can be best appreciated by electron microscopy (EM). Recently, mitochondrial antibodies have been used in this differential diagnosis. On the other hand, bcl-2 expression has been related to prognosis in renal cell carcinoma, although results of different authors are still contradictory. The aim of the present study is to investigate the different patterns of bcl2 expression in eosinophylic renal cell tumours, as this feature could be used both for differential diagnosis and for prognostic purposes in this setting.

**Methods**: Eighteen epithelial renal cell tumours are the subject of this study: 5 RGC, 5 CP with papillary architecture, 3 CBC, and 5 OC. All cases were confirmed by EM. Monoclonal bcl-2 antibody (BioGenex, San Ramón, CA, USA; dilution 1:200) was tested in paraffin sections after antigen retrieval in heated citrate buffer. Secondary antibody was combined with Envision reagent (Dako, Carpinteria, CA, USA). Tonsil was used as external control. Renal parenchyma and interstitial lymphocytes served as internal controls. Positivity was rated from 1+ to 4+. The amount of tumour cells showing every level of positivity, as well as the pattern of cytoplasmic distribution, were recorded in each case.

**Results**: Cells of RGC showed a very heterogeneous distribution and intensity of stain; irregular positive clumps outlined residual clear spaces; sarcomatoid areas were completely negative. CP cases varied from diffusely and intensely positive to weakly positive, and this seemed to be inversely related to the amount of histiocytes in the connective stalks of the papillary areas; in some cases, a fine positivity outlined vesicles that were identified as lipid droplets in UE. In CBC, 33% of cells were moderately positive, with granularity outlining very fine vesicles, and the remaining tumour cells were negative or weakly positive. OC cases showed the weakest bcl-2 positivity, with more strongly positive individual cells scattered in the tubules and cords of nearly negative cells.

**Conclusions**: bcl-2 expression is different, and shows characteristic distribution, pattern, and intensity of stain in the four groups of eosinophylic renal cell tumours tested. This finding could be helpful in their differential diagnosis and is probably related to their biology. The simultaneous use of bcl-2 expression as a diagnostic aid and a prognostic factor should be investigated separately in each of the renal cell carcinoma varieties.

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## P-328

### INVESTIGATION OF ADHESION MOLECULS INTERACTION AND INTEGRIN CHAINS DISTRIBUTION IN GLOMERULONEPHRITIS

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Glomerular cell proliferation and extracellular matrix protein deposition are key features of glomerulonephritis. Integrins are known to be involved in both of these processes since adherent cell types utilize integrins to bind and organize extracellular matrix proteins.

**Methods:** By immunohistochemical staining we have investigated expression and distribution of integrin chains beta$_1$, alpha$_1$ to $_6$ and alphaV in 30 human renal biopsies (different types of glomerulonephritis) and 5 normal kidneys.

**Results:** We have found strong correlation between expression of the alpha5 chain within theinterstitium, the alpha V chain on proximal and distal tubular epithelium and the presence of chronic histological damage. Staining for interstitial alpha 5 and tubular alphaV were also strongly associated with expression of adhesion molecules ICAM-1, VCAM-1, E-selection and L-selection, and the presence of macrophages within the interstitium which is linked with the degree of chronic histological damage and disease progression. There were strong positive associations between staining for alpha5 on glomerular endothelium and its expression on extraglomerular vascular endothelium as well as between both mesangial alpha1 and podocyte alpha3 and tubular staining for the common beta1 subunit. There were also positive association between staining for different integrins within the glomerulus such as mesangial cell staining for alpha2, glomerular endothelial cell staining for alpha5 and glomerular epithelial cell alpha3.

**Conclusion:** These results suggest that there is a coordinated upregulation of integrin expression both within the tubulointerstitium and the glomerulus and that they are associated with the expression of other adhesion molecules, macrophage infiltration and the presence of markers of disease progression (interstitial fibrosis and tubular atrophy).

## P-329

CYTOGENETIC FINDINGS IN RENAL CELL TUMORS, STUDY OF 13 CASES.

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**Aims**: In recent years cytogenetic investigations have demonstrated specific chromosomal abnormalities correlate with different histological subtypes in renal tumours (RTs). Our study reports cytogenetic findings in a series of 13 renal tumors (RTs) to further elucidate the relationship between cytogenetic abnormalities and various subtypes of RTs.

**Methods**: 13 RTs (8 clear cell, 3 chromophilic, 1 chromophobe and 1 oncocytoma) were cytogenetically analysed. Fragments of fresh tissue obtained from nephrectomies were disaggregated by collagenase II. The cells were plated on culture flasks in a growth medium supplemented with appropriate growth factors, serum and antibiotics. Chromosomes were G-banded with Wright's stain and karyotyped.

**Results**: The most common aberrations in 6 of the 8 renal cell carcinomas (RCCs) of the clear cell type involved chromosome 3, like deletion of the 3p13 region (1 case), loss of the whole chromosome 3 (2 cases) and unbalanced translocations (3 cases); trisomy of 5q, 7, 12 and loss of one sex chromosome were also observed as additional abnormalities. 2 of the 3 chromophilic RTs showed multiple aberrations including loss of Y and trisomy of chromosomes 3q, 7, 12, 16, 17 and 20. Two clear cell RCCs and one chromophilic RCC revealed a normal karyotype. Chromophobe RCC showed a deleted chromosome 2 along with the presence of a marker, while in the oncocytoma a rearranged chromosome 1 was observed.

**Conclusions**: Our results are similar to those reported in the literature, and confirmed the importance and the aid of cytogenetic investigations in characterization and differential diagnosis of different subtypes of RTs.

## P-330

PERIRENAL EPITHELIOID ANGIOMYOLIPOMA. PATHOLOGIC, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF 2 CASES.

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**Aims**: Renal epithelioid angiomyolipoma is a recently described entity. We report two new cases remarkable for their perirenal localization and their histological, ultrastructural and immunohistochemical features.

**Case reports**: Both tumors were discovered on ultrasound examination performed for abdominal pain in two 43 and 45 year-old women, without tuberous sclerosis. The lesions were homogeneous and enhanced after contrast injection on CT-scan. They were located in the retroperitoneal space, attached to the renal capsule by a thin stalk. Surgical enucleation was performed in both cases. There was no recurrence 1 and 1,5 years after surgery.

**Results**: The tumors measured 7 and 8 cm. They were firm, well-limited and whitish. Histologically, both tumors were made of cords of muscular epithelioid cells without nuclear atypia. The fibrous stroma contained numerous thick-walled blood vessels but no adipose component. The tumor cells were immunoreactive for vimentin, desmin, smooth muscle actin and few perivascular cells were positive for HMB-45. Most of the cells demonstrated nuclear positivity for progesteron and estrogen receptors. Some cells contained dense granules compatible with melanosoma on electron microscopy.

**Comments:** Epithelioid angiomyolipoma is a rare benign renal tumor composed of round muscular « epithelioid » cells. It differs from atypical angiomyolipoma by its lack of necrosis, mitotic activity and nuclear atypia. Despite the absence of adipose component in our two lesions, immunoreactivity of some tumor cells for HMB-45 and their negativity for epithelial markers facilitate their differential diagnosis from renal carcinoma. Our two cases are remarkable for their unusual perirenal localization. The positivity for progesteron and estrogen receptors in both tumors could suggest their hormonodependent character.

## P-331

CRITICAL EVALUATION OF IGA PATHOLOGIC CLASSIFICATION

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**Aims**: this study was performed to assess the prognostic value of the Haas morphological classification in IgA nephropathy. In order to improve the prognostic value of pathologic features, we additionally study tubular and vascular semiquantitative scales.

**Methods**: Univariate and multivariate analyses of 194 patients with primary IgA nephropathy diagnosed from 1985 to 1995 was realized.

**Results**: At the time of the biopsy, 65 patients (33.5%) have developed chronic renal failure and at the end of the follow-up period, 33 patients (17%) requiring hemodialysis. The mean age of the patients was 37.8 +/- 18.9 with predominance of males (sex-ratio = 3.12). Patients were followed for a mean period of 43.2 +/- 37.2 months post-biopsy. Univariate analysis revealed that hypertension ($p<10^{-4}$), nephrotic syndrome ($p = 0.01$) and crescents ($p = 0.02$) were significant to predict renal failure instead of subendothelial topography of the IgA deposits ($p = 0.05$) and proteinuria ($p = 0.05$). Hematuria is a protective factor ($p = 0.03$). Multivariate analysis shows that tubular index grade 2 (relative risk, RR = 5.5) and grade 3 (RR = 28.8) were the most relevant factors to predict renal failure. The Haas morphological clasification, emphasizing the glomerular changes, is only significant in the univariate analysis.

**Conclusions**: This study underlines that tubulo-interstitial evaluation is the highest relevant pathologic parameter to evaluate renal outcome of IgA nephropathy.

## P-332

INTRARENAL P-GLYCOPROTEIN AND ENDOTHELINS IN CHRONIC CYCLOSPORIN-INDUCED NEPHROTOXICITY IN RATS

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P-glycoprotein (P-gp) acts physiologically as an efflux pump to expel hydrophobic substances from cells. Our group and others have shown that cyclosporin A (CsA), among other actions in the kidney, induces P-gp overexpression (Am J Pathol,1995). Endothelin 1 (Et1) is widely expressed in the kidney in a variety of physiological or pathological situations, the latter of which can progress to sclerosis. Exposure to cyclosporin A (CsA) of mesangial, endothelial and renal tubule cells induces Et1 overexpression. The pathophysiological function of Et3 in the kidney is currently being debated; Et3 may be involved in the regulation of water reabsorption through the action of type B tubular receptors.

110 male Sprague-Dawley rats fed with a maintenance diet were divided into three groups: two control groups, one inoculated with 0.9% of sodium chloride (SC) and other with solvent used for CsA injection, and an experimental group treated with 25 mg CsA per kilogram of body weight per day during 28 or 56 days. We evaluated the expression levels of P-gp mRNA by using the RT-PCR technique. Prepro Et1 and Et3 mRNA was determined by northern blot (NB).

Chronic treatment with CsA induced an increase in the expression of P-gp mRNA in a dose and time dependent manner, more evident by 58 days (0.725 vs. 0.251, $p<0.01$, Newman-Keuls test.) (Table 1). A remarkable finding was that the upregulation of P-gp mRNA was inversely related to the incidence of hyaline arteriopathy (Spearmints test, $r=-0.3819$, $p<0.01$). Levels of preproEt3 mRNA were greatly increased from post-treatment day 28, whereas preproEt1 mRNA levels were increased from post-treatment day 56 (Table 1). On day 28 renal lesions correlated clearly with levels of Et3 mRNA. However, on day 56 the key finding was the strong correlation of preproEt1 mRNA levels in CsA nephrotoxicity with the most important analytical, histological and IHC findings.

Table 1

| Group: | 28 Days (x±SD) CsA | 28 Days (x±SD) SC | 56 Days (x±SD) CsA | 56 Days (x±SD) SC | Significance Two Way ANOVA |
|---|---|---|---|---|---|
| P-gp(RT-PCR) | 0.47±0.33 | 0.24±0.07 | 0.72±0.36 | 0.25±0.22 | P<0.01 |
| Et1-NB | 0.20±0.10 | 0.23±0.05 | 0.34±0.09 | 0.15±0.05 | p<0.05 |
| Et3-NB | 0.48±0.30 | 0.07±0.11 | 0.36±0.10 | 0.10±0.04 | p<0.01 |

The increased expression of P-gp mRNA in CsA treated rats compared with untreated group and the inverse relation of P-gp mRNA levels with hyaline arteriopathy are in agreement with previous analytical, histological and immunohistochemical findings, suggesting an important role of P-gp in the prevention of pharmacological nephrotoxicity by CsA, acting as a detoxicant in renal cells. Our results support the hypothesis that clinical and morphological phenomena related with CsA nephrotoxicity are related with hypersecretion of endothelins in the progression to interstitial-fibrosis induced by CsA. The changes are first evident in Et3 expression and angiotensin II accumulation, and are later reflected in Et1 expression

## P-333

**COMBINED ADRENAL ADENOMA AND MYELOLIPOMA ASSOCIATED WITH RENAL CELL CARCINOMA.**

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**Aims:** We report a non previously described combination of myelolipoma and adrenal adenoma in a patient simultaneously with a renal cell carcinoma.

**Methods:** CT scanning revealed a left kidney tumor ; left adrenal mass and an enlargement of the prostate. Left adrenal gland and kidney were resected simultaneously.

**Results:** The adrenal tumor consisted predominantly of clear zona-fasciculata-type cells arranged in nests and irregular cords. Scattered islands of fat containing active bone marrow elements were present . Microscopically, the renal tumor cells were large, with clear cytoplasm and sharply outlined boundaries

**Conclusions:** Combination of myelolipoma and true adrenal adenoma is a very rare event. We have been able to find only two of such cases described in the literature. In the other hand there is only one report in the literature of adrenal myelolipoma associated with renal cell carcinoma. The interest of our case is the combination of these two rare associations in the same patient. As far as we know, it has never been reported previously.

## P-334

KIDNEY GRANULOMA IN WHIPPLE'S DISEASE

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**Aims** : Whipple's disease (WD) is a rare, multiorgan disease with prominent intestinal manifestations. The WD is also an unusual cause of granulomatous interstitial nephropathy (GIN), with only one case report associated to chronic renal insufficiency. Detailed descriptions of clinical manifestations and histopathology are lacking. We describe 2 case reports of GIN in WD.

**Methods** : Four renal biopsy from 2 adults (51 and 53 year old respectively), with weight loss, asthenia, arthralgia and recently discovered renal failure (serum creatinine : 132 and 190 µmol/l ) were studied. In both cases, blood pressure was normal; urinalysis showed micoscopic hematuria, with no or mild proteinuria. Respectively 8 and 2 months after last renal biopsy, jejunal biopsy showed a WD histologic aspect, with periodic acid Schiff (PAS) positive, Ziehl-Neelsen (ZN) negative, large macrophages in the lamina propria.

**Results** : Initial renal biopsies showed large noncaseating tuberculoid interstitial, peri-tubular granulomas in a mild interstitial fibrosis. Control biopsy (25 and 43 months later) exhibited a dense fibrosis with same granulomas. All stains (PAS, ZN, Gram and Warthin-Starry) were negative. Tropheryma whippelii specific PCR was positive on renal biopsy (Patient 1). For Patient 2, bacteria were not found on electron microscopy; no renal tissue was available for PCR analysis. Routine direct immunofluorescence yielded non remarkable findings.

**Conclusions** : 1)We suspect that WD is responsible for renal lesions in our 2 cases. 2) Kidney granulomas may allow unsuspected WD diagnosis. 3) Based on the literature, WD may be included in differential diagnosis of GIN along angiitis, immunopathologic (ie. sarcoidosis) and transmissible agent disorders.

## P-335

**IMPAIRMENT OF CD95 (APO-1/FAS)-MEDIATED APOPTOSIS: A DETERMINANT OF MULTIDRUG RESISTANCE IN HUMAN RENAL CELL CARCINOMAS**

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**Aims:** Resistance of renal cell carcinoma (RCC) to anticancer drug-induced apoptosis has been related to the expression of P-glycoprotein and effective drug detoxification. Since the CD95 system has recently been identified as a key mediator of drug-induced apoptosis, we analyzed the role of the CD95 system in chemotherapy-induced apoptosis in four newly established RCC cell lines.

**Methods and Results:** 1. By RT-PCR and flow cytometry, expression of CD95 receptor and ligand was found in all RCC cell lines. 2. Exposure to topotecan or bleomycin resulted in induction of apoptosis and a significant dose-dependent ($p<0.05$) reduction of cell number. The effects of topotecan were seen at clinically relevant concentrations, whereas the $IC_{50}$ values of bleomycin were far beyond clinically achievable dose levels. 3. By flow cytometry, exposure to topotecan or bleomycin resulted in increased expression of CD95 ligand in all cell lines. Increase of CD 95 receptor expression was observed in three RCC cell lines, including one p53-mutated cell line, whereas another p53-mutated cell line showed no or only a weak upregulation after exposure to topotecan or bleomycin. 4. Despite the upregulation of CD95 receptor and ligand, *antagonistic* $F(ab)'_2$-anti-APO-1 antibody fragments, which interfere with CD95 receptor/ligand interaction, failed to inhibit apoptosis induced by topotecan or bleomycin in all cell lines.

**Conclusions:** Anticancer drugs induce upregulation of CD95 receptors and ligands in human RCCs. Despite this upregulation, however, the CD95 system was shown *not* to be involved in drug-induced apoptosis, thereby suggesting an impairment of CD95-mediated apoptosis in renal cancer. This impairment of CD95-mediated apoptosis, however, might play a key role for the multidrug resistance phenotype of renal cancer.

## P-336

PATHOLOGICAL STUDY IN CLEAR-CELL RENAL CARCINOMAS: A CYTOGENETIC AND FLUORESCENCE IN SITU HYBRIDIZATION (FISH) IN TEN TUMORS

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**Aims:** The most frequent cytogenetic finding in RCCs of the clear cell type is a deletion or unbalanced translocation involving the short arm of chromosome 3, usually in 3p14 or 3p21. Involvement of the long arm of #3 has rarely been described. Also a (partial) trisomy of #5, especially the 5q22-qter segment, is frequently found in the clear-cell tumors as well as trisomy 12 and 20, loss of #8, #9, #13 and #14 and structural abnormalities of the long arms of #6 and #10.

The aim of this study was to investigate the relationship between chromosome aberrations detected by FISH and/or cytogenetics, and tumor grade and stage in ten clear cell carcinomas.

**Methods:** Tumor specimens were minced with scalpels and then disaggregated for 15-20 min in collagenase II. Cells were harvested for cytogenetic and FISH (interphase nuclei) analysis after culture of 5-10 days. GTG staining banding was obtained as standard method and karyotypes were described according to ISCN (1995). Tel 3p DNA probe (Oncor) was used for FISH.

**Results:** Clear cell carcinomas showed a solid, tubular or tubulocystic growth pattern. Groups of cells having granular, eosinophilic cytoplasm were focally present in four cases.

By cytogenetic analysis all cases showed a modal chromosome number, diploid or hypodiploid. Common clonal abnormalities included: -3 or del 3p or der 3 in 6 cases. In some tumors, other clonal aberrations were observed in chromosomes 3, 7, 9, 11, 12, 13, 14, and 17. By FISH study, monosomy or loss in 3p segment was found in 8 cases.

**Conclusions:** These results suggest that the incidence of chromosome 3 aberration detected by FISH is more frequent than detected by conventional cytogenetic methods. Deletion in 3p was the most common aberration in clear-cell carcinomas. Finally, no relationship between chromosome abnormalities and tumor grade and stage were found.

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## P-337

**RENAL ONCOCYTOMA AND CHROMOPHOBE RENAL CELL CARCINOMA: INCIDENCE OF APOPTOSIS, CELL PROLIFERATION, P53, AND BCL-2 EXPRESSION IN 32 CASES.**

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Objectives: To study the cell cycle and its relationship with the incidence of apoptosis, bcl-2, p53 immunostaining and the proliferation index (MIB-1) in 18 renal oncocytomas (ROs) and 14 chromophobe renal cell carcinomas (RCCs).

Methods: We have used the TUNEL assay to detect apoptotic nuclei, immunohistochemistry for expression of bcl-2, p53 and MIB-1 in an automated immunostainer with heat induced epitope retrieval from paraffin blocks. Flow cytometry study was also undertaken from the paraffin blocks.

Results: ROs did not show significative apoptotic activity, against 61% of positivity in chromophobe RCCs. Neither ROs nor chromophobe RCCs overexpressed p53. Bcl-2 was not detected in ROs but 25% of chromophobe RCCs showed positivity. The proliferation indexes were different. Thus, ROs did not react for MIB-1 against 39% of chromophobe RCCs. With flow cytometry, 29% of the ROs were aneuploid with high S phases.

Conclusions: Bcl-2 and MIB-1 may be useful in the differential diagnosis between oncocytomas and chromophobe renal cell carcinomas. We propose an alteration in the mechanism of apoptosis as the possible pathogenesis for renal oncocytomas.

## P-338

**ULTRASTRUCTURE OF GLOMERULAR DEPOSITS IN CRYOGLOBULINEMIA**

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**Aims:** Cryoglobulins are serum immunoglobulins precipitating at lower temperatures. They have been described as having a crystalline/fibrillar configuration. Such a description is not in accordance with our experience. Therefore, the aim of this study was to reevaluate systemically our kidney biopsy material with a particular emphasis to the ultrastructure of glomerular immune deposits in patients with primary and secundary cryoglobulinemia.

**Methods:** In our archived bioptic material of 853 kidney biopsies there are 7 biopsies of 5 patients with primary mixed IgG-IgM cryoglobulinemia (210-3600 mg/l) and 23 biopsies of 14 patients with systemic lupus erythematosus (SLE) and secondary IgG-IgM cryoglobulinemia (124-3300 mg/l). For electron microscopy (EM), tissue samples were fixed in OsO4, embedded in Epon 812 and stained with uranyl acetate and lead citrate. EM analysis of immune deposits containing IgG and IgM by immunofluorescence was performed on highly magnified photos.

**Results:** Deposits of various electron density were observed in glomeruli of all the biopsies. In primary and in SLE associated cryoglobulinemia glomerular capillary wall deposits were always found homogeneous or finely granular, with no evidence of any fibrillary structure. In the mesangial matrix, however, microtubules of 10-12 nm in diameter were proven in all the biopsies studied. However, in 2 patients with SLE besides homogeneous, fingerprint deposits were also demonstrated. In one HbsAg positive female patient with primary cryoglobulinemia globular virus-like structures were also observed.

**Conclusion:** Our detailed EM study on kidney biopsy specimens after direct fixation with OsO4 and Epon embedding reveals that glomerular immune deposits in patients with primary and secondary mixed IgG-IgM cryoglobulinemia can be of various electron density but usually homogeneous or finely granular. Furthermore, we assume that cryoglobulin deposits display an organised substructure with crystalline/fibrillar configuration, described originally in 1977 by Feiner and Gallo and frequently cited as characteristic, only occasionally in a minority of patients with cryoglobulinemia.

## P-339

**IS METANEPHRIC ADENOMA A PRECURSORY TUMOR OF PAPILLARY RENAL CELL CARCINOMA?**

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**Aims**: The renal metanephric adenoma (RMA) is an uncommon entity, recently described among benign epithelial kidney tumors, with some histopathological features similar to the Wilm's tumor and the type I of papillary renal cell carcinoma (PRCC). Only one reported case called metanephric-like adenoma ("atypical") died with metastases. Simultaneous chromosome 7 and 17 gain and sex chromosome loss provides evidence that RMA is related to PRCC.
We report one case of RMA in 55 year-old women, and we analyzed the DNA content in two distinct tumor components (glomeruloid like bodies and papillary infolding).

**Methods:** DNA content was quantified by cell image analysis (CAS 200, Becton Dickinson) in the glomeruloid like bodies and papillary infolding.

**Results:** The inmunohistochemical profile (mainly cytokeratins and vimentin) shows some differences according to the growth pattern; however, the distribution of DNA content yielded similar aneuploid histograms in both components (DNA index: 1.38).

**Conclusions:** Differences were not observed in the DNA content between glomeruloid like and papillary growth of the RMA suggests one possible pathobiological relation with the PRCC.

## P-340

**CALCIUM OXALATE PRECIPITATE IN A RENOMEDULLARY INTERSTITIAL CELL TUMOUR**

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Deposition of calcium oxalate crystals in different types of human tissues may occur as a result of hereditary or acquired hyperoxalemia and in altered tissues without a concomitant hyperoxalemia. In the kidneys, crystals of oxalates have been identified in cases of hereditary oxalosis, glycol nephrosis and chronic renal disease with uremia, but also in patients with no evidence of renal or systemic disease. We report a case of calcium oxalate deposition in a renomedullary interstitial cell tumour (RICT) in an AIDS patient. The patient, aged 29 and homosexual, had died of bacterial bronchopneumonia and HIV encephalitis.
Using a partially polarized light, aggregates of large, plate-like birefringent crystals were visible in the central portion of the tumour. Crystals layed in an acellular fibrous area and were surrounded by a rim of elongated spindle shaped stromal cells. The precipitates were stained black in Yasue's silver nitrate-rubeanic acid method, a stain considered to be specific for calcium oxalate. No other deposits were observed in multiple samples of the kidneys.
RICT is a common incidental autopsy finding in kidneys of patients older than age 50. These lesions are round to ovalar, unencapsulated, up to 7 mm in diameter, pale gray to yellow, and located in the midportion of the medulla. Histologically, they are composed by ovoid to spindle-shaped stromal cells which lack distinct margins and contain abundant cytoplasmatic lipid droplets and a prominent Golgi apparatus. There is abundant intercellular collagen and mucopolysaccharyde, but not reticulin or elastin.
The combination of crystals of calcium oxalate and RICT has not been reported before in the literature. In a previous study, the authors found an increased incidence of oxalosis in an AIDS population subjected to a complete post mortem examination.

## P-341

DIAGNOSTIC AND PROGNOSTIC VALUE OF IMMUNO-HISTOCHEMICAL EXPRESSION OF INTERMEDIATE FILAMENTS IN RENAL CELL CARCINOMA

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**Aims:** Analysis of expression of intermediate filaments in Renal Cell Carcinoma (RCC) in order to determine their efficacy in the routine pathologic diagnosis of renal tumors and their metastases, as well as their prognostic connotations.

**Methods:** We studied immunohistochemical expression of keratins (polyclonal keratin and CAM 5.2 cytokeratin) and vimentin in 112 RCC, 25 samples of peritumoral kidney and 10 samples of ganglionar metastases, all paraffin-embedded. These tumors were previously classified according to cellular type, grading and staging. Ten-year survival curve of Kaplan and Meier was obtained for 90 patients and also applied in relation to immunohistochemical results.

**Results:** Polyclonal keratin and CAM 5.2 stained all peritumoral kidneys, but only 61 and 52 tumors, respectively. Coexpression of both keratins was seen in 33 tumors. There was a significant tumoral loss of staining against keratins in relation to peritumoral kidneys. Staining of ganglionar metastases against polyclonal keratin and CAM 5.2, was in accordance with primary tumor in 6 and 7 cases, respectively. Vimentin was expressed in 36% of peritumoral kidneys and in 40% corresponding tumors, but the stain was discordant between both. Staining of ganglionar metastases was positive in 8/10 cases, of which 5 corresponding tumors were also positive. There was coexpression of keratins and vimentin in 24 tumors. Vimentin expression was statistically related with high grade tumors. Poor survival was also related with patients who had vimentin expression in their tumors.

**Conclusions:** Coexpression of keratins and vimentin is not a frequent disgnostic finding in RCC, thus this may not be a primordial diagnostic criterion. In metastases of unknown origin, the loss of keratin expression can not rule out a renal origin. Poor prognosis of tumors that express vimentin, associated with high grade, do not permit us to consider vimentin as an independent prognostic factor.

## P-342

GLOMERULAR MORPHOMETRY AND DIGITAL IMAGE ANALYSIS OF INTERSTITIAL FIBROSIS IN PATIENTS WITH DIABETES MELLITUS

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Diabetic nephropathy is characterised by the appearance of glomerular, interstitial and vascular lesions that initially produce no measurable renal dysfunction. In insulin-dependent diabetes mellitus (IDDM), one of the most important morphological changes occurs in the basal membrane. This study was designed to use an automated technique to quantitatively evaluate these early changes, and to establish possible differences between patients with and without albuminuria (EAU).

We studied 23 kidney biopsies from patients diagnosed as having IDDM (15 without EAU [IDDMNA] and 8 with EAU [IDDMA]), and 8 control kidney biopsies. Sections were stained with Sirius red and immunostained for type IV collagen. The Fibrosis HR® image analysis system (Master Diagnóstica, Granada, Spain) was used to measure histomorphometric parameters. This system automatically extracts morphological areas of interest in concordance with stained zones, and automatically quantifies interstitial, periglomerular and mesangial expansion, the size of tufts and the size of glomeruli. The resulting morphometric values are expressed as percentages and absolute values in $\mu m^2$.

The table below summarises some of the results. The system is able to identify structural changes in incipient diabetic nephropathies. In particular, the percentage of periglomerular fibrosis and glomerular area were found to be significantly different ($p < 0.01$, Newman-Keuls test) between patients with and without albuminuria. In general, the results for type IV collagen were similar, although the degree of significance was lower, especially for the quantification of glomerular structures.

| Sirius red staining | % total fibrosis | % interstitial fibrosis | % periglomerular fibrosis | Mesangial area $\mu m^2$ | Glomerular area $\mu m^2$ |
|---|---|---|---|---|---|
| IDDMNA | 26.0±5.8 | 20.9±5.0 | 35.1±7.9 | 3876.2±3283.0 | 18772.0±3896.8 |
| IDDMA | 28.7±4.2 | 22.1±2.5 | 41.8±7.0 | 4951.2±2757.1 | 24583.5±5358.5 |
| Control | 17.2±2.9 | 12.9±2.5 | 24.6±3.3 | 1050.3±734.6 | 18967.5±3072.1 |
| ANOVA | p< 0.001 | p< 0.001 | p< 0.001 | p< 0.05 | p< 0.01 |

The method reported here can identify and quantify early renal lesions in IDDM, and is potentially of great value for following the progression of diabetes.

## P-343

REDUPLICATED BASAL LAMINA IN RENAL ONCOCYTOMA: AN IMMUNOHISTOCHEMICAL AND ELECTRON MICROSCOPY STUDY.

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**Aims**: Reduplicated Basal Lamina (BL) in Renal Oncocytoma (RO) is rarely described, and limited data on their immunohistochemical (IHC) and Electron Microscopic (EM) studies exist. The objective of the present study was to examine the IHC and EM appearance of the BL of 5RO.

**Methods**: Light microscopy was performed with 10% formalin-fixed and paraffin-embedded tissue stained with hematoxylin-eosin and periodic acid-Schiff (PAS) with and without diastase digestion. Immunohistochemistry was performed on paraffin-embedded sections by using the labelled streptavidin-biotin (LSBA) method and a mouse monoclonal antibody to Collagen IV (Dako). Electron microscopy was performed on each of the 5 tumors. Tissue had been fixed at the time of accession in 2.5% buffered glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Polarbed resin. Ultrathin sections were stained with uranyl acetate followed by lead citrate and examined with a Zeiss EM-109 at 80kV.

**Results**: Histologically, the tumors were composed of large uniform eosinophilic cells with prominent granular cytoplasm. Collections of hyaline material were disposed around and within nests of tumor cells in 4 cases. Finger-like projections of basement membrane connecting to the balls of hyaline material were noted. Prominent reduplication of the BL was seen in 4RO, whereas it was absent in 1 tumor. The distribution of hyaline material in PAS sections and of IHC staining for type IV Collagen correlated with the ultrastructural observations.

**Conclusions**: Our findings suggest that these cylindromatous changes in RO are most likely the result of an accumulation of BL material in protrusions of basement membrane into concavities in the plasmalemma. Since such projections occur in the normal distal tubule, our results support origin of RO from distal tubular epithelial cells.

## P-344

INFLUENCE OF IMMUNOSUPPRESSORS AND CYTOSTATICS ON EXTRACELLULAR MATRIX PRODUCTION IN EXPERIMENTAL NEPHROPATHIES.

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One of the key features of progressive glomerular injury is the extracellular matrix (ECM) accumulation. The influence of cytostatics and immunosuppressors on ECM production is poorly understood.

**Aim**: The study of methylprednisolone (MP), cyclophosphamide (CP) and cyclosporine A (CsA) action on ECM components accumulation in nephrotoxic nephritis (NTN) and puromycin aminonucleoside nephrosis (PAN).

**Methods**: PAN and NTN were induced by puromycin aminonucleoside and antibodies to GBM accordingly in Wistar rats (80-100g). On day 50 (NTN) and on day 70 (PAN) after induction of experimental nephropathies rats received 3 MP pulses (70mg/kg) during 3 days (1st group), 2 CP peroral pulses (15mg/kg) once a week (2nd group), (CsA) by intraperitoneal (15mg/kg) during 9 days (3rd group). 4-5 μ cryostat sections of kidneys and mesangial cell culture were studied by immunoperoxide method. The semiquantitative method was used to estimate the results.

**Results**: MP increased the content of laminin in vivo, in vitro; decreased the type IV collagen accumulation in vitro, but not in vivo (CP, CsA shown the same effect). CsA suppressed the laminin production, increased the level of cell-associated and plasmatic fibronectin, especially in vitro. Unlike the other medicines CP did not show any influence on the laminin production. It decreased the fibronectin accumulation in vivo and in vitro, except for the small increase of plasmatic fibronectin in vitro (PAN).

**Conclusions**: Special features of the extracellular matrix structure in different forms of chronic glomerulonephritis should be taken into consideration during the use of MP, CsA and CP in its treatment.

## P-345

GDNF EXPRESSION IN DEVELOPING AND MATURE HUMAN KIDNEYS.AND IN DYSPLASIA

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**Aims**: Renal dysplasia is a developmental aberration frequently associated with obstructive uropathy. Glial cell line-derived neurotrophic factor (GDNF) has been shown in rodents to be crucial in kidney development and ureteral arborisation.We investigated GDNF expression and apoptosis in developing and dysplastic human kidneys.

**Methods**: Four groups of human kidney specimens were studied: 7 fetal normal kidneys, 5 fetal dysplastic kidneys, 10 normal and 10 dysplastic pediatric kidneys. GDNF protein was detected by immunohistochemistry and the GDNF m RNA analysed by RT-PCR. Apoptosis was studied using the in situ end-labelling technique.

**Results**: In fetal kidney, GDNF was present in condensed blastema and ureteric buds, and their derived epithelial structures. In mature kidney, GDNF expression was present only in some tubes including collecting ducts. In both fetal and pediatric dysplastic kidneys, strong GDNF expression was found in dysplastic tubules whereas the peritubular mesenchyme was negative. Apoptosis was virtually absent in structures strongly expressing GDNF in normal and dysplastic kidneys. The presence of GDNF protein was associated with positive RT-PCR.

**Conclusions**: These are the first studies which localize GDNF expression in developing and mature human kidneys. Our results suggest that the role of GDNF is not limited to renal development but also extends to mature and dysplastic kidney. GDNF expression and apoptosis show an inverse correlation and support the role of GDNF as antiapoptotic factor in the human kidney.

## P-346

MULTILOCULAR CYSTIC RENAL CELL CARCINOMA. REPORT OF THREE CASES AND REWIEW OF THE LITERATURE.

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**Aims:** Multilocular cystic renal cell carcinoma is a rare form of cancer that is often included in the description of renal cysts, but it appears to be a distinct subtype of renal cell carcinoma with characteristical gross and microscopic features.

**Methods:** We revised three cases in our hospital, as regards, their clinical, gross and microscopic features. Patients were a 28-year-old male with a complicated renal cyst of 7 cm and two males of 69 and 48 years old with multicystic renal masses of 1.5 cm and 3cm, respectively. All tumors were diagnosed between June and September, 1998 and are free of disease nowadays.

**Results:** Macroscopically all tumors were cystic, with clear or gelatinous fluid, except for that of the 28-year-old male, which was hemorrhagic fluid. There were small areas of a variegated, yellowish, solid component, which constituted less than 10% of the entire lesion.

Microscopically, lesions were represented by multilocular cysts lined by a single layer of cuboidal epithelium and consisting of clear cells with small nuclei paracentrally oriented , with inconspicuous or no nucleoli (low grade). In the hemorrhagic case the epithelium was attenuated, and demanded examination of many areas to identify characteristical clear cells. Mitoses were very rare or absent and lacked atypical features. In some portions the tumor is clear cells formed sheets within the septa or walls of the cysts, with occasional microcysts areas that were separated by fibrovascular stroma.

**Conclusion:** In conclusion, these tumors are difficult to differenciate from nonneoplastic lesions by radiologic, cytologic, and frozen-section examination. However, we believe, it is important to recognize them because of their low malignant and metastatic potential, which can leads us to avoid radical nephrectomy when treating these lesions, in favour of a partial removal of the affected kidney.

## P-347

HISTOLOGICAL CHANGES IN PERITUMORAL TISSUE OF RENAL CELL CARCINOMA AFTER THE CHERNOBYL ACCIDENT IN UKRAINE

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**Aims:** During the 13 year period subsequent to the Chernobyl accident the morbility of RCC in adults has increased from 4.7 to 7.0 per 100,000 in Ukraine. Cesium 137 is responsible for 80-90% of the incorporated radioactivity in people exposed to long-term, low doses of ionizing radiation and 80% of the more labile pool of Cesium is excreted via the kidneys. We investigated the prevalence and morphological peculiarities of dysplasia and CIS in kidneys with RCC, that could be associated with radiation exposure in the Ukrainian population.

**Methods:** Histological study from peritumoral tissue samples of RCC obtained from 180 patients, operated after the accident were selected for 5 groups. The control Ukrainian group I consisted of all 26 patients who inhabited clean (without radioactive contamination) areas. Group II consisted of all 55 patients who lived in less radiocontaminated areas, including Kiev-City, operated in 1993-1996. Group III consisted of all 66 patients, who lived close to Group II areas, but were operated during 1997-1998. Group IV consisted of all 14 patients who lived in the more radiocontaminated areas and were operated in 1993-1996. Group V included all 20 patients who lived close to Group IV areas but were operated during 1997-1998. The control Spanish Group VI consisted of all 25 patients selected in Valencia for present study.

**Results:** The incidence of dysplasia and CIS was 50%, 67%, 88%, 73%, 94%, 8%, in groups I, II, III, IV, V, and VI respectively. A significant increase of cortical and especially medullar predominantly moderate dysplasia was found in groups II, III, IV and V, as compared to group VI. Moderate and severe irradiational-like lesions with the multiple areas of apoptosis and regeneration of collecting ducts epithelium in medulla of Groups V, IV and III in combination with large areas of dysplasia were found.

**Conclusions:** Since the irradiational lesions have been shown to be the strongest in Groups II, IV and V, that have been associated with increased incidence of dysplasia, the present results suggest that long-term low dose ionizing radiation may be associated with RCC and their accelerated progression.

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## P-348

**TUBULAR AND INTERSTITIAL EXPRESSION OF ICAM-1 IN IgA NEPHROPATHY: RELATIONSHIP WITH RENAL DYSFUNCTION AND DISEASE PROGRESSION.**

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**Aims:** To analyze the relation between tubular and interstitial ICAM-1 expression and the renal dysfunction in IgA nephropathy (IgAN).

**Methods:** ICAM-1 expression in tubular epithelial cells and interstitial inflammatory cells was assessed in renal biopsies from 32 patients with IgAN, using the monoclonal antibody CD54 with the avidin-biotin peroxidase technique, and correlated with proteinuria (Pr), creatinine level in serum (Cr) and blood pressure (BP) at the moment of the renal biopsy and after 2.4±2 years. An increase ≥50% over the initial levels of Pr or Cr was considered as progressive disease.

**Results:** Tubular epithelium was positive for ICAM-1 in 13 biopsies, the median value being 0.11±0.18 $mm^2/mm^2$ of tubule. Pr was 2.7±1.5 g/24h in patients with tubular ICAM-1 expression versus 1.5±1.8 g/24h (U=44, p=0.005) in patients without tubular expression. Correlation was found between ICAM-1 tubular expression and Pr (r=0.4059, p=0.02). The average number of ICAM-1+ interstitial leukocytes was 234±307 /$mm^2$ of interstitium. In patients with high BP, ICAM-1+ interstitial leukocytes were 379.3±371 /mm2, versus 108±164.3 /mm2 (U=44, p=0.03) in patients with normal BP. Correlation was found between ICAM-1 interstitial expression and Cr (r=0.6343, p<0.001). In patients with increase ≥50% in Cr, ICAM-1+ interstitial leukocytes were 516±360 /$mm^2$, versus 66±87.8 /$mm^2$ (U=16, p=0.004) in patients with stable Cr.

**Conclusions:** Tubular and interstitial ICAM-1 expression would reflect the severity of the renal disturbance in IgAN. Interstitial ICAM-1, more than tubular ICAM-1, can play a role as a marker of progression in this disease.

## P-349

THYMOSIN BETA-10 EXPRESSION IN HUMAN RENAL TUMORS

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**Aims:** Thymosin beta-10 has been shown to be major intracellular actin-binding protein that inhibits actin polymerization and increase cell mobility. Thymosin beta-10 is overexpressed at both the mRNA and protein levels in human tumors and recent research links thymosin gene expression and cancer progression. In this study we describe the results of immunohistochemical expression of thymosin beta-10 on paraffin embedded human renal tumors.
**Methods:** We analyzed 65 renal cell carcinomas (RCCs) of various histological subtypes, and surrounding normal tissues. A newly described polyclonal antibody anti-TB10(38-43) against the carboxy-terminal peptide fragment (amino acids 38-43) of thymosin beta-10 was applied to the tissue sections using the streptavidin-biotin-peroxidase method. Staining score was 0 if the tumor cytoplasm stained no stronger than the background, 1+ if the stain was slightly greater, 2+ for intermediate level of staining and 3+ for very intense staining.
**Results:** Thymosin beta-10 cytoplasmic immunoreactivity was detected in 83% (54/65) of RCCs. In 57% (37/65) of carcinomas the stain was moderate to strong. Tumors with high nuclear grade exhibited higher thymosin beta-10 immunoreaction and this relationship was found statistically significant ($p<0,01$). Papillary carcinomas showed no immunoreactivity. No relationship was observed with tumor stage. Some benign tissues (15%) surrounding tumors expressed weak cytoplasmic reactivity, confined mainly to proximal convoluted tubules. In the rest of the cases immunoexpression of the renal parenchyma was constantly negative.
**Conclusions:** Thymosin beta-10 is highly upregulated in malignant renal tissue and its association with nuclear grade may suggest a potential prognostic value.

## P-350

P-GLYCOPROTEIN, TGF-BETA AND ENDOTHELINS IN CHRONIC TRANSPLANT NEPHROPATHY. A MOLECULAR STUDY.

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In kidney transplants molecular procedures have contributed to define the role of specific cytokines such as perforin, granzyme B, IL-2, IL-7, IL-10 and IL-15 in triggering acute transplant rejection. In chronic transplant nephropathy, only increase of TGFß-1 mRNA has been related with the genesis of interstitial fibrosis that characterises this process.
We present the study of 33 kidney biopsies from patients having chronic transplant nephropathy based on the use of RT-PCR to determine the expression levels of the most important mediators in this lesion (i.e., TGFß-1, endothelin 1 (Et1), endothelin 3 (Et3), angiotensinogen) and P-glycoprotein (P-gp), as a regulator of nephrotoxicity of immunosuppressive drugs.The ethidium bromide staining gels for each marker were analysed semiquantitatively by densitometry using ß2-microglobulin mRNA as an internal marker.
Our study made it possible to establish differences in the amounts of mRNA for each marker between control and patients diagnosed as chronic transplant nephropathy. All markers showed an increased expression in this group compared with the control group, being more evident in P-gp and TGFß-1 (0.447 Vs 0.255, and 0.607 Vs 0.319, $p<0.05$, respectively). On the other hand, there was a direct correlation between markers, both in chronic groups and all groups together, showing considerable overlap in all cases, especially between P-gp and ET1 (Spearman' s test, $r=0.734$, $p< 0.001$). A finding of note was the clear inverse correlation between mRNA levels of each marker and Banff score :patients expressing the highest levels of lesion markers had the lowest Banff score, principally for P-gp and Et-1.
The use of molecular biology techniques to quantify mRNA of markers of chronic transplant nephropathy lesions is a promising approach in the diagnosis of this pathology. The notable differences between patients in the levels of mRNA for each marker studied here raise the possibility of using specific pharmacological treatments for each (e.g., ACE inhibitors for excess levels of angiotensinogen mRNA, or in the near future, type A endothelin receptor blockers). The notable inverse correlation between each marker studied here and Banff score suggested a soon study of these markers in order to address the origin of these lesions and furthermore to refine the prospective analysis of chronic transplant nephropathy and possibly its response to treatment.

## P-351

**LIVER ADENOMATOSIS. CASE REPORT AND LITERATURE REVIEW.**

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**Aims:** Liver adenomatosis is a extraordinarily rare lesion, of unknwon pathogenesis, defined arbitrarily by Flejou et al, as the existence of more than 10 hepatic cell adenomas within a normal hepatic parenchyma. Clinical manifestations are abdominal pain, hepatomegaly, intratumorous and peritoneal bleeding. We report the case of a 25 year-old healthy man, that suffered at work a mild trauma. Soon after he collapsed and died. He had no history of hormone medication.
**Methods:** A forensic autopsy was done. Samples of liver tissue were stained with H.E., Masson and Wilder. The liver adenomatosis cases published are reviewed.
**Results:** At autopsy of the case reported, a 1.5 l hemoperitoneum was found. The liver had a multinodular surface with a 5 cm laceration of the capsule in the left lobule. The parenchyma showed 20 well circumscribed yellowish nodules, of 0.5-8 cm in diameter, with focal hemorrhagies in the biggers and normal hepatic parenchyma between the nodules. Liver histology showed encapsulated and unencapsulated nodules, that consisted of normal hepatocytes arranged in cords of various sizes, with no portal triads or bile ducts, but with abundant venous structures and peliotic cavities. One of this cavities opened through the capsule. A liver adenomatosis diagnosis was done. There are only 24 cases published (19 female-5 male, mean age of 37 year-old). In ten cases is associated with the use of oral contraceptives. Bleeding occurs in tumours of 4 cm or more.
**Conclusions:** The outcome of patients with liver adenomatosis is not bad, except those with massive intraperitoneal bleeding, causing unexpected death. In no case was a malignant transformation.

## P-352

**COMPARATİVE ANALYSİS OF C VİRUS HİSTOLOGY B HEPATİTİS**

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**Aims:** Rate of certain histopathologic features of hepatitis C were looked for in hepatitis B materials and evaluated statistically in Turkish population.
**Method:** Presence of lymphoid aggregate, steatosis, ductal lesion, sinusoidal inflammation and portal iron deposition were searched in 80 C and 104 B hepatitis specimens and evaluated using chi-square test. Pan T and B cell markers were used for lymphoid aggregate analysis. P53 was applied for the possible impact of steatosis on cell biology.
**Results:** All mentioned parameters excluding iron deposition were found significantly higher in C hepatitis samples ($p<0.05$). Copresence of lymphoid aggregate and ductal lesion was found meaningful in C hepatitis also. Distribution of T and B cells was similar in both groups. P53 was found negative in samples showing steatosis.
**Conclusion:** Our results except portal iron deposition were thought to be in correlation with the reports in the literature. Absence of iron deposition might be related to geographic differences that could be observed as a feature of C virus.

## P-353

**CORRELATION BETWEEN A CLINICAL SCORE AND HISTOLOGICAL FINDINGS IN HEPATIC TRU-CUT BIOPSY OF ASYMPTOMATIC HCV-INFECTED PATIENTS.**

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**Aims:** A clinical score (CS) based on clinical, analytic and ecographic data and defined over a previous retrospective series is used in our Hospital for follow-up of asymptomatic HCV-infected patients. The aim is to correlate CS, histological diagnosis (HD) and fibrosis pattern (FP) of hepatic tru-cut biopsy (HTB) in a prospective series.

**Methods:** In 134 asymptomatic HCV-infected patients with alteration of hepatic analytic values HTB was performed. CS was worked-out and HD (grouping chronic hepatitis/suspicious or definite cirrhosis) was emitted. 6 progressive FPs were determined (1, 2, 3: none, occasional and some bridges with preserved architecture, 4: segmentary presence of well-defined nodules, 5: bridges delimiting parenchyma with nodular contours, 6: definite cirrhosis) and evaluated until agreement by two pathologists without any information. Statistical analysis was made.

**Results:** CS mean values increment linearly with progressive FP. When FPs are grouped as (1+2+3), and compared with (4+5+6), CS means are significantly different with $p<0.001$. FP4-SC mean is significantly different from FP(1+2+3)-CS, but not from FP(5+6)-CS (ANOVA, T-test). Correlation between HD and FP (1+2+3)/FP (4+5+6) groups is statistically significant with $p<0.001$ (chi-square).

**Conclusions:** Clinical score (CS) is a good tool for evaluation of asymptomatic HCV-infected patients, as it increases with hepatic fibrosis and has significantly different mean values between FPs consistent with chronic hepatitis and FPs suspicious or consistent with cirrhosis. FPs have good correlation with HD.

## P-354

EPSTEIN-BARR VIRUS ASSOCIATED CHOLANGIOCARCINOMA WITH LYMPHOEPITHELIOMA-LIKE COMPONENT

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**Aims:** Epstein-Barr virus (EBV) associated cholangiocarcinoma with lymphoepithelioma-like component has only been described in a female Chinese patient. To date its has been poorly characterized as a distinct tumor entity.

**Methods:** Immunohistochemistry (IHC) was performed on paraffin-embedded sections by using the labelled streptavidin-biotin (LSBA) method. The antibodies used in the study included anti-LCA (CD-45), UCHL1 (CD45 R0), L26 (CD 20), LMP-1 (CS1-4), p53 (D0-7), and bcl-2 (Clone 124); all were obtained from Dako. Cytokeratin cocktail (CK22 Biomeda) and cytokeratins (AE1, 5D3 Biogenex). Paraffin-section in situ hybridization (ISH) was performed using the EBV-encoded small RNAs (EBERs) with an EBER1 oligonucleotide probe labelled with fluoresceine-isothiocyanate (Dako).

**Results:** A 19-year-old female Spanish patient, received an extended left segmentectomy for a huge hepatic tumor of 5,5 cm in diameter. Light microscopy revealed a cholangiocarcinoma composed of both well-differentiated adenocarcinoma and lymphoepitheliomatous undifferentiated carcinoma components. By IHC, the tumor showed strong and difuse expression for cytokeratin AE1, 5D3, and CK22. The small lymphocytes in the stroma showed staining for LCA. An admixture of UCHL1+ and L26 + lymphocytes was found, and the former was predominant. The tumoral epithelial cells were positive for p53 in more than 75% of the cells, but negative for bcl-2 ad LMP-1. Abundant EBV EBER1 was detected in both tumor components, but not in the lymphoid stroma and the nontumor liver.

**Conclusions:** These findings imply that EBER1 ISH is more sensitive than LMP-1 IHC on paraffin sections in detecting carcinoma-associated virus. The results suggest that unusual cholangiocarcinoma in Western patients may share similar EBV-related pathogenesis with that of Taiwanese Chinese.

## P-355

HEPATOCELLULAR CARCINOMA OCCURRING IN NON-FIBROTIC LIVER: IS NON-TUMORAL LIVER NORMAL? ANALYSIS OF 80 CASES.

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**Aims:** Carcinogenesis of hepatocellular carcinoma (HCC) occurring in non-cirrhotic liver remains unclear. The aim of our study was to assess the histopathological changes in the non-tumoral liver of patients developping HCC without cirrhosis.

**Methods:** Out of 330 cases of HCC surgically resected in our institution between 1985 and 1998, we retrospectively analysed 80 (24%) cases (53 men, 27 women; mean age: 51±16yrs) in which the non-tumoral liver showed no (n= 28) or minimal (n= 52) portal fibrosis without any septal fibrosis. Sixty patients (82.5%) had no risk factors for development of HCC; 11 were HBsAg positive; 2 had anti-HCV antibodies; 1 had homozygous C282Y mutation of HFE.

**Results:** Among the 80 tumors, 67 (83.5%) had trabecular, acinar or solid growth patterns, 8 (10%) were fibrolamellar HCC and 5 (6.5%) were hepato-cholangiocarcinomas. Mean tumor size was 10±5cm (range from 2 to 25 cm). In the non-tumoral liver, lobular architecture was normal; large and small liver cell changes were noted in 3 and 1 cases, respectively; others lesions are indicated in the table:

| | Absent (%) | Mild (%) | Moderate (%) | Severe(%) |
|---|---|---|---|---|
| Portal inflammation | 22 | 56 | 17 | 5 |
| Periportal necrosis | 88 | 9 | 3 | 0 |
| Lobular necrosis | 86.5 | 12 | 1.5 | 0 |
| Steatosis | 45 | 34 | 14.5 | 6.5 |
| Iron overload | 43 | 37 | 15 | 5 |

**Conclusion:** in patients with HCC occurring on non-fibrotic liver, the non-tumoral part of the liver shows non specific minimal changes without evidence of regeneration. Dysplasia is very rare. Such findings emphasize the need of molecular studies to better characterize mechanisms involved in hepatocarcinogenesis in such patients.

## P-356

**EVALUATION OF IMMUNOHISTOCHEMICAL MARKERS AND ALBUMIN RNA *IN SITU* HYBRIDIZATION FOR THE DIAGNOSIS OF HEPATOCELLULAR CARCINOMA**

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**Aims**: The differential diagnosis of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) or metastatic carcinoma of the liver may be difficult. Immunohistochemistry is a useful tool to differentiate between these tumors. The aim of this study was to evaluate different antibodies and *in situ* hybridization for albumin as marker for the diagnosis of HCC.

**Methods**: Formalin-fixed, paraffin-embedded liver biopsies were analyzed by immunohistochemistry using antibodies against alpha-fetoprotein (AFP), the carcinoembrionic antigen (CEA), cytokeratin 19 (CK19), the epithelial membrane antigen (EMA) and the monoclonal antibodies hepatocyte (HEP) and D-11, directed against hepatocyte proteins. In addition, *in situ* hybridization for albumin RNA using a riboprobe and signal amplification by biotinylated thrymine were performed.

**Results**: A total of 40 tumors were analyzed, including 22 HCC, 5 CC, 3 mixed HCC-CC and 10 other carcinomas. All 22 HCC showed reactivity to the D-11 antibody and 14 (63.6%) were positive for the HEP marker. AFP and the expression of albumin RNA were found in 15 (68.2%) and 6 (27.3%), respectively. Four HCC were positive to the D-11 antibody only. Reactivity to CEA, EMA and CK19 was observed in 27.3%, 18.2% and 18.2% of HCC, respectively. In contrast, D-11, HEP, albumin RNA and AFP were never positive in the CC or the other carcinomas analyzed. In the 3 tumors with histological features of a combined HCC-CC, 1 showed reactivity for D-11, Hep and albumin RNA and one to D-11 and AFP, respectively. CK19 and EMA were positive in all 3 of the HCC-CC as they were reactive in 11 (73.3%) and 7 (46.7%) of a total of 15 CC/other carcinomas, respectively.

**Conclusions**: Our data show that the D-11 antibody has a high, the HEP antibody and *in situ* hybridization for albumin RNA a considerable sensitivity. and all three marker show a specificity of 100%. HCC-CC, as expected for this composite tumor, reveals a broad spectrum of reactivity.

## P-357

BENIGN CYSTIC TUMOURS OF THE PANCREAS. PATHOLOGIC OBSERVATION.

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**Background:** Cystic pancreatic lesions comprise relatively frequent pseudocysts, congenital and retention cysts, as well as rather uncommon cystic neoplastic growths with benign (cystadenoma), borderline malignant (mucinous cystic-MCT and solid-pseudopapillary tumour-SPT), and malignant potential (cystadenocarcinoma and solid-pseudopapillary carcinoma). Since clinical manifestations are not characteristic, pathohistological and immunohistochemical analysis is essential in defining these growths exactly.

**Methodology**: Two patients with MCT and three patients with SPT (4 women, 1 man, aged 49-72yrs.) were studied. Conventional pathohistological examination was completed with immunostaining. Differentiation between these benign appearing growths and ductal carcinoma was morphometrically evaluated by mitotic rate, nuclear atypias and proliferative activity (PCNA, Ki67).

**Results:** 7-15cm large well demarcated tumours involved the head, body or tail of the pancreas. Due to encapsulation and no invasion into the surrounding tissue they were completely resected. The **MCTs** consisted of several cysts lined by tall columnar epithelium with papillary projections and focally dysplastic cells. Among cylindric epithelial cells producing acid mucin (PAS and AB positive) some endocrine cells were present (NSE and serotonin positive). Cytokeratins, CEA, and Ca 19-9 were weakly positive, vimentin was negative. The **SPTs** expressed diverse staining characteristics such as α-1 antitrypsin positivity and diffuse reaction for vimentin, keratins (positive in two cases), and S100 (positive in one). According to the histologic criteria for malignancy our cases were proclaimed as benign.

**Conclusion:** The cystic tumours of the pancreas represent heterogeneous growths evaluated by histologic phenotyping and functional identification. Their pathohistological features suggest cellular differentiation into both exocrine as well as endocrine lines.

## P-358

**HISTOLOGICAL FEATURES OF A SERIES OF 94 PATIENTS WITH NON ALCOHOLIC STEATOHEPATITIS (NASH).**

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Sporadic patients without alcoholic habit suffer a chronic liver disease (LD) clinicopathologicaly similar to the alcoholic hepatitis which is called NASH.

**Aim**:To analyse the histological features of a series of biopsies with EHNA.

**Patients, Material and Methods**: Clinical criteria to diagnose possible NASH in 94 patients were: x2 increase normal seric level of ALT and AST for >6 months, echografic studies consistent with steatosis (bright liver), no ethiological markers for LD (seronegativity for VHB, VHC, and non organ specific antibodies) and exclusion of ethanol abuse (unequivocal denied by the patient and relatives plus nule or low level of seric desialated transferrin). Confirmation was achieved by histological criteria in the needle liver biopsies (steatosis, hidropic liver cell degeneratións associated with some neutrophilic infiltate). Qualitative *items* were evaluated by 2 pathologists as in Table.

| RESULTS: | 0 (%) | minimal(%) | mild (%) | moderate() | intense (%) |
|---|---|---|---|---|---|
| Fibrosis(stage) | 25,5 | 57,5 | 10,6 | 6,4 | 0 |
| Lobular inflam.activity | - | 75 | 19,6 | 5,4 | 0 |
| Hidropic change | - | 45,4 | 42 | 9,6 | 0 |
| steatosis (grade) | - | 11,7 | 20,2 | 33 | 35 |
| steatosis (type) | Macro | Micro | mix. >macro | mix.>micro | -------- |
| | 32,9 | 0 | 63,8 | 3,2 | |
| steatosis (loc.) | 63,8 (Z3) | 2,1(Z1) | 34(difuse) | | -------- |

**Conclusions**:1) Some patients were in cirrhotic stage at diagnosis and 2) all fibrotic stages were found, but most of the patients(83%) were identified in the very early phase of the disease. 3) These findings suggest that NASH is an evolutive disease and 4) biopsy is mandatory for confirmation and staging.

## P-359

IMUNOCYTOCHEMICAL AND MORPHOMETRIC ANALYSIS OF HEPATIC CARCINOMAS. A STUDY OF 53 CASES FROM CONTINENTAL CHINA

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**Aims:** In addition to several environmental factors, molecular and biological events have been involved in the genesis and progression of hepatic carcinomas. These factors include mutations of the p53 gene and infection with HbB virus, as well as activation and over-expression of hepatocyte growth factor (HGF), its receptor C-Met and the BCL-2.

**Methods:** We have studied the immunohistochemical expression of VHBc, VHBs, p53, CK7, CK19, HGF, C-Met and BCL-2 in 53 cases of primitive hepatic carcinoma: 39 hepatocellular carcinomas (HCC), 8 cholangiocarcinomas (CC) and 6 hepatocholangiocarcinomas (cHCC-CC), from the files of the Department of Pathology, Cancer Institute, Beijing, China. Immunocytochemical was carried out on paraffin-embedded tissues following the ABC peroxidase method and antigen retrieval procedures.

**Results:** Hbs Ag expression was mainly correlated with HCC and was not seen in the other groups. C-Met and HGF were ubiquitously detected independently of the degree of differentiation, with loss of the expression of HGF in the poorly differentiated HCC. BCL-2 expression was found in the poorly differentiated neoplasms (HCC degree IV) more than in better-differentiated HCC. The cytokeratin 19 expression was detected in all CC of our cases, as opposed to cHCC-CC and HCC. The cytokeratin 7 was detected in all tumours and non-neoplastic hepatic cells. Regarding DNA content, aneuploidy was found in almost all cases of hepatic carcinoma, compared with the diploid content of the normal hepatic tissue.

**Conclusion:** The incorporation of these markers could be useful for establishing the diagnosis (and differential diagnosis) and prognosis of HCC. The presence of hepatitis B viral antigen seems to be important in the development of hepatocellular carcinoma, particularly in countries with high incidence of the disease, such as China.

## P-360

HEMOPHAGOCYTIC SYNDROME : A UNRECOGNIZED CAUSE OF MULTIORGAN FAILURE WITH TYPICAL LIVER PATHOLOGY.

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Hemophagocytic syndrome (HS) is a uncommon and severe clinico-biologic entity characterized by systemic proliferation and activation of benign monocyte-macrophage cells (M/M), with hemophagocytic properties. Most HS are secondary to viral or immune diseases, neoplasia or drugs.

**Aims** : To report cases of HS, with special reference to the clinical context and to liver pathology.

**Methods** : Review of liver biopsies performed in a context of unexplained liver test disturbances, fever, cytopenia and progressive multiorgan failure.

**Results** : 5 cases of HS were found, based on classical clinical, biological and histological criteria.They developed in 2 males (59 and 60 yr-old) and 3 females (9, 35 and 60 yr-old). There was a history of lymphoproliferative disorder in all of them (2 NK lymphomas, one cutaneous B lymphoma, one Hodgkin's disease, one EBV-related atypical lymphoproliferation). All cases had cholestasis and some degree of cytolysis ; one patient presented with fulminant hepatitis. EBV hybridization was positive in 4/5. Liver biopsy showed various degree of cholestasis, some apoptotic hepatocytes and a striking dense sinusoidal infiltrate made of activated CD 68 positive M/M with microvacuolar cytoplasm and features of hemophagocytosis, in the absence of specific tumoral infiltration. The same typical features of HS were also observed in the lung (n=2), bone marrow (n=2), duodenum, spleen, and lymph nodes (n=1, each). Four patients out of 5 rapidly died of disease.

**Conclusion** : HS, is a severe condition which must be suspected in the presence of prolonged pyrexia, cytopenia, multivisceral failure and liver function test disturbances, specially in predisposed patients. Liver biopsy, like myelogram, is very contributive to establish the diagnosis at this time .

## P-361

HISTOMORPHOLOGY OF HCV INFECTION IN CHRONIC HEMODIALYSIS PATIENTS

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**Aims:** In hemodialysis patients, the prevalence of anti-HCV positivity is from 5 to over 50%, with 0.7% incidence per year. Previous blood transfusions, mode and duration of therapy, and nosocomial transmission appear to be the main causes. The aim of our study was to evaluate the histologic changes in the livers of renal transplant candidates infected with HCV, and to compare their liver damage with liver lesions in HCV infected patients without kidney failure.

**Methods:** HCV positivity was determined by type specific PCR analysis. 60 patients were divided into two groups: HCV positive without (50) and with (10) end stage kidney failure. Necroinflammatory changes (activity) of chronic hepatitis and structural alterations (staging) were graded according to the proposed criteria (Ishak). The scores generated for each separate grading component were compared. Statistical analysis was performed using a nonparametric Wilcoxon rank test with the level of significance at $p<0.05$.

**Results:** 40% of patients with end stage kidney failure had chronic mild (persistent) hepatitis, and 60% had chronic active hepatitis of moderate intensity. Between the both groups of patients no statistically significant differences comprising periportal or periseptal inflammation and fibrosis, intralobular inflammation, the presence of focal necrosis, and apoptosis were observed.

**Conclusions:** Kidney failure itself seems not to increase the damaging influence of HCV on the liver. However, it seems reasonable to perform liver biopsy in patients with end stage kidney and HCV infection since no normal liver histology was found in any of such patients included in our study.

## P-362

PHENOTYPIC ANALYSIS OF ATYPICAL DUCTULAR REACTION IN HUMAN LIVER

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**Aims:** Atypical ductules in human liver are regarded similar to the oval cells in rodents. While oval cells have been studied intensively for decades our knowledge about their human counterparts is quite limited. Therefore we decided to study the phenotype of these structures.

**Methods:** 10 specimens were collected from regenerating human livers after submassive necrosis, containing atypical ductular structures. Immunohistochemical analysis was performed with special emphasis on the growth factor/receptor molecules, which are thought to drive the oval cell reaction in rat liver.

**Results**: Atypical ductules are decorated by CK-7 but no AFP and CD-34 could be demonstrated in these cells.

The following growth factors/receptors could be found in the studied livers: HGF - c-met; TGF-α - EGFR; TGF-β - TGF-β RII; SCF.

Urokinase type plasminogen activator was also expressed in the ductular structures and they had a close relationship with SMA positive activated myofibroblasts.

**Conclusions:** Although phenotypic differences could be observed between the human and rat ductular cells, the most important growth factors/receptors which participate in the regulation of the oval cell reaction are also expressed in the human specimens. Our results further support that the atypical ductular cells are functionally similar to the rat oval cells.

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## P-363

**CHANGES IN THE BILIARY- AND HEPATIC-LIPID COMPOSITION AND HEPATIC LESIONS IN HYPERCHOLESTEROLEMIC RABBITS. EFFECT OF THE DIETARY FAT TYPE (OLIVE OIL, SUNFLOWER OIL, FISH OIL).**

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**Aims:** We seek to ascertain whether the administration of diets with different fatty-acid profiles (MUFA, PUFA) having been proven a hypolipemic effects influence the content of biliary lipids, the hepatic-lipid composition and hepatic lesions in rabbits with hypercholesterolemiac diet.

**Methods:** ***Animals design***. 30 male New Zealand rabbits were divided into 5 groups. 4 of them were fed on atherogenic diet for 50 days. The fifth one was fed on standard diet for the same period (control group C). After this period 3 atherogenic groups were fed on during 30 days with different diets what differed only in their lipid source: fish oil (F), olive oil (O) and sunflower oil (S). C continued being fed on the chow diet for the same period. ***Analytical procedures***. Triglycerides (TG), total cholesterol (CT), free cholesterol (FC), and phospholipids (PL) were measured in liver and bile. Biliary acids were also measured in bile. ***Histology***. We evaluated the steatosis (macro-microvesicular), its distribution, bile deposits, cellular atypia, fibrosis, inflammatory infiltrate, ductular proliferation and vascular pathology in liver.

**Results: 1.- Bile**. The atherogenic diet raised the molar percentage of FC and PL but decreased the biliary acids (BA) excretion vs C. The percentage of BA rose only in O group reaching values similar to C group. The molar percentage of CT significantly fell in O and S groups vs atherogenic. **2.- Hepatic composition**. Hypercholesterolemia gave rise to hepatic steatosis with sharp increases in the biochemical parameters.During the recovery period, the diets rich in PUFA lowered hepatic CT and PL more than did the diet rich in MUFA **3.- Hepatic lesions.** The fatty distribution was high in zones 3 and 2 (type microvesicular). In O group the pericellular fibrosis was significantly lower than the other groups although the steatohepatitis was higher in O and F groups. **Conclusions**: The intake of olive oil removed less CT and PL from liver producing a bile no litogenic and inducing less fibrosis in liver. However this diet provokes more lobular inflammation in this organ.

## P-364

**ADENOSQUAMOUS CARCINOMA OF THE LIVER : REPORT OF A FATAL JUVENILE CASE**

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**Aims :** Adenosquamous carcinoma (ASC) of the liver is an exceptional neoplasm wich occurs generally in adults. We report the first juvenile case. Using immunohistochemical findings, primary or secondary origin and nomenclature pathogenesis are discussed.

**Case Report :** A 15-year-old woman presented with a 1 month history of weight loss, abdominal pain and enlarged liver. Computerized tomography revealed a large mass occupying right and left lobes of the liver and three bilateral pulmonary masses. Coelioscopic specimen was resected for pathologic examination. She died 6 months after surgery.

**Pathologic findings :** The resected specimen contained a tumor composed of both an area of adenocarcinoma (AC) and an other area of squamous cell carcinoma (SCC). The tumor consisted predominantly of stratified polygonal malignant cells with prominent nuclear atypia. Some bridges, many concentric stratifications with keratinization and glandular differentiation with Alcian blue positive mucin-producing substances were observed. A transition area between AC and SCC was recognized. Using antibodies of cytokeratin (CK) 7, 8, 18, and CEA, AC component were positive with CEA, keratinazing zone with CK8,18 and both AC and SCC were positive with CK7.

**Conclusion :** Thirty-six cases of ASC have been reported including the present one.The 2 components AC and SCC were positive for CK7 wich in the liver is specific for the bile duct epithelium and may suggest that ASC in the liver develops from a squamous change of a cholangiocarcinoma. The prognosis is poorer than that of patients with common type of cholangiocarcinoma.

## P-365

*IN SITU* DETECTION OF HEPATITIS C VIRUS (HCV) IN CIRRHOSIS BY IMMUNOHISTOCHEMISTRY (IHC) AND PARALLEL TISSULAR QUANTITATIVE PCR (qtRT-PCR).

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**Aims:** i) Detection and semi-quantitative evaluation of HCV Ags in cirrhotic liver by IHC, ii) Quantitation of HCV RNA by qtRT-PCR in the same tissue samples, and iii) Comparison of the results of both methods.

**Methods:** Twenty five frozen cirrhotic samples from patients HCVAb+ in the serum who underwent liver transplantation were tested. For IHC, we used a FITC-conjugated spontaneous human polyclonal Ab† and Dako EnVision amplification system. Normal human Ig, omission of the primary Ab, HCV negative and positive tissues were used as controls. The staining was quantitated according to the % of + cells (1 : < 10%, 2 : 10-50%, 3 : >50%) and its intensity (low, moderate, strong). For qtRT-PCR, Roche Amplicor kit was used and the results were expressed as a number of HCV copies/µg of total extracted RNA. The % of + cells and the intensity of staining were compared with PCR levels with appropriate statistical tests.

**Results:** 76% (19/25) and 84% (21/25) of cirrhosis were found positive by IHC and qtRT-PCR respectively. The immunostaining was only found in hepatocytes, quite exclusively in the cytoplasms, with various intensity, and no zonal distribution. Controls were satisfactory. Amongst the 6 negative cases by IHC, 4 were PCR negative and 2 had a very low viral copy number. Furthermore, there was a significant correlation between the % of + cells (1, 2, or 3) and the amount of RNA in the tissues. The intensity of staining also grew parallel with the amount of RNA, but this was not statistically significant.

**Conclusions:** The IHC method has a good feasibility, reproducibility and sensitivity as compared with qtRT-PCR. The % of + cells detected by IHC correlates with the amount of HCV RNA in the cirrhotic tissues. IHC could provide complementary data about HCV pathobiology.

*† Ballardini et al. Hepatology 1995; 21: 730-4.*

## P-366

**UTILITY OF DECADES-OLD ARCHIVAL PARAFFINED LIVER BIOPSIES FOR HEPATITIS C VIRUS (HCV) RETROSPECTIVE STUDIES BY RT-PCR.**

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**Aim:** Until now, archival liver biopsies have only been used for histopathological diagnosis and, as a consequence, HCV retrospective studies were limited to the availability of patient serum. Drawbacks of RNA extraction in paraffin embedded tissue, such as RNA fragility, or small sample size or archival biopsy age has been overcome with an improved RT-PCR method and assessed in over 25 year-old archival liver biopsies.

**Methods**: RNA was extracted from 88 archival paraffined liver biopsies from 1971 to 1996: 27 explant liver samples from 27 patients (16 serum HCV-RNA positive and 11 HCV-RNA negative) who underwent liver transplantation from 1988 to 1996 and 61 Trucut liver biopsies from 1971 to 1985 (mean age 20.2 ± 3.7 years) from patients diagnosed at the time of sampling as non-A, non-B (NANB) chronic hepatitis (n = 12), alcoholic fibrosteatosis (AF) (n = 41) and normal histology (n = 8). HCV-RNA was amplified with primers from 5' non-coding region. Albumin mRNA was used as housekeeping gene in all analysis.

**Results:** HCV-RNA was amplified in 27 samples: 16/16 (100%) HCV-RNA positive patients, 8/12 (75%) NANB chronic hepatitis and 3/41 (7.3%) alcoholic fibrosteatosis patients. According to sample age and histological or serological diagnosis, sensitivity of the assay was :

| 1971-1975 | 1976-1980 | 1981-1985 | 1986-1990 | 1991-1996 |
|---|---|---|---|---|
| NANB: 2/4 (50%) AF: 2/11 (18.1%) | NANB: 2/4 (50%) AF:0/13 (0%) | NANB:4/4 (100%) AF: 1/17 (5.9%) | HCV: 6/6 (100%) | HCV: 10/10 (100%) |

**Conclusions:** This methodology can be a useful tool to carry out retrospective studies of HCV infection in archival paraffin liver biopsies.

## P-367

PROGNOSTIC FACTORS IN CURATIVELY RESECTED COLORECTAL LIVER METASTASES

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AIM: To establish prognostic factors in curatively resected colorectal liver metastases.

METHODS: A series of 111 patients with colorectal liver metastases were resected with curative intention in Lund from 1971 to 1995. The impact of several clinical and the following morphological parameters was determined, namely number of tumors, extrahepatic tumor, tumor replacement of liver volume, diameter of largest metastasis, unilateral vs bilateral tumor, satellite metastases, grade of liver tumor, venous vascular invasion, degree of tumor necrosis in a peripheral section, degree of mucin in the tumor, degree of inflammatory cells, degree of irregularity of tumor margin, degree of fibrosis around the tumor and immuno-histochemistry of, so far, p53, MIB-1, CEA, p27, metallothionein and DCC (deleted in colorectal carcinoma).

RESULTS: In the univariate analysis significant positive morphological determinants were in descending order, a clear resection margin, high degree of fibrosis around the tumor, high expression of p27, absence of extrahepatic metastases, low/moderate grade of liver tumor. In the multivariate analysis determinants were in descending order grade of liver tumor, absence of extrahepatic tumor, few intraoperative blood transfusions, low preoperative serum CEA level.

CONCLUSIONS: Several morphological parameters are of prognostic importance in curatively resected colorectal liver metastases. Such an old method as grade of tumor had the lowest p value.

## P-368

**CLOSE CORRELATION BETWEEN β-CATENIN GENE ALTERATIONS AND NUCLEAR ACCUMULATION OF THE PROTEIN IN HUMAN HEPATOCELLULAR CARCINOMAS**

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***Aims and methods:*** Several lines of evidence indicate that β-catenin acquires oncogenic activity when its intracellular concentration increases as a result of either mutation in the β-catenin gene itself or inactivation of the adenomatous polyposis coli (APC) gene. In an attempt to elucidate the molecular mechanisms underlying hepatocellular carcinogenesis, we have studied the frequency of β-catenin gene alterations in exon 3, a region known to represent a mutation hot spot, and its inappropriate protein expression by immunohistochemistry in 73 hepatocellular carcinomas (HCCs). The results were correlated with different clinical and pathological data, particularly with the presence or not of an associated cirrhosis.

***Results:*** Fourteen (19%) HCCs showed β-catenin gene alterations with missense mutations in 9 cases and interstitial deletions in 5 cases. These genetic alterations were present in both cirrhotic and non-cirrhotic groups. By contrast, we did not find any β-catenin gene alterations in the 9 fibrolamellar carcinomas we examined. Nuclear accumulation of the protein was observed in eighteen of them (25%). Remarkably, these included ten of the fourteen tumors harbouring somatic mutations in the β-catenin gene ($p<0.001$).

**Conclusions:** Our results indicate that accumulation of β-catenin resulting from genetic mutations is a frequent event in non-fibrolamellar type hepatocellular carcinoma. The close association between increased β-catenin protein stability and mutation indicates that immunohistochemistry may be a powerful method for the detection of the mutated protein in future clinical practice.

## P-369

DISTRIBUTION OF HCV-RNA-POSITIVE HEPATOCYTES AND ITS RELATIONSHIP TO FIBROSIS IN CHRONIC HEPATITIS C. A MORPHOMETRIC ANALYSIS USING in situ HYBRIDIZATION

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As yet, the morphogenesis of cirrhosis from chronic hepatitis is not fully understood. As reported at 16th ECP, we found that in chronic hepatitis B, HBcAg-positive hepatocytes distribute along the septa, suggesting the pattern of septum formation determined by the antigen localization. In view of this, we extended the study to hepatitis C to analyze the distribution of HCV positive hepatocytes and its relation to that of fibrosis using 2-D distance distribution analysis (2-DDDA), a technique of morphometry we established. Liver specimens from twelve patients with chronic hepatitis C were obtained at hepatic resection or by open liver biopsy; these were confirmed to be positive for hepatitis C virus either by second generation antibody studies or RT-PCR for hepatitis C-RNA on patient serum. Each of the fresh specimens was trimmed into a block up to 1X1X0.5 cm instantly, and was fixed in 4% neutral buffered PFA solution for several hr and embedded in paraffin. Sections 5μm thick were cut and in situ hybridization was performed on MicroProbe staining system (Fisher Scientific) by use of Brigati-tailed oligo probe and manual capillary action. Hybrid detection was visualized with streptoavidin-horse raddish peroxidase method and was observed microscopically. Positive signal was obtained in cytoplasm of hepatocytes in specimens from six of the twelve patients. In two, in which the signal was the clearest, color prints were prepared at X50, which were subjected to morphometry with 2-DDDA. The analysis confirmed that in one of the specimens, HCV-RNA-positive hepatocytes are located with statistically significant proximity to areas of fibrosis, although this was not confirmed in the other. We can say that at least in some cases of chronic hepatitis C, the distribution pattern of HCV-RNA-positive hepatocytes is essential in deciding the pattern of septum formation.

## P-370

BRONCHIAL HYALINIZING CLEAR CELL CARCINOMA

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One case of hyalinizing clear cell carcinoma (HCCC) developped from bronchial sero-mucosal glands is reported. To our knowledge this tumor recently described in salivary glands, has not been previously reported in bronchial localisation.

We report a case of a 67-year-old non smoking woman without history of pulmonary disease, who presented productive cough of two years duration. The chest radiograph revealed left lung atelectasis. At the fiberoptic bronchoscopy, the left main bronchus was obstructed by a round, beige mass. Biopsy examination displayed an epithelial infiltrating clear cell proliferation suggestive of a low grade neoplasm of seromucosal glands.

On the pneumonectomy specimen, the left lower lobe central zone was destructed by a well limited, steady iceberg-tumor (6x4cm) which appeared withe-nacreous homogeneous on cross-section. Two peribronchial lymph nodes (10L and 11L) contained tumor metastasis. Microscopic study showed a massive and trabecular proliferation without squamous nor glandular differentiation remarkable by its fibrohyaline stroma. Tumor cell cytoplasms had sharp outlines, were clear and stained by periodic acid of shiff. Nuclei were central with a fine chromatin and inconspicuous nucleoli; mitotic figures were rare.

An immunohistochemical study was performed. The tumor cells expressed epithelial markers (KL1 and EMA) and were negative for chromogranin, actin, PS100, amylase and HMB45, excluding endocrine, myoepithelial, acinic and sugar tumors.

The prognosis of HCCC needs to be better defined. Nevertheless, in bronchial localisation, it appears as a low grade neoplasm with locoregional aggressivity, like in the salivary gland.

Milchgrub & al. HCCC of salivary gland. Am J Surg Pathol 1994;18:74-82.

## P-371

P27 AND CYCLIN D1 PROTEIN EXPRESSION RELATED TO ULTRASTRUCTURAL DIFFERENTIATION IN NON- SMALL-CELL LUNG TUMORS

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**Aims**: Overexpression of cell cycle regulators p27 and cyclin D1 (CCND1) has been demonstrated in lung neoplasms, although relationship to tumor differentiation or advanced disease is controversial.

**Methods:** A series of 127 tumors was investigated for immunohistochemical expression of p27 and CCND1 upon formalin-fixed paraffin embedded archival tissue and ultrastructural study of glutaraldehid-fixed sections. 66% of patients were in stage I, 31% in stage II , 2% in stage III and 1% in stage IV. Positivity for p27 was considered when 10% or more of nuclei stained and for CCND1 when 5 % or more . Ultrastructural differentiation (adenoid, squamous and neuroendocrine) was scored 0-3. Tumors with scores 2 or higher were considered well differentiated.

**Results**: 23% showed mixed adenosquamous differentiation. 70% were well differentiated tumors. 84% (57/68) of them were p27 positive and 59% (40/68) CCND1 positive. Adenoid differentiation (score 2-3) was found in 56 cases: 83% (39/47) were p27 positive and 64% (30/47) CCND1 positive . All bronchioloalveolar carcinomas (13/13) were p27 positive, 69% (9/13) were CCND1 positive and showed coincidental high p27 and CCND1 expression. Most of the CCND1 negative bronchioalveolar carcinomas were of the Clara cell type (75%). Squamous differentiation (score 1-2) was found in 2 cases. 69% (9/13) were p27 positive and 69% (9/13) were CCND1 positive. Almost all neuroendocrine tumors (8/9) were p27 positive and CCND1 negative, and all of them showed high p27 expression.

**Conclusions:** p27 and CCND1 expression is a frequent event in most lung tumors unrelated to ultrastructural differentiation, histological type or stage in non-small-cell lung carcinomas.

## P-372

IMMUNOHISTOCHEMICAL REACTIVITY IN MALIGNANT MESOTHELIOMA: A STUDY OF 36 CASES.

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**Aims:** Immunohistochemistry is a powerful diagnostic adjunt in the diagnosis of malignant mesothelioma. As a specific marker for mesothelioma has not yet been recognised, the diagnosis of this tumour has been based on the use of a battery of antibodies. The purpose of this study is to present our experience with malignant mesothelioma and to determine the practical value of immunohistochemical markers.

**Methods:** Retrospective study of 36 malignant mesotheliomas diagnosed between 1989-1999. In all of these cases, the diagnosis of mesothelioma was made using histologic criteria, combined with immunohistochemical and clinical features. The immunoreactivity of keratin (K116), vimentin, carcinoembryonic antigen (CEA), epithelial specific antigen (ESA), CD15 (Leu M1) and calretinin was investigated using semiquantitative evaluation; 0: no immunostained cells; 1+: ≤25%; 2+: >25-≤50%; 3+: >50-≤75%; 4+: >75% of cells.

**Results:** Between 1989-1999, 36 mesotheliomas were diagnosed, 33 of the pleura and 3 peritoneal. The patients were 28 males and 8 females with a mean age of 64 (range: 45-86). Histologically 16 cases (45%) were epithelial, 16 (45%) biphasic and 4 (10%) sarcomatoid. Immunohistochemical results: K116 positive in 35 cases, only one case was negative (biphasic mesothelioma). Vimentin positive in 35 cases, only one case negative (epithelial). ESA negative in 31/36, five cases positive (14%) (epithelial and biphasic). None of the mesotheliomas reacted with CEA or Leu M1. Calretinin was performed in 12 cases, all of them exhibited reactivity. However, in 5 cases immunoreactivity was focal (3 sarcomatoid, 1 biphasic and 1 epithelial).

**Conclusions:** In our experience CEA and Leu M1 were constantly negative. All mesotheliomas were immunoreactive for calretinin, however 42% showed focal positivity, most of them in the sarcomatoid group. Fourteen per cent of mesotheliomas were positive for ESA, the majority were epithelial mesothelioma.

## P-373

**UTILITY OF EPITHELIAL MEMBRANE ANTIGEN AND p53 IN THE DIFFERENTIAL DIAGNOSIS OF BENIGN REACTIVE PROCESSES FROM MALIGNANCY IN PLEURAL BIOPSY SPECIMENS**

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**Aims:** To determine the value of epithelial membrane antigen (EMA) and p53 immunostaining in the differencial diagnosis between benign reactive mesothelial lesions (BR), mesothelioma (ME) and metastatic pleural adenocarcinoma (AD).

**Methods:** Immunostaining of EMA and p53 each were applied to the histologic sections of 36 pleural biopsies including 12 AD, 12 ME, and 12 BR. We also studied the staining pattern of EMA (citoplasmic versus membranous). We considered positives in p53 the cases with ≥ 10% cell positivity. Statistical analysis was made by using $\chi 2$ analysis and Fisher exact test.

**Results:** EMA was positive in 10/12 ME and 12/12 AD, whereas it was negative in the majority of BR (2/12). Immunostaining for p53 was positive in 5/12 ME, 5/12 AD, and negative in all BR (0/12). There were differences statistically significant between benign and malignant lesions for EMA (p=0.00003) and p53 (p=0.031). The sensitivity and specificity in diagnosis of ME were: EMA (83% and 83%) and p53 (42% and 100%).
There also were statistically significant differences (p=0.0007) in EMA staining pattern between ME (membranous pattern in 8/10) and AD (citoplasmic pattern in 11/12). The sensibility and specificity in diagnosis of ME by EMA pattern was 80% and 92%.

**Conclusions:** This study confirms the usefulness of EMA and p53 in the differential diagnosis between malignant and benign mesothelial lesions. The results also suggest the value of EMA immunostaining pattern (citoplasmic versus membranous) in the differential diagnosis between AD and ME.

## P-374

**PULMONARY CARCINOID TUMORS. IMMUNOHISTOCHEMICAL STUDY OF 14 CASES.**

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**Aims.** To study the immunohistochemical expression of p53, bcl-2, cerbB2 and Ki67 in pulmonary carcinoid tumors, and to relate to classical and other histopathologic features.

**Methods**. Fourteen primary carcinoid neoplasms of bronchopulmonary origin, 11 typical and 3 atypical were studied, according WHO criteria. Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue sections. Staining for p53 (DO7), Ki67, bcl-2 protein, c-erbB2, synaptophysin, citokeratins, antimitochondrial, ACTH and calcitonin were applied.

**Results.** Patients ranged in age from 25 to 71 years (mean 50), 8 women and 6 men. The tumors ranged in size from 1.5 to 5 cm (mean 2.8 cm). All cases were strongly positive for synaptophysin and cytokeratins, and in three a prominent dot pattern was observed. Two cases with oncocytic morphology were strongly positive for antimitochondrial antibody. Only two tumors showed p53 positivity, one typical (in 2% of tumor cells) and one atypical (in 5% of tumor cells). Ki 67 immunostain ranged 1 to 30 % of tumor cells (mean 6 %), with 2.6 % of mean (SD 3.8) in typical and 18.7 % (SD 11.0) in atypical ($p<0.05$). Immunoreactivity for c-erbB2 were observed in two cases (1 typical and 1 atypical). None of the tumors showed positivity with bcl-2 protein.

**Conclusions.** A significant correlation for the proliferative activity and histological classification of pulmonary carcinoids was found. Although is a short series of cases our results indicate that not differences are observed in p53 protein expression between typical and atypical carcinoid pulmonary tumors. Bcl-2 protein and c-erbB2 does not play an important role in the genesis of this tumors.

## P-375

**STUDY OF RNA AND PROTEIN EXPRESSION OF TELOMERASE SUBUNITS IN HUMAN PLEURAL MESOTHELIOMA.**

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**Aims:** Using the 'telomeric repeat amplification protocol' (TRAP), we detected telomerase activity in over 90% of mesotheliomas (MM) but not in normal mesothelial cell cultures (MCC) [Dhaene et al. (1998), Thorax 53:915-918]. Similar activity studies are infeasible on archived materials. In search for surrogate markers to study telomerase activity *in situ* in archived pre-malignant serosal lesions, we here determined which component of the telomerase holo-enzyme parallels enzyme activity best.

**Methods:** Transcript expression of the telomerase template component (hTERC), telomerase-associated protein (hTEP1), and telomerase catalytic subunit (hTERT) was examined by reverse transcriptase polymerase chain reaction (RT-PCR) on frozen samples of 16 MMs, 4 MM-derived cell lines and 6 MCCs. Applicability of the L-20 anti-hTERT antibody [Santa Cruz Biotechnology Inc, USA] was tested by immunofluorescence, combined with telomere fluorescence *in situ* hybridisation and by immunoblotting on normal and neoplastic mesothelial cells.

**Results:** RT-PCR analysis revealed that only hTERT mRNA expression parallels the activity status documented by the TRAP assay. hTERC and hTEP1 mRNA are expressed in all tumoural and non-tumoural serosal cells and tissues. Three alternately spliced hTERT transcripts were detected in all samples positive for telomerase activity, whereas neither variants could be detected in mesothelial cells. Detection of the hTERT protein with the L20 antibody was not successful.

**Conclusions:** Our results indicate that hTERT expression is rate-limiting for human telomerase activity and that re-activation, rather than up-regulation, of hTERT expression can play a role in MM oncogenesis. Awaiting suitable anti-hTERT antibodies, our results provide information for the design of full-transcript hTERT mRNA-specific *in situ* probes to study the role of telomerase in archived pre-malignant serosal lesions.

## P-376

**TNF-α TGF-β EXPRESSION IN NON SMALL CELL LUNG CARCINOMA (NSCLC): RELATIONSHIP WITH NEOANGIOGENESIS AND PROGNOSIS.**

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Recent in vivo and in vitro studies have demonstrated a wide spectrum of biologic activities of cytokines in the pathogenesis and progression of malignancy. Tumor necrosis factors (TNFs) and TGF-beta have emerged as two of the many host-derived mediators that appear to interfer with both antiproliferartive and tumorigenic effects in malignant tumors including lung cancer. However, their association with tumor prognosis or prognostic factors has as yet not been completely clarified. In this study, we assessed TNF-alpha and TGF-beta mRNA expression by PCR technique in 61 NSCLC samples, demonstrating the presence of TNF-alpha and TGF-beta mRNA in 55.74% and 54% of cases, respectively. We also evaluated the expression of the two distinct transmembrane TNF-receptors, TNFR-I and TNFR-II, with a PCR-positive signal in 70.49% and 65.57% of cases, respectively. In 49 of the 61 cases, we evaluated the prognostic impact of the two growth-inhibiting factors using the Kaplan-Meier analysis. TNF-alpha and TGF-beta were significantly associated with a favorable prognosis in terms of both overall and disease free survival (OS p=0.01 and 0.03; DFI p=0.01 and 0.05). Since our previous studies demonstrated a significant association between NSCLC behaviour, neoangiogenesis and bcl2 expression, we investigated the putative relation among TNF-alpha and TGF-beta, on the one hand, and vascular count (as a measure of tumor angiogenesis) and bcl2 protein expression, on the other hand. Our results show a significant direct association between TNF-alpha and bcl2 (p=0.05) and an inverse association between TNF-alpha and microvessel count (p=0.036). Moreover, as previously demonstrated, we observed an inverse significant correlation between bcl2 protein expression and vascular count (p=0.04) suggesting that the favorable effect of TNF-alpha on clinical outcome may be related to a bcl2-mediated low neo-vascular development.

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## P-377

**PULMONARY ALVEOLAR PROTEINOSIS AND NONASBESTOS PNEUMOCONIOSIS**

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Pulmonary alveolar proteinosis (PAP) appears as idiopathic disease or secondary to occupational exposures to a variety of dusts or fumes. The **aim** of this study is to investigate the prevalence of PAP in patients with nonasbestos pneumoconiosis (NAP) by **means** of pathological analysis of a large number of autopsy cases in Japan, in order to evaluate a pathogenetic relationship between the two conditions. Unselected consecutive autopsy cases with a pathologic diagnosis of NAP were collected from all over Japan (N=1200; age 69.5±8.5 yrs) and reviewed histopathologically to look into the prevalence of PAP as well as its relationship to underlying pneumoconiosis which includes silicosis and mixed dust pneumoconiosis (MDP). Immunohistochemistry for surfactant apoprotein was performed in a small number of cases to identify the lesion. There were no cases of acute silicosis.

As a **result**, 44.9% of cases with silicosis showed evidence of PAP irrespective of its extent. In most cases, PAP developed in and around the confluent silicotic nodules and/or massive fibrosis, associated with deposition of dust, interstitial inflammatory infiltration and fibrosis. On the other hand, MDP was associated with a lower prevalence of PAP (26.4%; $p < 0.000001$ ).

In **conclusion**, crystalline silica is likely to play an important role in the development of PAP. The concept 'silicoproteinosis' should not be confined to acute disease but entail chronic conditions including classical silicosis.

## P-378

**OCCURRENCE OF HUMAN PAPILLOMAVIRUS DNA IN PRIMARY LUNG SQUAMOUS CELL CARCINOMAS**

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**Aims :** The aim of the study is to detect the occurrence of human papillomavirüs (HPV) DNA in primary squamous cell carcinomas (SCC) of lung.

**Methods :** In situ hybridization technique and commercially available dig-labelled DNA probes to HPV was used.

**Results :** HPV DNA was found in five of 34 (%14.7) carcinomas. HPV DNA expression by means of infected cell quantity and signal intensity in the late stage carcinomas was stronger compared to the other HPV DNA positive cases.

**Conclusion :** In this ongoing study, the high prevelance of HPV DNA in primary lung SCC suggests virus subtyping for 6/11, 16/18 and 31/33/35 may be of prognostic importance in relation to the development of high grade squamous cell carcinomas.

## P-379

**p53 EXPRESSION IN NON SMALL CELL LUNG AND SMALL LUNG CARCINOMAS: RELATIONSHIP WITH PROLIFERATING CELL NUCLEAR ANTIGEN AND CIGARETTE SMOKING**

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**Aims:** Our purpose is to investigate the relationship of p53 gene expression and proliferating cell nuclear antigen (PCNA) in smoking and nonsmoking lung cancer patients. And also to find out if there was any correlation between expression of these markers and the clinical status and histopathologic findings..

**Method:** We investigated the immunohistochemical expression of p53 protein, PCNA, and smoking history of 30 patients with non-small cell lung carcinoma (NSCLC) and 30 patients with small cell lung carcinoma (SCLC).

**Results:** We found p53 expression in 13 (43.3 %) NSCLC cases and in 6 (20 %) SCLC cases. PCNA nuclear staining was detected in 30 (100 %) of 30 NSCL cases and in 22 (73.3 %) of 30 SCLC cases. We found a significant correlation between p53and PCNA expression in NSCLC cases (p:0.047). No correlation was found between either the PCNA index or the level of the p53 expression and sex, age, histopathologic findings, tumor stage, tumor size. Significant correlation was not found between PCNA index and a smoking history. None of the twelve nonsmoking patients showed p53 nuclear positive staining whereas 13 of 18 smoking patients showed. A relation was found between p53 immunostaining and smoking history in the group of NSCLC (p:0.005).

**Conclusion:** These data suggest that the p53 gene could be a target of tobacco -associated lung carcinogenesis and the relation between PCNA and p53 expression in NSCLC cases indicates the PCNA is slightly up-regulated by p53.

## P-380

CALRETININ AND P53 AS MARKERS FOR DIFFERENTIATION BETWEEN REACTIVE AND NEOPLASTIC PLEURAL LESIONS?

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**Aims:** Until now, no reliable immunohistochemical marker for the distinction between reactive and neoplastic pleural lesions has been found. The value of calretinin as a marker for mesotheliomas has been demonstrated earlier. The p53 tumor suppressor gene has been shown to be frequently mutated in a wide range of neoplasms. This is accompanied by increased levels of P53 protein which become immunologically detectable. Its diagnostic value is in discussion. The aim of the study was to test if calretinin expression and overexpression of P53 in pleural mesothelia may help differentiate reactive and primary as well as secondary neoplastic pleural conditions.

**Methods:** Paraffin embedded pleural biopsies in each 40 cases of pleural metastases of carcinomas and benign pleural changes were investigated by APAAP immunohistochemistry using antisera directed against calretinin (SWant) and CM1 against P53 using a DAKO TechMate.

**Results:** Calretinin was demonstrated in reactive pleural mesothelia over pleural metastases of adenocarcimas in nearly all cases. In about 30% calretinin could be demonstrated in the non-neoplastic pleural lesions, while adenocarcinoma cells were negative. Some of the non-neoplastic mesothelial cells showed a clear nuclear P53 staining. In reactive mesothelia a disseminated single cell labeling comparable to mesotheliomas could be documented.

**Conclusions:** Calretinin is a well established, very sensitive and specific marker for the differential diagnosis between mesotheliomas and adenocarcinomas, but calretinin expression and P53 accumulation are features not only of pleural mesotheliomas. They may also be detected in reactive mesothelial changes and therefore can not be recommended as a specific and reliable marker for differentiating between malignant and reactive, non-neoplastic mesothelial alterations.

## P-381

**IDIOPATHIC PULMONARY FIBROSIS SURVIVAL BASED ON THE PATHOLOGICAL CLASSIFICATION**

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**AIM**: To retrospectively determine differences of survival in the cases of idiopathic pulmonary fibrosis (IPF) when using the Katzenstein's classification in pathological diagnosis: Usual interstitial Pneumonia (UIP), Desquamative interstitial Pneumonia (DIP), Respiratory Bronchiolitis Interstitial Lung Disease (RBILD), Acute Interstitial Pneumonia (AIP) and Nonspecific interstitial Pneumonia (NSIP).

**METHODS**: From 1977 to 1994, 45 patients were diagnosed of IPF in our hospital. We found 21 of them with surgical lung biopsy useful for histological diagnosis, and a minimum follow up of 5 years if alive. All patients were treated by corticosteroids. In a blind fashion (without knowing clinical data), using HE and Masson's trichrome stains and by consensus of two pathologists, we diagnosed the patients following the Katzenstein's classification.

**RESULTS**: From these 21 patients, 14 were diagnosed of UIP (8 males and 6 females) and 7 of NSIP (2 males and 5 females). The UIP patients were slightly older (63±7 year-old) than patients with NSIP (62±6 year-old) (p=n.s.). The survival (from the onset of the symptoms till the death of the patient or till the time of the present abstract) of patients with NSIP was longer (100±54 months) than patients with UIP (48±33 months) ($p<0.05$, U Mann-Whitney). Four NSIP patients are still alive, while all of the UIP patients are dead.

**CONCLUSIONS**: Our results support the 1994 Katzenstein's statement of better prognosis for NSIP compared with UIP. Therefore, we believe that anatomopathological diagnosis in all cases of IPF is warranted. On the other hand, no differences in age were found in our group, contrary to original Katzenstein's study.

## P-382

PROGNOSTIC SIGNIFICANCE OF CATHEPSIN D IN SQUAMOUS CELL CARCINOMA OF LUNG. AN IMMUNOHISTOCHEMICAL STUDY.

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AIMS:.To investigate immunohistochemical expression of cathepsin- D (CD) in squamous cell carcinoma of lung (SCC) and its probable prognostic value.

METHODS: 56 surgically obtained SCC were studied. Tumor differentiation, nuclear pleomorphism, mitotic index, pleural and thoracic invasion and lymph node metastasis were recorded. Clinical data and follow-up (nov 1989-nov 1998) were available in all cases. Standard immunohistochemical study on paraffin-embedded tumoral samples was performed. Primary antibody was cathepsin D (1/500) (from Dako Denmark). Semiquantification score of expression of CD was done. Tumors with positivity in less than 5% of tumor cells were considered negative. Macrophages were used as an internal positive control.

A SPSS statistical package was used to analyze the results. Overall survival was performed using the Kaplan-Meier method.

RESULTS: CD inmmunostaining was negative in 44 tumors. Tumor with non-expression of CD showed higher mitotic index (mean 1.09) than tumors with high CD expression (mean 0,59) ($p=0.01$). No other statistical relationship was observed between CD expression and other pathological and clinical parameters studied. Strong association was found between CD immunoreactivity and overall survival. Longer survival was observed in patients with non-CD expression ($p< 0.0042$).

CONCLUSIONS: Non-expression of CD immunohistochemical staining has a prognostic value in squamous cell carcinoma of lung, although the mitotic index was higher in these cases with better survival.

## P-383

IMMUNOHISTOCHEMISTRY AND ELECTRON-MICROSCOPY IN AN APUD CELL TUMOR SHOWING DUAL DIFFERENTIATION

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**Aims**: About 3-5% of surgical pathology specimens requires ancillary diagnostic techniques over traditional histological stainings. We are going to report a peculiar case in which both immunohistochemistry /IHS/ and electronmicroscopy /EM/ were necessary to establish the correct diagnosis.

**Methods**: A 42 year old male had clinically inoperable retroperitoneal neoplasm, a tumorous nodule was removed from the mesentery for diagnostic purpose. The patient died a few days after operation. At autopsy a solitary lung tumor was found. The retroperitoneal mass proved to be a metastatic lymph node conglomerate. In addition to routine histological stainings IHS and EM were performed from the mesenteric nodule and from the lung tumor as well.

**Results**: In both specimens epithelial and neuroendocrine morphological features were observed with all applied techniques. Furthermore very unusual endoplasmic reticular patterns: tubules of different shape, surfactant granules, lamellar bodies were found in the cytoplasm of the lung tumor cells. The diagnosis was atypical carcinoid of the lung with metastasis in the mesentery.

**Conclusion**: Electron microscopy and immunohistochemistry should be viewed not as competing with each other but rather as complementary methods to recognize the tumor phenotype. In our case the electronmicroscopy beyond the confirmation of the diagnosis revealed a rare ultrastructural variant.

## P-384

**AUTOPSY STUDY OF THE ROLE OF THE NATIVE LUNG IN THE UNIPULMONARY TRANSPLANTATION**

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**AIM**: To retrospectively determine, in unipulmonary transplantated patients, the role of the native lung in the cause of death of the patient, and in the fate of the transplanted lung.

**METHODS**: From 47 unilateral lung transplantations carried out in our institution, 20 have died, and in 6 cases we performed the autopsy study. The cause of left lung transplantation in these 6 patients was: emphysema (EMP) in 2 patients, usual interstitial pneumonia (UIP) in 2 patients, lymphangioleiomyomatosis (LAM) in 1 patient, and pulmonary hypertension thrombotic type (PHTT) in 1 patient.

**RESULTS**: The overall survival of these patients was 20±22 months (13 months in EMP, 10±5 in UIP, 10 in LAM and 64 in PHTT). The two patients with UIP died due to invasive fungus infection originated in the native lung with less affection of the transplanted lungs. One patient with EMP died because of invasive aspergillosis originated in the transplanted lung without affection of native lung. The patient with LAM died due to Streptococcus pneumonia in the transplanted lung, again, without affection of the native lung. The patient with PHTT died because of chronic rejection. The remaining patient with EMP died due to Enterococcus sepsis of gastrointestinal tract origin.

**CONCLUSIONS**: Our results show that native lungs with UIP were the source of fatal fungal infection in those cases, suggesting that pulmonary fibrosis may restrict the therapy effectiveness against fungus more than emphysema, LAM or PHTT. In addition, fungus infection in UIP causes affection of transplanted lung and septicemia. On the contrary, native lungs with EMP and LAM seem to have better response to aspergillus and streptococcus therapy, being more resistant to that pathology than the transplanted lungs.

## P-385

**EXPRESSION OF CATENINS (α, β AND γ) IN NON-SMALL CELL LUNG CANCER AS RELATED TO CLINICOPATHOLOGICAL FACTORS AND SURVIVAL**

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**Aims:** The purpose of the study was to evaluate the expression of different catenins (α, β and γ) in lung cancer specimens with special emphasis on their prognostic significance.

**Methods:** The study consisted of 261 patients with resected non-small cell lung cancer (163 squamous cell carcinomas, 68 adenocarcinomas and 30 anaplastic/large cell carcinomas). The localization and signal intensity of catenins in carcinoma cells was immunohistochemically analyzed. Also proliferative activity was evaluated by using Ki-67 (MIB1) antibody.

**Results:** The normal membranous staining was inversely correlated with grade ($p<0.007$ for all) and nuclear staining pattern (β- and γ-cat), ($p<0.003$). Also the expression of catenins was related to tumour type ($p=0.0001$ for all). The tumours showing high Ki-67 positivity had more often the reduced staining patterns of β-and γ-catenins, ($p<0.005$ for both). In the survival analyses catenins did not have any prognostic significance. The most significant predictors of disease free and overall survival were stage ($p=0.004$) and tumour type ($p=0.001$).

**Conclusions:** Our results emphasize the role of different catenins in the differentiation and progression of non-small cell lung cancer. However, they provided not any prognostic information beyond the well known prognostic factors.

## P-386

**RESPIRATORY BRONCHIOLITIS ASSOCIATED INTERSTITIAL LUNG DISEASE: DIAGNOSIS BY TRANSBRONCHIAL LUNG BIOPSY.**

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**AIMS**: The respiratory bronchiolitis-associated interstitial lung disease (RBILD) is a lung interstitial disease (ILD) seen in cigarette smokers. Histologically it shows peribronchiolar fibrosis and numerous macrophages within the peribronchiolar spaces. The clinical symptoms and the chest radiograph are similar to ILD. RBILD does not progress to end stage fibrosis and symptoms resolve with cessation of cigartte smoking and steroid therapy for acute exacerbations.

**METHODS**:We show the histological findings of 6 transbronchial biopsies corresponding to 6 cases of BRILD diagnosed in our Hospital during the last two years. Multiple slides were performed and stained with hematoxiline-eosine, Masson, PAS and Iron stains. In all cases a clinical, radiographic and histolgic correlation was made.

**RESULTS**: All biopsies shown intraalveolar fine brown pigment macrophages. Furthemore, 2 biopsies showed peribronchiolar fibrosis and 4 biopsies showed thickening of alveolar septa and pneumocytes hyperplasia.

**DISCUSSION**: The presence of numerous macrophages within the peribronchiolar spaces is a typical morpholgic finding in BRILD but it is not specific.This finding with a very similar clinical and radiographic manifestation appears in descamative interstitial pneumonia (DIP). They differ in the distribution of lesions: in the former the findings are patchy, bonchiolocentric and nonuniform and we can make this diagnosis, whereas in the latter the findings are diffuse, widespread and unifom and we need an open lung biosy. In the ILDinterstial we must do a correlation betwen the clinical, radiographic and histological findings for a suitable diagnosis.

## P-387

**BENIGN LYMPHOCYTIC ANGIITIS AND GRANULOMATOSIS (BLAG) OF THE LUNG : ITS SEPARATION FROM WEGENER'S GRANULOMATOSIS AND LYMPHOCYTIC LYMPHOMA**

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Benign Lymphocytic Angiitis and Granulomatosis of the lung (BLAG) is a controversial entity whose differential diagnosis includes Wegener's granulomatosis and lymphocytic lymphoma. We present clinicopathologic observations in 18 cases of BLAG seen over the last 25 years. Patients were adults (mean age: 57 years) and both sexes were equally represented.About 60 percent of them were asymptomatic and the lesions were discovered by chance in the chest x-ray. They usually consisted of a single, or multiple sharply circumscribed nodules or masses suggesting tumor.Other patients had malaise, low grade fever and the chest x-ray showed bilateral, irregular infiltrates. Histologically, there was a background of alveolar-filling disease by histiocytes, lymphocytes, plasma cells and granulomata. Bronchiolitis obliterans and a lymphocytic infiltrative vasculitis of both arteries and veins was a constant feature of the lesion. Hyperplasia of BALT was frequently noted. Necrosis of the lesions and extrapulmonary involvement were not features of BLAG. Stains for microorganisms and cultures for bacterial, fungi and viruses were negative. Immunophenotypic studies revealed a mixed T and B cell population with polytypic plasma cells, and genotypic studies in a few cases failed to reveal expansion of a clonal cell population. Chlorambucile, 8 mg/24 hours for up to 12 months resulted in resolution of extensive infiltrates. Surgery was the treatment of choice for localized lesions.

## P-388

MMP-Expression in primary squamous cell carcinomas of the lung, their lymph node metastasis and in malignant pleural effusions

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Aims: Matrix-Metalloproteinases (MMPs) are endoproteases affecting stromal invasion and tumor cell settlement of malignant tumors. MMP 1, 2, 3 and 9 are supposed to be produced by tumor cells and surrounding stromal elements. We examined the cellular source of MMP-expression in primary squamous cell carcinomas of the lung, corresponding lymph node metastasis and associated malignant effusions by Western blotting and immunohistochemistry. Special emphasis was layed on comparision of MMP-pattern in the primary tumor and in tumor associated processes.

Methods: Immunoperoxidase staining for MMP 1, 2, 3 and 9 was performed on 10 resection specimens of primary squamous cell carcinoma of the lung including 5 cases with lymph node metastasis. Double immunostaining for MMP 1, 2, 3 and 9 and for the mesothel-specific marker HBME-1 was carried out on pleural effusions.

Results: About 90% of the tumor cells in the primary tumor and in the lymph node metastasis displayed an immunoreactivity for MMP 1, 2 and 9, whereas 50% of the stromal and normal epithelial cells close to the primary tumor showed an immunostaining for MMP 1, 2 and 9. 50% of the tumor cells and about 20% of the surrounding epithelial and stromal cells exhibited an immunoreactivity for MMP3. In malignant pleural effusions nearly all tumor cells were immunoreactive for MMP 1, 2 and 9, 30% were immunostained for MMP3. 25% of the mesothelial cells were positive for MMP 1, 2, 3 and 9. Western blotting showed an identical pattern of MMP-distribution as immunhistochemistry.

Conclusions: The expression of MMP 1, 2, 3 and 9 in tumor and stromal cells pictures a common production of endoproteases by elements of tumor and also stromal tissue. Immunohistochemistry and Western blotting analysis showed a lower expression of MMP3 compared to MMP1, 2 and 9. The identical MMP-pattern within the primary tumor, the lymph node metastasis and the malignant pleural effusions almost exclude a change of MMP-expression in the process of metastasis.

## P-389

**AN UNUSUAL CASE OF PARTIALLY INDEPENDENT EPITHELIAL CELL MESOTHELIOMA.**

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We report the case of a 84-year-old woman, presenting a voluminous mediastinal tumor that invaded by contiguity the left lung and was associated to bilateral lung nodules. Despite the absence of asbestosis exposure, a mesothelioma diagnosis was suspected on two fine needle lung biopsies. The patient died fifteen days later. At autopsy, tumor occupied the whole mediastinum with multiple pleural localization and a deep extension into the left lung. There was a tumor hernia through the diaphragm orifice into the abdominal cavity, with pancreatic invasion and liver metastasis. Histological analysis revealed a proliferation composed partially with clear vacuolated independent cells. A more mesothelial characteristic pattern with papillary features was found in pleura.

| Tumor pattern | PAS | Oil red'O | Kl1 | Vimentin | HMBE1 | ACE | CD15 |
|---|---|---|---|---|---|---|---|
| Independent cells | - | + | + | + | + | - | - |
| Papillary | - | - | + | + | + | - | rare |

Despite atypical clinical and morphological features, a diagnosis of mesothelioma was performed, and validated by the french group of mesothelioma pathologists « MESOPATH ». To our knowledge, no case of epithelial mesothelioma harboring independent cells have been described. This case point out that the diagnosis of mesothelioma must be searched by an immunohistological study in all mediastinal tumors, even if the morphological pattern could lead to other diagnosis, such as independent clear cell carcinoma or liposarcoma.

## P-390

**METALLOTHIONEIN EXPRESSION IN LUNG CANCER**

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**Aim:** Metallothioneins (MTs) are cytosolic proteins rich in cysteine appearing to play a physiological role in the absorption, transport and metabolism of trace elements, mainly zinc and copper. Recently over-expression of cellular MT has been linked with the progression of malignant tumors. The aim of this study was to assess metallothionein expression in human lung carcinoma.

**Methods:** Archival pathologic specimens and medical records were reviewed from 43 patients with lung carcinoma (14 cases of adenocarcinoma, 18 cases of squamous cell carcinoma and 11 cases of small cell carcinoma). Sections of paraffin embedded tissues were stained immunohistochemically by the streptavidin-biotin peroxidase technique, using a mouse ($IgG_{1k}$) monoclonal antibody (Clone E9, Zymed, San Francisco, CA, USA) that recognizes a common epitope for both MT isoforms (I,II).

**Results:** Intense cytoplasmic staining for MT was prominent in 16 out of 18 patients with squamous cell carcinoma (89%) and in 5 out of 14 patients with adenocarcinoma (36%), while no immunoreactivity for MT was noted in the lung of patients with small cell carcinoma. Intense MT expression was also prominent in stromal cells. Statistically significant difference for MT expression was noted between the histological types of lung carcinoma ($p<0.001$). Non statistically significant difference was found between MT intensity of staining and histological grade of squamous cell carcinoma, while a negative correlation was found between MT intensity of staining and histological grade of adenocarcinoma ($p<0.001$).

**Conclusion:** Our work for first time describes the differential distribution of MT in the histological types of human lung carcinoma, providing evidence for the participation of this protein in the biological mechanisms underlying the carcinogenic evolution in the lung

## P-391

A MORPHOMETRIC ANALYSIS ON HUMAN LUNG ALLOGRAFT RECIPIENTS

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**Aims:** Morphometry previously performed on autopsy lungs have demonstrated severe structural parenchymal changes in emphysema and COPD, but the degree of lung damage in patients who are lung transplanted has never been described.

**Methods:** With unbiased stereological methods lung volume, bronchial- and vessel volume, capillary length and alveolar surface area were estimated in 23 patients: cystic fibrosis (CF) = 8 patients, COPD = 6 patients, $\alpha_1$-antitrypsin deficiency (A1AD) = 7 patients, primary pulmonary hypertension (PPH) = 2 patients and 6 unused donor lungs. All the patients had been thoroughly examined that allowed comparison to functional status.

**Results:** The patients with A1AD showed an increase in lung volume to 138% compared to normal lungs, while the lung volume of patients with COPD was not enlarged. The patients with CF had a bronchial volume of 246% compared to normal ($p<0.001$). The emphysema and PPH patients had bronchial volumes equal to normal. The mean alveolar surface area density was in patients with A1AD reduced to 68.4% ($p<0.001$) and in COPD patients to 73.2% ($p=0.001$). The capillary length density was in patients with A1AD and COPD patients reduced to 68.5% compared to the control group ($p<0.001$). In a previous study on autopsy materials the alveolar surface area density was reduced to 67.1% and capillary length density to 68.4%.
In patients with CF and PPH the capillary length and length density and alveolar area and area density were equal to the control group. The patients with CF and PPH had a significant better diffusion capacity compared to the emphysema patients ($p=0.001$).

**Conclusion:** The lung transplanted patients with A1AD and COPD have severely and irreversibly altered parenchymal structure in accordance with significant reduced diffusion capacities. The degree of changes in lung transplanted emphysema patients equals the end-stage situation in patients dying of emphysema. CF and PPH patients showed a better preserved architecture in accordance with a better functional status.

## P-392

PCR ANALYSIS IN THE PATHOLOGIC DIAGNOSIS OF WHIPPLE'S DISEASE

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**Aims:** To assess the value of PCR analysis in the pathologic diagnosis of Whipple's disease (WD) in intestinal and extraintestinal surgical specimens.

**Methods:** PCR analysis of species specific bacterial 16S rRNA gene of Tropheryma whippelii was done in 10 cases of WD. In seven of them, PCR was done in the diagnostic intestinal biopsy, whereas in the other three cases, (two that began with a lymphadenophaty as initial clinical presentation and one post- mortem case of disseminated WD), PCR was performed in lymph node specimens. Follow up biopsies after antibiotic therapy were evaluated in two patients.

**Results:** The specific bacterial DNA of Tropheryma whippelii was detected in all ten cases, both intestinal and extraintestinal specimens. The two follow up biopsies were negative.

**Conclusion:** PCR is a very useful tool for the pathologic diagnosis of both, intestinal and extraintestinal WD, even when the morphologic appearance is not yet typical. It is also the most precise technique for the monitorization of therapeutic success.

## P-393

**FREQUENT DETECTION OF KAPOSI'S SARCOMA HERPES VIRUS IN BONE MARROW SAMPLES**

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**Aims:** To study the presence of Kaposi's sarcoma herpes virus (KSHV) in 38 fresh bone marrow samples from 11 patients with multiple myeloma, 9 with monoclonal gammapathy of undetermined significance, and 18 with other pathological conditions like leukemia, lymphoma, carcinoma, melanoma, and anemia.

**Methods:** We analyzed the presence of KSHV in the DNA extracted from the fresh bone marrow samples using a nested PCR with primers KS3-4 and KS1-2. Specificity of the PCR amplified fragments was assesed using three different restriction endonuclease digestions with Rsa I, Mbo I, and Hpa II.

**Results:** We detected the presence of KSHV in 9 of 11 myeloma patients, in 7 of 9 MGUS patients, and in 11 of 18 control patients.

**Conclusions:** These findings suggest that the using of DNA extracted from fresh bone marrow samples together with a highly sensitive PCR allows the detection of KSHV in a significant percentage of bone marrow samples from patients with and without myeloma. These results are in disagreement with previously reported data and show that infection by KSHV may be ubiquitous in the general population.

## P-394

MUTATION ANALYSIS OF THE PTEN GENE IN ENDOMETRIAL CARCINOMAS

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**Aims:** To assess the frecuency of somatic mutations in the PTEN tumor suppressor gene in a series of endometrial carcinomas. To correlate the presence of PTEN mutations with the clinicopathological features and the microsatellite instability (MI) phenotype of the tumors.

**Methods:** DNA of 40 patients with endometrial carcinoma was extracted from fresh frozen tumor biopsies and from blood lymphocytes. Each of the nine exons and intro-exon boundaries of the PTEN gene were amplified by PCR. PTEN mutations were screened by single stranded conformation polymorphism (SSCP) and by sequencing. Matched normal DNA from all cases were analyzed by SSCP to determine the germ-line status of PTEN.

**Results:** Mutations were detected in 17 (43%) endometrial carcinomas. Of the 17 mutation positive tumors, 10 were MI positive and 7 MI negative. Mutations were detected in seven of the nine exons; only exons 2 and 9 lacked mutations. The majority of mutations ocurred in exons 5 and 8. The absence of DNA alterations in constitutional DNA proved the somatic nature of these changes.

**Conclusions:** Our results support the hypotesis that PTEN gene may be a preferential target in endometrial tumors with the microsatellite instability phenotype.

## P-395

**DIFFERENTIAL GENE EXPRESSION IN ENDOMETRIAL CANCER**

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**Aims:** Identification of genes that may be involved in the oncogenesis and progression of endometrial cancer, or may have a diagnostic or prognostic implications.

**Methods:** Tumor and non-tumoral endometrial samples were obtained immediately after sugery. All of them were endometrioid carcinomas with similar FIGO stages. Epithelium was isolated from stroma by colagenase digestion followed by mechanic disruption. After extraction of mRNA a differential display PCR was performed, obtaining an electrophoretic band pattern different in each sample. Some of the bands obtained were present in normal tissue but not in the tumor. These bands were cut out of the gel and then reamplificated, subcloned and finally analyzed with an automatic sequencer. The sequences obtained were compared with those of Gene Bank and studied informatically.

**Results:** A first differential display PCR (arbitrarily-primed RT-PCR) was performed, and several bands were repeatedly present in different normal samples but were absent in tumoral tissue, and vice versa. 2 of these bands have been sequenced and are being analyzed informatically.

**Conclusions:** The method used for isolation of the epithelial component of normal and tumoral tissue, as well as the study of both samples from the same patient by differential display PCR, can help us in finding new genes involved in endometrial carcinoma. Further studies are required to know the importance of the sequences found.

## P-396

**MUTATIONS AT CODING MONONUCLEOTIDE REPEATS IN ENDOMETRIAL CARCINOMAS WITH MICROSATELLITE INSTABILITY**

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**Aims:** Microsatellite instability (MI) has been observed in endometrioid adenocarcinomas of the endometrium (EC). Genes known to contain mononucleotide short tracts in their coding sequence are regarded as targets for mutations in these tumors. The objective of this study, was to verify associations between MI at dinucleotide repeats and mutations at coding mononucleotide runs within the *TGF-β RII, IGFIIR, BAX, hMSH3* and *hMSH6* genes.

**Methods:** DNA of 26 patients with EC was extracted from blood and from fresh-frozen and paraffin-embedded tumor tissue. For MI analysis, microsatellite loci on chromosomes 4, 5, 10, 12, 17 and 18 were amplified by PCR. Detection of *BAX, TGF-β RII, IGFIIR, hMSH3* and *hMSH6* frameshift mutations in the coding regions were detected by single strand conformation polymorphism (SSCP) analysis and sequencing.

**Results:** MI at 3 or more loci were detected in 13 cases. The *BAX* poly(G)$_8$ appeared to be much more frequently mutated than the other targets, resulted to be the most frequently altered among the coding mutational targets. *BAX* frameshift mutations were detected in seven MI+ tumors (53.8%), but in none of the 13 MI- neoplasm. In two cases, identical *BAX* frameshift mutations were detected in different areas of the neoplasm, whereas in the other five BAX mutations were heterogeneously distributed throughout the tumor. Immunostaining with antibodies against the carboxy terminus of BAX protein was very useful in assessing the heterogeneous distribution of *BAX* frameshift mutations in the neoplasms.

**Conclusions:** We described the multistep tumor progression of a mutator phenotype from mismatch repair insufficiency to frameshift mutations of cancer-related genes. Our results suggest a key role of *BAX* in the mutational cascade leading to the progression of MSI+ in endometrial carcinomas.

## P-397

**ANIMAL MODEL IN THE STUDY OF COLORECTAL TUMORIGENESIS**

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**Aims:** Experimental animal models of neoplastic diseases are important in understanding etiological and pathophysiological processes also in humans. In order to investigate whether the mechanism of genomic instability is associated with chemically induced colorectal tumorigenesis in the rat, 45 microdissected mammary carcinomas were analyzed with 9 different microsatellite markers from chromosomes 1p, 2p, 4q, 5q, 11p, 17p and 18q.

**Methods:** One hundred and fifty Wistar rats (males 220-280g and females 140-180g) were used in the study. Colorectal tumors were induced by means of 15 s.c. applications (20mg/kg) of dimethylhidrazine (DMH). The animals were left to live 10 weeks after completed DMH injection, and there upon sacrified by CO2 inhalation. On autopsy, all internal organs except central nervous system were examined. All intestinal lesions were assessed by histological criteria used in human pathology. DNA was extracted from rat neoplastic lesions and adjoining microscopically normal tissues from the same slide, amplified by PCR, using different polymorphic DNA markers. PCR - reaction products were analyzed for microsatellite instability with non-isotopic method.

**Results:** More than 95% of animals survived the duration of experiment. Forty five tumors were found in the large intestine 32 of these in males and 12 in females, i.e. in 30% of all animals. In 10 animals multiple primary tumors were found. Histologically 24 tumours were adenocarcinomas, 14 signet-cell carcinoma and 7 adenomas. In 12 adenocarcinomas (27%) microsatellite instability was found at a minimum of 1 locus. Four tumors (9%) showed microsatellite instability at more than one loci.

**Conclusions:** The results of our experiment suggest that genomic instability is an important molecular event in the pathophysiology of DMH induced colorectal carcinogenesis in rats.

## P-398

**IDENTIFICATION OF NEW FACTORS IMPLICATED IN ENDOMETRIAL CARCINOMA**

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**Aims:** The molecular mechanisms reponsible for the stablishment and progression of endometrial cancer are not well understood. The aim of this study is to use differential dislpay for the identification of genes involved in these processes.

**Methods:** Test for differential gene expression were carried out on mRNA samples obtained from both tumoral and healthy tissue collected from the same patient. The study was carried out in patients undergoing hysterectomy for endometrial cancer stage I. Paraffin included samples from the last 5 years are also included in this study. In all cases the clinical follow up of patients and hystolpathological analysis of samples was carried out. The molecular study include: 1- Identification of unknown markers of endometrial carcinoma, 2- confirmation of the differential expression of the above selected markers by Northern blot and in situ hybridization and 3- cloning and sequencing of the above gene products.

**Results:** Nine bands were selected for their identification and characterization. Three of them were specific for atrophic epithelium and absent in the corresponding tumor samples from the same patient. Six additional bands did not show significant similarities to any known gene.

**Conclusions:** We have found novel genes wich could be involved in the adquisition of phenotypes related to the cell growth in endometrial carcinoma. We are currently studing the nature of the proteins encoded for some of these genes.

## P-399

SIGNIFICANCE OF NASOPHARINGEAL CARCINOMA AND EPSTEIN-BARR VIRUS ASSOCIATION WITH LATENT MEMBRANE PROTEIN-1 DELETION.

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**Aims**: The relationship between the Epstein-Barr virus (EBV) and nasopharingeal carcinoma (NPC) has been recently confirmed by demonstrating EBV presence in NPC tissue samples through molecular studies with high sensibility, such as Polimerase Chain Reaction (PCR), and techniques with high accuracy in tumour location such as In Situ Hibridation (ISH) for EBV-RNA. The aim of this study is to assess the relationship among NPC, EBV and Latent Membrane Protein-1 (LMP-1).

**Methods**: We analysed clinical, histological and immunohistochemical (viral protein LMP-1 expression) characteristics of 30 cases of NPC. EBV sequences detection by ISH for EBERs, molecular study by PCR of the TC60-61 gene to locate EBV-DNA and of the LMP-1 en to identify deletioned strains (LMP-1 del) were made.

**Results**: We found 24 cases of histological undifferentiated NPC, being 9 of them of high grade (type A). LMP-1 was positive in 13 cases (43.3%). By contrast, EBERs expression was observed in 29 cases (96.7%). EBV detection by PCR was possible in 28 cases (93.3%) and the presence of LMP-1 gene deletion was detected in 64.3% of the cases, which differed significantly with the control group ($p<0.0001$).

**Conclusions**: The NPC showed a strong association with EBV infection in our population, with a type II latency pattern (EBERs and LMP-1), but with low LMP-1 expression. The most sensible method to detect EBV presence was ISH for EBERs. There were 65% of cases with deletioned strains of EBV which could have a higher oncogenic potential.

## P-400

ABSENCE OF p16 GENE *(CDKN2)* DELETIONS IN MICRODISSECTED PRIMARY BREAST CARCINOMA SPECIMENS

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**Aims**: The p16 gene (CDKN2), a tumour suppressor gene located on chromosome 9p21, has been demonstrated to be mutated or deleted with high frequency in a variety of tumour cell lines, including breast cancer cells. While previous studies have not demonstrated CDKN2 mutations in primary breast carcinomas, it is possible that gene deletion in neoplastic DNA was masked by the presence of contaminating normal stromal DNA in breast carcinoma specimens. The microdissection technique allows us to separate pure populations of carcinoma cells from formalin-fixed, paraffin-embedded, stained breast tissue, and in this way minimise the risk of contamination by normal stromal tissue

**Material and methods**: A total of 12 primary infiltrating breast tumour specimens with an abnormal immunohistochemical expression of p16 were included in this study. DNA from microdissected carcinoma and normal tissue pairs from specimens were analysed by PCR for homozygous deletion of CDKN2 gene.

**Results**: We detected no deletions or mutations of the p16 gene.

**Conclusions**: CDKN2 is not deleted with high frequency in primary breast carcinomas and the role of the p16 gene in breast carcinogenesis might be via some other mechanism.

## P-401

LOSS OF HETEROZYGOSITY ON CHROMOSOME 13q IN EPITHELIAL OVARIAN TUMORS.

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**Aims**: To assess the frequency of loss of heterozygosity (LOH) on chromosome 13q in a series of sporadic ovarian tumors. To correlate LOH results with clinicopathological features of the tumors.

**Methods**: Genomic DNA of 52 patients with epithelial ovarian tumors (EOT) was isolated from peripheral blood lymphocytes and from fresh-frozen tissues. PCR was performed using seven polymorphic microsatellite markers (D13S217, D13S260, D13S171, D13S267, D13S263, D13S153 and D13S259) and visualized with autoradiography. LOH was ascertained by comparison of allelic patterns to corresponding normal tissue in heterozygotes and was scored by the absence or reduction of the signal to less than 50% of one of the alleles in the tumor.

**Results**: LOH for at least one polymorphic marker from a 3 cM region containing the BRCA 2 gene, were identified in 17 of 52 (33%) tumors, (0 of 10 benign, 1 of 7 bordreline and 16 of 35 malignant. Among ovarian cancers LOH was detected more frequently in serous carcinomas (8/17) than in mixed endometrioid-serous (3/6), endometrioid (2/4), clear cell (2/5) or muscinous (0/2) carcinomas, one carcinosarcoma also exhibited LOH. The D13S153 microsatellite, localized within the retinoblastoma gene, was lost in 9 of the 17 tumors (53%) with LOH in BRCA2 region. Patients with LOH + tumors were older (mean age 67) than those with LOH - neoplasms (mean age 56).

**Conclusions**: Results support the hypothesis that LOH on 13q may be associated with development of malignant ovarian tumors in elderly patients, particularly serous carcinomas.

## P-402

p53 MUTATION AND GENETIC INSTABILITY IN THE EARLY STAGES OF MULTIFOCAL ESOPHAGEAL SQUAMOUS CELL CARCINOMA: ANALYSIS USING MICROMANIPULATOR-BASED MICRODISSECTION AND PCR TECHNIQUE

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Aims: In esophageal squamous cell carcinoma (SCC), tumor multicentricity occasionally occurs synchronously. In this study, we analyzed *p53* gene mutation and genetic instability within individual lesions of multifocal SCC.

Methods: We studied 3 cases with surgically resected, multifocal esophageal SCC (1 mucosal, 2 submucosal carcinoma). DNA was extracted from the paraffin sections that were microdissected from individual areas constituted with p53-positive cells of SCC, dysplasia, basal cell hyperplasia (BCH) and normal squamous epithelium (NS).

Results: Analyzing the *p53* gene mutation by direct DNA sequence, 3 cases showed the missense mutations at identical codon that is contact mutation, in areas of SCC and BCH (case 1), 2 different SCCs and BCH (case 2), and NS (case 3). Case 2 showed heterogeneity for *p53* mutation in 3 areas noted above. Loss of heterozygosity (LOH) at *TP53* locus was detected in the same areas of case 2 and 3. Analyzing the replication error (RER) at five microsatellite loci, RER (+) phenotype was observed in most of the microdissected samples of 3 cases including areas of NS.

Conclusion: Our results suggested that: (*a*) concerning tumor multicentricity of esophagus, *p53* gene contact mutation together with LOH is, at least in part, a key event in early phase of multistage esophageal carcinogenesis, and (*b*) genetic instability may play an important role in development of multifocal primary esophageal SCC.

## P-403

**Highly Sensitive *In Situ* Hybridization Technique: Tyramide Signal Amplification (TSA™) or Catalyzed Reporter Deposition (CARD)**

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**Aims**: Non-isotopic *in situ* hybridization (ISH) is a useful technique broadly applied in histopathology. Its goal is the reliable and sensitive *in situ* detection of genetic nucleic acid sequences of interest. One drawback of conventional ISH is the fact that its sensitivity is often too low. Until recently, the microscopic demonstration of single copies of DNA or RNA was only possible by *in situ* polymerase chain reaction (IS-PCR). This technique, however, is costly and often lacks reliability, frequently giving false-positive and false-negative reactions. New modifications of ISH and its combination with catalyzed reporter deposition (CARD; also termed TSA) for the first time allowed the detection of single or very few copies of DNA-viruses and is also highly efficient for RNA detection. The ultrasensitive tyramide-amplified ISH technique can be easily combined with labels other than peroxidase, e.g. streptavidin-Nanogold™ (Nanoprobes, NY), fluorochromes or alkaline phosphatase. CARD-based ISH proved to be more sensitive than conventional ISH techniques in various applications.

**Methods:** 61 squamous cervical carcinomas from all grades were investigated for the detection of HPV 16/18. Routinely-processed and paraffin-embedded tissue sections were used. Conventional ISH with peroxidase, Nanogold-ISH and CARD-NG-ISH were compared.

**Results:** About 40 percent of all cases were positive by conventional ISH. The number of positive cases increased using NG-ISH to 45 %, and the highest sensitivity was achieved by CARD-NG-ISH with 65 % positive cases. For validiation of the overall sensitivity. SiHa cells derived from human cervical carcinoma, known to contain 1-2 copies per cell of HPV 16 (SiHa) were used. Condylomata acuminata routinely stained with a conventional ISH evaluated as negative or slightly positive for HPV 16/18 turned out to be clearly positive after performing CARD.

**Conclusions:** In our test system, more cellular structures were labeled with CARD in combination with peroxidase, streptavidin-Cy3 or Nanogold-gold-ISH. Furthermore, it was possible to reach single-gene-copy detection within 5-6 hours only, using an automated staining system (Ventana).

## P-404

DIAGNOSIS OF PAPILLARY THYROID CARCINOMA IS FACILITATED BY USING A RT-PCR APPROACH ON LASER-MICRODISSECTED ARCHIVAL MATERIAL TO DETECT RET ONCOGEN ACTIVATION

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**Aims:** The purpose of this study was to investigate the value of the expression of the RET oncogene (*RE*arranged during *T*ransfection) in papillary thyroid carcinomas (PTC) in the differential diagnosis of thyroid neoplasias. RET oncogene activation by chromosomal rearrangements have been exclusively implicated in PTCs.

**Methods:** To establish the incidence of RET activation in PTC's we used 5 µm sections from archival paraffin blocks. Either parts of the tissue slices were manually dissected or a few distinct cells were microdissected by laser-mediated manipulation with the ROBOT-MICROBEAM SYSTEM. RNA was extracted from paraffin-embedded thyroid tumors and the corresponding normal tissue. RT and nested PCR was performed using primers for RET/PTC1. –2, and –3, or for RET exons 12 and 13. PCR products were resolved by gel electrophoresis.

**Results:** We detected RET transcription in approximately 25% of the PTCs including follicular variants and in isolated cells of the same tissues, but not in non-malignant thyroid tissue.

**Conclusions:** Our method may serve as an additional diagnostic tool to type ambiguous papillary thyroid carcinomas and additionally allows to analyze expressed genes from routine histopathological tissue slides or single cells. Large retrospective studies can also be performed with this method.

## P-405

MOLECULAR ALTERATIONS OF P16/MTS1 GENE IN EWING'S FAMILY TUMOURS

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**Aims**: The cytogenetic translocation t(11;22)(q24;q12) is specifically associated with histopathologically diagnosed Ewing (ES) and peripheral primitive neuroectodermal tumours (pPNET), but it is possible that a less specific genetic event may occur that could be involved in the progression of this type of tumours. This study attempts to clarify whether molecular alterations of p16 tumour suppressor gene are implicated in the pathogenesis of ES/pPNET tumours.

**Methods:** We analysed 24 samples (biopsies and/or nude mice xenotransplants) collected from 18 patients with ES/pPNET tumours for the loss of p16 expression by RT-PCR and for the homozygous deletions and methylation status of the p16 gene by differential PCR and a methylation specific PCR (MSP) assay respectively.

**Results:** Samples from 16 of 18 patients had the cytogenetic translocation t(11;22)(q24;q12). Tumours of 7 patients showed no expression of p16 (39%), whereas 7 patients had tumours with a high level of p16 expression (39%) and the tumours from the remaining patients presented low levels of p16 expression (22%). Deletions of the p16 gene were present in tumours of 3 patients (17%) that correlated with the loss of p16 expression. Hypermethylation of the p16 gene occured in 3 cases (17%) correlated with a low expression of p16. In three samples of the same patient low p16 expression was observed in the primary tumour, whereas no expression and homozygous deletion of the p16 gene were observed in the nude mice xenotransplant from the primary tumour and in a metastatic lesion.

**Conclusions:** 1) The loss of p16 expression is a frequent event in ES/pPNET tumours. 2) This loss of p16 expression is due to homozygous deletions or hypermethylation of the gene, but some cases present this loss without deletion or hypermethylation, indicating that other mechanisms are implicated in the loss of p16 expression. 3) The loss of p16 expression confers a proliferative advantage to tumour cells that is maintained through the progression of ES/pPNET tumours.

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## P-406

**EXPRESSION OF HEAT SHOCK PROTEINS-70 IN HUMAN MESOTHELIAL CELLS IN VIVO AND IN VITRO**

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**Aims:** This study asked whether mesothelial cells respond to the injurious microenvironment during inflammation by an increased expression of heat shock proteins, which have a known cell protective role. The inflammatory reaction constitutes of coordinated sequele of cellular and humoral events leading to the neutralization of an insult and return to normal control of tissue function. Studies have reported that mesothelial cells and the cytokines release during peritonitis play a central role in the initiation and progression of the inflammatory process. We questioned wether the cytokine network controlling peritoneal inflammation play a role in the regulation of the HSP-70-family in human mesothelial cells too.

**Methods:** In vivo, HSP72 and HSP73 expression of mesothelial cells was investigated by immunohistochemistry on paraffin embedded tissue sections from appendices vermiformis with and without peritonitis. In vitro experiments were performed on human peritoneal mesothelial cells (HPMC). HPMCs were exposed to heat shock (42°C, 90 minutes), pro-inflammatory cytokines TNF-α (500 µg/ml) or IL-1β (50 µg/ml) for 2, 6, 12 hours. The HSP-expression were determined by immunoblotting.

**Results:** Immunohistochemistry of mesothelial cells during peritonitis showed an increased expression of HSP73 and HSP72 in comparison with resting peritoneum. As revealed by immunoblotting, TNF-α and IL-1β stimulation caused an increased in HSP72 and HSP73 expression in HPMCs after 6 hours incubation, similar to the heat shock response after exposure to 42°C.

**Conclusion:** We conclude that during peritonitis mesothelial HSP72/73 expression is enhanced. This effect may be explained in part by the inductive effect of proinflammatory cytokines TNF-α and IL-1β. We suggest that the described HSP induction in mesothelial cells during peritonitis ameliorate mesothelial injury from activated polymorphonuclear leukocytes.

## P-407

**MOLECULAR GENETIC ANALYSIS OF TUMOR SUPRESSOR GENES AND ONCOGENES IN SPORADIC RENAL CELL TUMORS**

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**Aims:** The accumulations of multiple genetic changes are essential steps in the progression of human cancers including the most common malignancy in the adult kidney, renal cell carcinoma (RCC). In a present study we analyzed 145 renal cell tumors of non-papillary and papillary growth pattern and 9 oncocytomas for genomic instability, methylation pattern and mutations in tumor suppressor genes (VHL, p53, mlh1, msh2) and oncogenes (K-Ras and Met).

**Methods:** Normal and tumor tissue was obtained from patients undergoing nephrectomy for renal cancer at Ljubljana University Clinical Center. High molecular weight DNA was isolated from frozen tumor tissue by phenol-chloroform extraction and ethanol precipitation. PCR - non-isotopic conformation analysis (CA) was used for mutations analysis followed by a direct sequencing of CA positive cases.

**Results:** Somatic mutations were identified in 41 tumors (46%) in the coding region of the VHL gene. They were twenty seven deletions, nine insertions, seven missense, six nonsense and two splice mutations. Thirty-four of these mutations predicted to truncate the VHL protein. Furthermore in eleven tumors hypermethylation of 5' CpG islands was found. In eleven RCC microsatellite instability was detected while in eight tumors missense mutations were identified in p53 gene, but no mutation was found in K-ras gene.

**Conclusions:** Our results showed that inactivation of the VHL gene as a critical "gatekeeper" gene is the most common molecular genetic event and was found in more than 60% of non-papillary RCC. Together with cytological and histological data the presence of VHL mutations in RCCs should lead to a better understanding of how renal cell growth is regulated and should aid in methods of diagnosis and in guidelines for therapy.

## P-408

DCC GENE ALTERATIONS IN GASTRIC CARCINOMA

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**Aims:** Determination of the incidence of loss of heterozygosity (LOH) of DCC gene and expression of its protein in gastric carcinoma (GC) and valoration of the prognostic usefulness.

**Methods:** Tumoral and no tumoral tissues criopreservated at -120°C and formalin-fixed, paraffin-embedded tissue from 35 gastrectomy by GC diagnosed at the HSCSP. Sex: 19M, 16F; age range: 39-87 years; histological classification: 17 intestinal (48.5%), 12 diffuse (34.2%), 4 mixed (11.4%), 2 unclassified (5%); follow-up: 10 patients alive without disease (25.5%), 18 dead by GC (51.4%), 5 dead by other causes (14.2%), 2 lost to follow-up (5.7%); stage I: 5 (14.2%), II: 8 (22.8%), III: 19 (54.2%), IV: 3 (8.5%). Immunohistochemical detection of DCC protein with monoclonal antibody DCC, clone G97-499 (Pharmingen, San Diego, CA) on paraffin-embedded tissue. Positive control: normal gastric mucosae counterparts (cytoplasmic reactivity in fundic, pyloric, and intestinal metaplastic glands). DNA extraction was obtained from microdissected frozen tissue. LOH was assessed by 5 markers at 18q21: extragenic D18S58, D18S69, D18S851, intragenic M2 and VNTR sequence. Statistical analysis: chi-square test.

**Results:** Only two tumors showed focal immunoreactivity (6%). LOH incidence: D18S58 27% (6/22), D18S69 15% (4/26) , D18S851 17% (5/29), M2 0% and VNTR 4% (1/24). There were LOH on 1 or more markers in 12 tumors (34%): 5 intestinal (29.4%), 4 diffuse (33.3%), 2 mixed (50%) and 1 unclassified (50%).

**Conclusions:** LOH of DCC gene is frequent (34%) but is not associated with survival (p=0.117), stage (p=0.220) or histological type (p=0.839) in GC. Lack of DCC protein immunoreactivity in GC is a common phenomenon independent of LOH at the DCC locus.

## P-409

**HPV AND HHV-8 DNA IN LOW GRADE AND HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESIONS OF THE UTERINE CERVIX.**

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**Aims :** Can HPV DNA be detected by PRINS method in paraffin embedded formalin fixed tissue?

**Methods :** formalin fixed paraffin embedded tissue of cervical neoplasia (CIN1: n=20 , CIN2: n= 20 , CIN3: n=20 , Squamous carcinoma: n=4) was investigated for the presence of HPV and HHV-8 DNA using PRINS technique with the following primers : HPV screening primers MY 09 and MY11 and with HPV genotypic primers for HPV 6, 11, 16, 18 and 33. HHV-8 DNA was detected by the KS 330 primer. The in situ synthesized DNA was visualized immunohistochemically. Conventional PCR was used as a confirmatory test.

**Results :** HPV DNA was present in all investigated cervical lesions. Nuclear signal was found with both the MY09 aand MY11 primer in the PRINS reaction. Both superficial as well as basal and suprabasal squamous cells were reactive. Subtyping with genotypic primers revealed HPV 6 and HPV 11 in 10 % of the specimens with CIN I. HPV 16, 18 and 33 DNA were found in 60 % of the CIN I lesions and in 85 % of the CIN II and CIN III lesions. The investigated carcinomas were positive for HPV 16 (75%) or HPV 18 (25%). HHV-8 DNA was not found.

**Conclusions :** The PRINS method is able to detect and subtype viral HPV DNA in formalin fixed paraffin embedded tissue. The results are comparable with those obtained by conventional PCR. The reactivity for both superficial and suprabasal squamous cells with the PRINS technique as well as the detection of HPV DNA in high grade lesions and carcinoma with this technique suggest a sensitivity comparable to PCR. HHV-8 DNA was not found in our series.

## P-410

**EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN-1 ONCOGENE DELETIONS IN SPANISH PAEDIATRIC HODGKIN'S DISEASE.**

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**Aims:** To study the distribution of Epstein-Barr virus (EBV) Latent membrane protein-1 (LMP-1) 30- base pair (bp) deletions in childhood HD as well as in healthy children.

**Methods:** We have analyzed by PCR 24 cases of EBV-positive pediatric HD and 39 normal controls to determine the prevalence of the 30-bp deletion in both populations. EBV typing was also performed.

**Results:** The 30-bp deletion was identified in 19 of 24 pediatric HD cases (79.2%), in 7 (29.2%) the deleted fragment was the only present, whereas in the remaining 12 (50%) it was accompanied by a nondeleted band. Conversely, the 30-bp LMP-1 deletion was found in 8 of 22 (36.4%) EBV-positive healthy children, in 2 (9.1%) the deleted fragment was alone, and in the other 6 (27.3%) the deleted LMP-1 gene was co-infecting with the nondeleted variant. Statistical analysis showed that the LMP-1 deletion was significantly associated with pediatric HD both including dual infections ($P$= 0.006) or excluding them ($P$= 0.01).

EBV typing showed that 25% of children with HD carried type 2 virus, whereas all controls harbored EBV of type 1. The analysis also revealed the presence of the 30-bp deletion in all specimens of pediatric HD that contained EBV of type 2.

**Conclusions:** These findings indicate that EBV strains carrying the 30-bp deletion in the LMP-1 oncogene may have a more important role in the pathogenesis of pediatric HD than EBV containing the full-size LMP-1. Dual infection by LMP-1 deleted and nondeleted EBV strains is a frequent event both in healthy children and in the pediatric HD population from Spain. Concerning EBV typing, type 2 EBV strain associated with pediatric HD since all healthy children carried EBV of type 1. Finally, the fact that all cases harboring EBV of type 2 showed the deletion suggests that a deleted type 2 EBV strain may be more tumorigenic than a nondeleted type 2 EBV strain.

## P-411

**ANALYSIS OF GENE DIVERSITY AND EXPRESSION PATTERNS IN CERVICAL CELL LINES**

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**Aims:** The following questions constituted the basis of this as yet incomplete study: 1) whether it is possible to use the micro-array to determine differential gene expression in normal cervical epithelia, 2) whether a gene-expression pattern is present and 3) to what extent can positive genomic DNA spots within the function groups be compared in terms of their intensity. Poly $A^+$ RNA cell suspensions from normal cervical epithelia and cervical carcinomas were used for differential gene expression.

**Methods:** The "Atlas array" method makes use of two identical nylon membranes with specially prepared cDNA spots. This enables a direct comparison of the expression profiles of two mRNA populations. In the first step, 2 µl of each Poly $A^+$ RNA population are catalyzed with the reverse transcriptase enzyme together with [$\alpha$-$^{32}$P]dATP. All complex radioactively-labeled cDNA probes are then individually hybridized on the "Atlas array", analyzed using autoradiography and semi-quantified. The level of cDNA expression of two different mRNA matrices can be estimated by comparing signal intensities.

**Results:** In normal and cancerous cells, the transcribed gene patterns demonstrate up/down-regulation in the function groups, similar to transcription of: 1) oncogenes, 2) tumor suppressor genes, 3) cell-cycle regulator proteins.

**Conclusions:** Changes in signal transduction in cellular neoplasias give rise to different levels of gene expression. In addition, variations in the gene expression patterns of transcription factors, cell cycle and apoptosis proteins can be observed. These disturbances of gene regulation are thus a central, molecular-biological characteristic in the cancerogenesis of malignant tumors.

## P-412

MOLECULAR ANALYSIS REVEALS EVIDENCE OF DISSEMINATED INVOLVEMENT IN THE AUTOPSY OF A CUTANEOUS T-CELL LYMPHOMA

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A polimerase chain reaction (PCR) amplification of T-cell receptor-γ (TCRγ) gene rearrangement was used for molecular staging in a primary cutaneous T-cell lymphoma (CTCL) with fatal evolution. Although initial evaluation was negative for systemic involvement, the patient died due to heart failure. Autopsy findings revealed lymphomatous myocardial infiltration of but any other tissues and organs examined including lymph nodes, liver, spleen, lung and bone marrow appeared to be free of disease. Interestingly, molecular analysis from frozen samples obtained during the initial evaluation as well as paraffin-embedded material obtained during the autopsy revealed the presence of clonal rearranged bands in all tissues examined except the bone marrow. Subsequent hybridization of PCR products with a tumor-specific oligoprobe confirmed the PCR results suggesting a widespread dissemination of the lymphomatous process. Therefore, the use of molecular analysis can add significant information about the extent of the disease in patients with CTCL and be helpful in the establishment of therapeutic options.

## P-413

**THE REPLICATION ERROR PHENOTYPE IN NERVOUS SYSTEM TUMORS**

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**Aims:** Microsatellite instability or replication error (RER) has been implicated in human carcinogenesis. We undertook this study to assess the incidence of RER in primary nervous system tumors using a fluorescence-based semiautomated DNA sequencer. **Methods:** Paraffin-embedded samples from 65 gliomas, 32 meningiomas and 11 schwannomas were obtained from the Department of Pathology and 10µ section were used for DNA extraction. We evaluated eight microsatellite loci (five tetra- and 3 dinucleotide repeats) on seven chromosomes. The PCR products were run on polyacrylamide gels using an ALFexpress® automated sequencer and the sizes of the resulting fragments were compared. Microsatellite instability (MIN) was defined by the presence of one or more additional peaks in the tumor not present in the corresponding normal DNA. We considered a tumor to exhibit the RER phenotype when MIN was present in ≥25% evaluated loci. MIN positive and MIN negative tumors were compared for histological type, tumor grade, location, size, duration of symptoms at diagnosis, sex, and patient age. **Results:** 17.9%, gliomas, 6.3% meningiomas and 18.2%, schwannomas were MIN positive. The RER positivity rate was 1,8%, 6,3% and 9,1% respectively. High grade gliomas showed more frequent MIN than low grade gliomas (p = NS). MIN positive meningiomas had a shorter clinical course (p = 0,0172). MIN was more frequent in spinal than cranial schwannomas ( p = 0,0182). **Conclusions:** Instability of microsatellites is an infrequent feature in primary human brain tumors. It is unlikely that mutations of the mismatch repair genes play an important role in the developement of these tumors. Larger series are needed to confirm the association between MIN and spinal localtion in schwannomas.

## P-414

PCR DETECTION OF THE t(14;18)(q32;q21) INVOLVING BCL-2 ONCOGENE IN FORMALIN-FIXED, PARAFFIN-EMBEDDED SPECIMENS OF FOLLICULAR LYMPHOMA

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**Aims:** The t(14;18)(q32;q21) chromosomal translocation involving bcl-2 oncogene and occurring in 80% of follicular lymphomas can be detected using the polymerase chain reaction. In order to improve molecular diagnosis of low-grade follicular non-Hodgkin's lymphoma we developed the PCR method for detection of t(14;18)(q32;q21).

**Methods:** We analysed 17 cases of low-grade NHLs, all of which were hystologically diagnosed as follicular centroblastic-centrocytic lymphomas as well as appropriate positive and negative controls. PCR analysis of t(14;18)(q32;q21) was performed with the use of two different primers complementary to the negative strand of major breakpoint region of bcl-2 oncogene (mbr1 and mbr2 primers) and consensus primer for Jh gene segments of the immunoglobulin heavy chain gene (IgH). All 17 cases were also analysed for the presence of IgH gene rearrangements with the use of primer complementary to the FRIII region of Vh gene segments and consensus Jh primer. PCR products were analysed by electrophoresis in 2% agarose and 12% polyacrylamide gels, stained with ethidium bromide and photographed under UV light.

**Results:** We detected the t(14;18)(q32;q21) in 7 of 17 analysed cases (41%) by the presence of amplified product of 170-250 bp in length. Seven cases were monoclonal by the IgH-PCR analysis and negative for the presence of the t(14;18)(q32;q21). The last 3 cases were polyclonal by the IgH-PCR analysis, but also negative by the mbr-PCR analysis.

**Conclusions:** We introduced the PCR method for detection of the t(14;18)(q32;q21) involving bcl-2 oncogene. Using described method we detected this chromosomal translocation in 41% of analysed cases of follicular lymphomas. In our hands the sensitivity of the method is lower than in other laboratories with detection rate of 50%.

## P-415

**SV40 LIKE DNA IN MALIGNANT MESOTHELIOMA.**

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**Aims :** Is SV40 like DNA detected by the PRINS method or SV40 viral protein a potential marker for neoplastic mesothelium ?

**Methods :** Formalin fixed paraffin embedded tissue of 25 pleural malignant mesotheliomas together with 25 specimens of reactive pleura and 25 pleural lung carcinoma metastasis were investigated for the presence of SV40 like DNA using PRINS technique with the following primers : PYVrev detecting a viral sequence common to SV40 virus, BK virus and JC virus and primer SVrev specific for SV40. Primed in situ labeling was performed with an annealing step at 52°C for 10 minutes followed by an elongation step at 72 °C during 30 minutes (500 µM dATP, 500 µM dCTP, 500 µM dGTP, 450 µM dTTP, 50 µM dig-dUTP in 50% glycerol and 2.5 U Taq polymerase). The synthesized DNA was visualized immunohistochemically. The presence of SV40 viral proteins was investigated immunohistochemically with the Pab 280 antibody recognizing a formalin resistant epitope of the SV40 small t-antigen.

**Results :** In the majority of mesothelioma specimens (60%) a strong nuclear signal was found with both the PYVrev and SVrev primer in the PRINS reaction. The amount of reactive neoplastic cells ranged from 5 to 95 %. In all the cases that were positive for viral DNA there was immunoreactivity for SV40 small t-antigen with the Pab 280. No SV40 DNA or SV40 small t-antigen was found in the non-neoplastic mesothelium or carcinoma metastasis.

**Conclusions :** The PRINS method is able to detect viral DNA in formalin fixed paraffin embedded tissue. SV40 like DNA and immunoreactivity for SV40 small t antigen might be potential markers for mesothelioma as non-neoplastic mesothelium and pleural lung carcinoma metastasis were negative for SV40 DNA or SV40 protein.

## P-416

PRIMARY CEREBRAL GLIOSARCOMA:A CASE REPORT.

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Gliosarcoma is an uncommon brain malignant tumor with a mixture of neuroglial and mesenshymal components. This entity rises questioning about its clinical features, its management and its histogenesis.

We report on a case of a 57 years-old male patient who souffered from intra-cranial hypertension during 3 months. MRI and CT scan showed a frontal expansive tumor process surrounded with edema. Histopathologic examination of a stereotaxic biopsy of the tumor evoked the diagnosis of meningioma. The patient underwent surgery for tumor resection but he died a week later in a comatose status.

Gross analysis showed a solid heterogenous tumor on cut sections.

Microscopic examination revealed a polymorphous malignant tumor made up of a mixture of a fusocellular proliferation (photo) rolling up arround thick vessels, foci of neuroglial cells (photo) and osteocartilaginous islands.

Immunohistochemistry staining showed a strong positivity of the different components with PS100 antibody (photo), positivity of the glial cells with GFAP antibody (photo) and a scatered positivity of the vessels and the fusocellular component with smooth muscle Actin antibody (photo).

The immunoreactivity of our tumor is in agreement with the suggested histogenesis which might be either from smooth muscle or via differenciation from a pluripotential cell precursor.

The aggressiveness of gliosarcoma is well demonstrated in our case since the whole medical course lasted no more than 4 months ending in the death of the patient a week after surgery.

## P-417

EPIDEMIOLOGICAL AND MORPHOLOGICAL ANALYSIS OF MENINGIOMAS AND ATTEMPT TO CORRELATE NUCLEAR MAGNETIC RESONANSE IMAGE WITH HISTOLOGICAL APPEARANCE

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We analyzed 544 cases of meningioma (out of 5615 neuropathologic specimens) diagnosed in 1989-1998. The male-to-female ratio was 1:3.18 (130 males and 414 females). There were 26 patients below 20 y, 54 aged 21-30 y, 127 aged 31-40 y, 132 aged 41-50 y, 122 aged 51-60 y, 72 aged 61-70, and 11 over 70 y (range 13-86 y).
In 89.5% of patients tumors were located in the cranial cavity and in 10.5% of patients in the spinal canal. Out of all intracranial tumors, 30.5% (34% in males and 27% in females) localized in the anterior fossa, 38% (42% in males and 34% in females) in the medial fossa and 21% (19% in males and 23% in females) in the posterior fossa.
The following histological types of intracranial meningioma were diagnosed: meningotheliomatous (116 cases, 34 males and 82 females), transitional (106 cases, 21 males and 85 females), fibroblastic (104 cases, 16 males and 88 females), psammomatous (24 cases, 3 males and 21 females), angiomatous (22 cases, 6 males and 16 females), atypical and malignant (46 cases, 31 males and 15 females). The remainder were microcystic, clear cell, chordoid, metaplastic, and papillary types. In the spinal canal the following types predominated: psammomatous (31 cases, 10 males and 21 females), fibroblatic (9 cases, 3 males and 6 females), mixed (6 cases, 2 males and 4 females), atypical and malignant (7 cases, 3 males and 4 females), and others (4 cases, 1 male and 3 females).
We also carried out comparative analysis of the histological appearance of tumors and their NMR images in 13 cases diagnosed in 1998.

## P-418

NEUROPATHOLOGICAL CLINICAL AND NEURORADIOLOGICAL FINDINGS IN TUMOURS ASSOCIATED WITH CHRONIC PHARMACORESISTANT EPILEPSIES.(C.P.E.)

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Aims: Describe the histopathology of tumours associated with CPE and relate to clinical and neuroradiological findings.
Methods: For each patient were recorded the following data. age, sex, site of the tumour, duration of seizures, type of resection, and results of neuroimaging. Tissue representative of neoplasia was included in paraffin and stained with E&E ,Kluver-Barrera, Nissl, GFAP, Synaptofisin , Neurofilament, Vimentin ,and MIB 1. Histopathological diagnosis were matched with clinical and neuroradiological records.
Results from June 1996 to February 1999 were operated 132 patients. In 37 cases (27.5%) a neoplasia was diagnosed. The most frequent diagnosis was disembrioplastic neuroepithelial tumour (DNT) (10 cases) followed by ganglioglioma (8 cases).All but two were low grade (grade I and II WHO).The most frequent site was temporal lobe . No difference was observed for age and sex for different histotype. The duration or seizures was longer for DNT (mean 12 years) and shorter for fibrillary astrocytoma (2 years).In all the patients neuroimaging was considered abnormal. A diagnosis of low grade neoplasia was rendered in the majority of cases with only few diagnosis of non-neoplastic lesion (e.g. cortical dysplasia).
Conclusion neoplasia associated with CPE are a particular setting of brain tumour with a high proportion of low-grade glioneuronal lesions. The knowledge of neuroimaging and of the length of clinical history is useful in avoiding diagnosis of high grade tumours.

## P-419

PROGRESSION OF HISTOPATHOLOGICAL AND GENETIC FINDINGS IN RECURRENT MENINGIOMAS

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**Aims:** Neoplastic progression in meningiomas exhibits different morphological changes and an increased tendency to recur after surgery. The morphological changes form are the basis of the World Health Organization (WHO) classification of tumors in benign, atypical and anaplastic (malignant) meningiomas. A number of cytogenetic studies have shown that atypical and anaplastic meningiomas, in addition to monosomy 22 or 22q deletions, usually show abnormalities of several other chromosomes.
**Methods:** We present a group of 12 recurrent meningiomas in five different patients. Morphological, immunohistochemical, cytogenetics and fluorescence in situ hybridization (FISH) studies have been performed. Semiquantitative methods have been used to determine cellular density and proliferation index (PCNA, Ki-67).
**Results:** Only one case presented benign morphological features and later evolved as an atypical tumor. In the other cases the first recurrence had atypical morphological findings and successively evolved as anaplastic morphological meningiomas.
The cytogenetic results showed that except in one case, where the complex karyotype was present from the beginning, in the other cases the first recurrence showed a normal karyotype or only monosomy 22, and the following recurrences showed complex karyotype with clonal and non-clonal anomalies. The loss of short arm of chromosome 1 in 1p36 is the most common cytogenetic anomaly found.
**Conclusions:** Analysis of morphological findings showed a progressive increase in successive recurrences of nuclear pleomorphism, mitosis, necrosis, proliferantion index and complex karyotypes. Deletion of chromosome 1p was the most important anomaly in the meningioma progression.

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## P-420

PITUITARY CARCINOMA: STUDY OF TWO CASES

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**Aims**: Pituitary carcinomas are rare adenohypophysial neoplasms. The inicial clinical presentation is usually that of a pituitary adenoma. Morphologic findings are no well-defined and are found in pituitary adenomas too. Only by presence of craniospinal and/or systemic metastases they can be diagnosed with certainty. The rarity of these cases do not allow valid conclusions concerning the pathogenesis and treatment. In general, patients have a poor prognosis.
**Methods**: We report a clinicopathologic study of two cases examined by light microscopy and immunohistochemistry. Pituitary hormones, proliferative activity (MIB-1) and p53 immunoreactivity were studied in primary and metastatic tumors.
**Results**: Case 1 a 36-year-old male presented with panhypopituitarism and visual deficit. He has a non-functioning tumor that is initially considered a macroadenoma with suprasellar extension. The time interval between the diagnosis of pituitary adenoma and metastatic carcinoma was 63 months with rapidly progress to death (10 months).
Case 2 a 18-years-old female presented with amenorrhea, hyperprolactinemia and visual deficit. A prolactin-producing tumor is initially considered a pituitary adenoma with suprasellar extension. The time interval between the primary tumor and metastatic carcinoma was 39 months with rapidly progress to death (12 months).
Both cases presented sellar tumour recurrence and disemination in subaracnoid space. Histopathological appearance of all tumors has not correlated with agressive behaviour. The proliferative activity and p53 expresion is increased in recurrent tumors and much more in metastatic neoplasms.
**Conclusions**: The high mitotic and MIB-1 labeling indices as well as p53 immunoreactivity may be of some diagnostic usefulness as markers of biologycally agressive behavior.

## P-421

PROGNOSTIC SIGNIFICANCE OF PROLIFERATION INDEX AND EXPRESSION OF GFAP IN MALIGNANT ASTROCYTOMAS

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The WHO and St.Anne-Mayo grading systems for astrocytic neoplasms, are the most frequently used for differential diagnosis between anaplastic astrocytoma (AA) and glioblastoma multiforme (GM). Both systems are based only on histological criteria and the agreement among pathologists is frequently low. Because of that we proposed a system based on the differentiation of tumoral cells, quantifing the cytoplasmic expresión of GFAP and the proliferation index (PI) by measuring the immunohistochemical expression of Ki-67.

We have studied 81 patients with malignant astrocytomas that were followed clinically until death due to tumor. In an univariate analysis we found prognostic value for, age, sex, necrosis, angiogenesis, expresión of Ki 67 and GFAP. But only did expressión of Ki 67 and GFAP show independent prognostic value using the Cox Stepwise regression model. We classified as GM the tumors with a PI higher than 15% of tumoral cells or with a weak GFAP expression and as AA the rest of malignant astrocytomas. We found statistically significant survival differences between these two groups of patients ($p<0,000$). In addition we found a lesser degree of overlapping (33%) with our system vs WHO grading system (41%).

Conclussion: The combination of PI and expression of GFAP in tumoral cells has prognostic value in malignant astrocytomas and it can be useful to differentiate between anaplastic astrocytoma and glioblastoma multiforme.

## P-422

PROGNOSTIC SIGNIFICANCE OF ANGIOGENESIS IN LOW-GRADE OLIGODENDROGLIOMAS

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The importance of angiogenesis as a prognostic factor in brain tumors recently has been reported. Based on the expression of CD-34 endothelial marker, tumors with low or high vascular density may be identified, but this factor had no influence on the survival rate of patients.

Nevertheless, the prognostic significance of intratumor angiogenesis must be evaluated in relation to the number of tumor cells. For this reason, in the present study, we analyzed the long-term prognostic significance of a morphometric score expressing the vascular area for every 1000 tumor cells in patients with a low-grade oligodendroglioma that has been treated surgically and irradiated

A series of 26 patients with a low-grade oligodendroglioma was followed clinically for 10 years or until death due to tumor progression. In each case, the tumor vascular surface index (VSI) was determined as the CD-34 immunostained endothelial surface in $\mu m^2$ per 1000 tumor cells. Survival was compared between patients with a VSI lesser or greater than 15 and the correlation between the VSI score and CT-scan tumor-enhancement was analyzed. Patients with a VSI of less than 15 ($n=12$) showed a survival at 5 and 10 years of 100% and 71%, respectively, versus a survival of 50% and 0% for patients presenting a VSI greater than 15 ($n=14$) ($P < 0.05$).

Our present findings show the usefulness of the VSI as a long-term prognostic factor in low-grade oligodendroglioma

## P-423

**DIAPHRAGMATIC MITOCHONDRIAL CRYSTALLINE INCLUSIONS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS. AN ULTRASTRUCTURAL STUDY.**

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**Background**: Impaired respiratory muscle function is thought to be a relevant pathogenetic mechanism in chronic obstructive pulmonary disease (COPD) patients. Although it has been attributed to mechanical overload, this disfunction could also be related to a primary defect in respiratory muscles, predisposing COPD patients to develop respiratory muscle fatigue. Mitochondrial crystalline inclusions (MCI) can be induced by oxidative stress, but are not specific for primary or secondary muscle disease. This study was carried out to determine the incidence and functional correlates of MCI in muscle fibers of COPD patients.

**Material and methods**: Diaphragm, latissimus dorsi, and deltoid muscle biopsies from 40 patients with COPD, undergoing surgery for other reasons, were the subject of the present study. Samples were processed for electron microscopy. Micrographs were taken from areas with high mitochondrial densitiy (MD) at constant, calibrated magnification of 5,700. MD was obtained by counting the number of mitochondrial sections per 100 $\mu m^2$ of muscle fiber. Relationship of MCI with MD, $FEV_1$, $FEV_1$/FVC, RV, RV/TLC, $PaO_2$, and $Pdi_{max}$ was investigated.

**Results**: MCI were found in diaphragm muscle fibers of 4 patients (10%). These inclusions consisted of four parallel layers, with periodic dots between them, and were located in the intercristal mitochondrial space. They were not found in latissimus dorsi nor deltoid muscle fibers in any of the cases. Presence of MCI was not correlated with age (64 ±12 years), MD (50±28 mit/100 $\mu m^2$; Control values= 46±15mit/100 $\mu m^2$), $FEV_1$ (60±34%ref), $FEV_1$/FVC (60±12%), RV (110±33%ref), RV/TLC (43±18%ref), $PaO_2$ (85±8 mm Hg) nor $Pdi_{max}$ (86±18 cm $H_2O$).

**Conclusion**: MCI can be found in the diaphragm of 10% of COPD patients. Their occurrence does not seem to be related to severity of disease, and it has not been observed in other muscles from these patients. Although MCI are probably a non-specific finding, they could be the reflection of a primary mitochondrial defect. Study of mitochondrial DNA and respiratory chain components in the diaphragm of COPD patients could be helpful in understanding respiratory muscle disfunction, as well as the biological meaning of these inclusions.

## P-424

CLINICO-PATHOLOGICAL ANALYSIS OF PILOCYTIC ASTROCYTOMAS & GANGLIOGLIOMAS IN CHILDREN.

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**Aims**: Clinico-pathological analysis of pilocytic astrocytomas (PA) & gangliogliomas (GG), connected with neuronal markers investigation in these two groups of neoplasms. Analysis of surviving.

**Methods**: The data from 69 patients in age from 4 months to 16 years with tumors of CNS, recognised as pilocytic astrocytoma (58cases) or ganglioglioma (11 cases) was retrospectively reviewed. The existing HE-stained hisopathological slides of all cases were reviewed too. All specimens were additionaly stained using antibodies to „GFAP, SYN & NFP". Historical, clinical, neuroimaging and intraoperative data was analysed carefully as well as median survival time in these groups of patients.

**Results**: During the retrospective review of 58 cases recognised as PA, 7 cases had characteristic, perikaryal synaptophysin positive immunoreactivity arround large neuron-like cells and 8 tumors had positive NFP reactivity in the cellular processes. Finally, 11 verified neoplasms, which previously carried diagnosis of PA, demonstrated strong, immunopositive reaction for SYN or NFP or both antibodies, These cases were reclassified as gangliogliomas. None of 11 tumors recognised as „GG" were reclassified as PA. After mentioned above verification 47 neoplasm carried diagnosis of pilocytic astrocytoma & 22 of ganglioglioma.

The overall 5-year survival was 88,89% in PA and 70,00% in GG group.

**Conclusions**: Pilocytic astrocytomas must be scrupulously differentiated from Gangliogliomas. Differential diagnosis should be made with help of immunohistopathological reaction on SYN & NFP.

The highest survival in the midst of children's CNS neoplasms is observed among PA-s and it is almost twice higher then among Fibrillary Astrocytomas. Survival in a group of ganglioglioma is a little less then in pilocytic astrocytoma and it is probably related to unfavourable lacalisation of the former.

## P-425

**EXPRESSION OF P53 IN LOW-GRADE GLIOMAS. PROGNOSTIC VALUE.**

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**Aims**: To determine overexpression of p53 in a series of low-grade gliomas and evaluate a possible relationship with tumoral agressive behaviour.

**Methods**: 48 low-grade gliomas (30 astrocytomas, 14 oligodendrogliomas and 4 oligo-astrocytomas) in 29 males and 19 females (age range 15-75) were examined for expression of p53, which was measured as the percentage of stained nuclei. Tumoral progression was assumed in cases of recurrence, rapid growth after incomplete surgical resection or histologically proved malignant transformation.

**Results**: 28 patients (37,5%) showed tumoral progression. For the entire group, 32 tumors (67%) showed positive p53 inmunoreactivity. p53 expression was observed in 16 astrocytomas (53%), 12 oligodendrogliomas (86%) and 4 oligo-astrocytomas (100%). Patients with p53 negative tumors showed longer periods without tumoral progression than patients with p53 positive ones, although the difference was not statistically significant. Further separation of positive tumors into two groups (p53 indices higher or lower than 10%), showed neither a significant difference.

**Conclusions**: p53 immunoreactivity is observed in a high number of low-grade gliomas. p53 expresses more frequently in tumors with an oligodendroglioma component, whether they are pure or mixed. Overexpression of p53 is not predictive of agressive behaviour in low-grade gliomas.

## P-426

**PREDICTION OF RECURRENCE OF BENIGN MENINGIOMAS USING ANTIBODIES AGAINST PROTEINS THAT REGULATE DNA REPLICATION**

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Aims: No features predictive of recurrence of benign meningiomas have been consistently identified to date. We have investigated the potential utility of antibodies against the minichromosome maintenance-2 protein (Mcm-2), a member of the DNA-binding pre-replicative complex, in predicting meningioma recurrence. MCM proteins are essential for eukaryotic DNA replication and are present throughout the cell cycle, but down-regulated in quiescence and cell differentiation, making them specific markers of proliferating cells.

Methods: We compared the Mcm-2 Labelling Index (percentage of positively-stained cells) of 14 recurrent meningiomas with 28 non recurrent meningiomas, measuring for each tumour both the average Labelling Index ($L.I._{Average}$)and the Labelling Index for the region of highest proliferative activity ($L.I._{Highest}$).

Results: Our results show that both the Mcm-2 $L.I._{Average}$ and Mcm-2 $L.I._{Highest}$ are significantly greater in the meningiomas which subsequently recurred compared to the nonrecurrent group. The values for Mcm-2 $L.I._{Average}$ show a large degree of overlap between groups, and therefore an individual value contains little information predictive of recurrence. However, values for Mcm-2 $L.I._{Highest}$ overlap much less between groups, suggesting that the individual values contain useful information for prediction of tumour recurrence.

Conclusions: The results from this study suggest that activity of the region of highest proliferation appears to be an important determinant of meningioma recurrence.

## P-427

GLUCOSE TRANSPORT OF ASTROCYTES DURING MICROSPHERE-INDUCED BRAIN ISCHEMIA IN RATS: AN ULTRASTRUCTURAL STUDY

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Aims: Brain infarction is the most common disease of the central nervous system. However, glucose metabolism of nerve cells and astrocytes during brain ischemia is not fully understood. To study the structural changes of nerve cells and astrocytes during brain ischimia, brain infarction was induced by injection of microspheres through the internal carotid artery, and surviving cells surrounding infarcted areas were observed by light and electron microscope.

Methods: Adult male Wistar rats were used in this study. The brain infarction was induced by injection of microsphares, 47.5 $\pm$ 0.5 $\mu$ m in diameter, through right internal carotid artery. Rats which showed hemiplegia were sacrificed at 3, 5 and 7 days after operation and the brains of these animals were observed by light and electron microscopy.

Results: At 3 days after microsphere injection, necrotic areas, variable in size and shape, were seen mainly in the parietotemporal cortex, corpus callosum, hippocampus, thalamus, and lenticular nucleus of the affected hemisphere. These necrotic areas showed liquefactive changes toward 14 days after operation. Perivascular narrow areas and cytoplasm of some glia cells were distinctly PAS positive in the survival areas on the 3rd day after operation. However, these PAS positive cells disappeared in the tissues on the 7th day. In electron microscopic observations, numerous glycogen granules accumulated in the cytoplasm of astrocytes on the 3rd day. The cytoplasm of astrocytes surrounding capillary vessels also contained numerous glycogen granules. Numerous small cytoplasmic processes containing glycogen granules were attached to the surfaces of the nerve cells. In contrast, glycogen granules could not be found in the cytoplasm of the nerve cells. The glycogen granules of astrocytes decreased in number by the 5th day, and were almost absent by the 7th day after operation.

Conclusion: Glycogen granules accumulated in the cytoplasm of the astrocytes during the early stage of brain infarction. These findings indicate that a) glucose is transported from astrocytes to nerve cells, 2) glucose utilization of nerve cells is impaired in the early stage of brain ischemia, 3) the function of glucose transport is not impaired in the astrocytes during brain ischemia, and 4) accumulated glucose is stored as glycogen granules in the cytoplasm of the astrocytes..

## P-428

**PATHOLOGICAL COMPARISON BETWEEN CHILDREN AND ADULTS WITH CORTICAL LOBECTOMY FOR INTRACTABLE SEIZURES**

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**Aims:** Many pathological surveys of partial resections of cerebral cortex for intractable seizures have been reviewed by several groups. Few studies, however, have been focused on the differences between childhood and adults. Herein we assessed the differences of pathologic features between adults and children.

**Methods:** One hundred and sixty-four lobectomies for intractable complex partial and generalized tonic clonic seizures were histologically analyzed. There were 28 children (less than 15 year) and 136 adults. We compared the frequency of histopathologic features, the distribution of involved cortex (temporal or extratemporal lobe), previous history of febrile sezure, and coexistence of other lesions (dual pathology) between two groups. Focal cortical dysplasia was graded into mild, moderate, and severe. Mild cortical dysplasia represented microdysgenesis. Polymicrogyria and/or white matter glioneuronal heterotopia belonged to moderate cortical dysplasia and the presence of balloon cells and/or neuronal cytomegaly to severe dysplasia. More than 8 heterotopic neurons per 200 $mm^2$ in white matter were regarded as single heterotopic white matter neurons.

**Results:** Pathologic alterations were encountered in 92 % of 164 patients. There were cortical dysplasia (n=97), hippocampal sclerosis (n=86), neoplasm (n=27), cerebral cysticercosis (n=3), cortical tuber (n=1), leukomalacia (n=1), and Rasmussen's encephalitis (n=1). Focal cortical dysplasia was more frequent lesion in adults (79 %) than in children (57%). A higher frequency of severe cortical dysplasia, neoplasia and extratemporal lobe involvement was found in children (31.2%, 25%, 50%) than in adults (1.2%, 11.7%, 24%). Subependymal giant cell astrocytomas and central neurocytoma were exclusively found in children. Glioblastoma, oligodendroglioma, gliomatosis cerebri and vascular malformations were found only in adults. Dysembryoplastic neuroepithelial tumors and gangliogliomas were observed in both group. Hippocampal sclerosis was associated with younger age of seizure onset and previous injury. Dual pathology was seen in 54% of 164 patients (5 children and 86 adults) and much more common in adults (63%) than in children (18%).

**Conclusions**: Based on our results, more frequent extratemporal lobe involvement, a higher frequency of neoplastic lesions and a lower incidence of dual pathology were common in pediatric intractable seizures.

## P-429

**MIXED GLIONEURONAL TUMORS ASSOCIATED WITH EPILEPSY: A SPECTRUM OF INTERRELATED LESIONS**

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**Aims:** Several types of tumors and hamartomas are distinguished by glial proliferation and the presence of neuronal cells. Meningioangiomatosis is characterized by meningeal desmoplasia and cortical infiltration by fibroblastic elements intermixed with glia and neurons. Ganglionic tumors frequently contain neurons of different sizes and glial cells with or without meningeal proliferation and desmoplasia.

**Methods:** Twenty-two lesions associated with drug-resistant temporal epilepsy were studied. They were diagnosed as meningioangiomatosis (n=6), ganglioglioma (n=10), gangliocytoma (n=3) or pleomorphic xanthoastrocytoma (n=3).

**Results:** Meningioangiomatosis was characterized by meningothelial and glial-neuronal proliferation, with variable proportions of the different components, as well as sclerosis and other more cellular lesions. All of them showed positivity for neurofilaments, synaptophysin and GFAP. The gangliogliomas and gangliocytomas had mixed neuronal and glial components and variable degrees of meningeal and vascular involvement. The pleomorphic xanthoastrocytomas presented signs of neuronal differentiation, fibrosis and pleomorphic glial cells.

**Conclusions:** The histological study of these lesions demonstrates that they all contain neuronal cells in different stages of maturation and astroglia or oligodendroglia. This fact suggests that meningioangiomatosis, gangliogliomas, gangliocytomas and glial tumors with divergent differentiation may all represent a single type of tumor, with variable proportions of the same cellular elements.

## P-430

DYSEMBRYOPLASTIC NEUROEPITHELIAL TUMOR. PRESENTATION OF TWO CASES, ONE IN A 37 YEARS MALE.

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**AIMS**: Dysembryoplastic Neuroepithelial Tumor (DNT) is an uncommon lesion considered quasihamartomatous and closely associated with embryogenesis by some authors. Generally it is diagnosed in children after a long history of seizures and has an excellent prognosis despite incomplete tumor removal. We present two cases of DNT in two patients aged 6 and 37 years. The tumors were located in temporal and frontal lobes and were considered clinically as low grade gliomas.

**METHODS**: Standard histological procedeure and immunohistochemical study were performed.

**RESULTS**: Microscopic examination showed two neuroepithelial tumors composed of mutiple nodules of oligodendrocite-like cells in a ribbon or targetoid pattern and microcysts containing mucoid basophilic material. The intervening cortex displayed only mild astrocytosis without displastic features. Well differentiated neurons occasionally within mucous pools and peripheral satellitosis were appreciated. These lesions closely resemble low grade oligodendrogliomas and must be differentiated also from pylocytic astrocytoma, ganglion cell tumor and central neurocytoma, particularly in frozen sections.

**CONCLUSIONS**: Dysembryoplastic Neuroepithelial Tumor is a complex lesion of a relative difficult diagnosis that should be cosidered in the differential diagnosis of seizure-causing neoplasms. Distinction from other tumors is important because of the good behavior of this lesion; its recognition will avoid unnecesary aggresive therapy.

## P-431

PRIMARY MALIGNANT MELANOMA OF CENTRAL NERVOUS SYSTEM WITH SUBARACNOIDAL INVASION. A CASE REPORT.

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We report a primary malignant melanoma of central nervous system (PMMCNS) with massive subaracnoidal invasion, and discuss differential diagnosis.

Male aged 42 with neurologic deficit and normal computerized tomography, who showed on magnetic resonance extracranial mass of 2,5 cm of diameter at Foramen Magnum level. At surgical resection it was a soft, black nodular mass and subpial invasion with black plaque appearance. Histologic examination reveald a pigmented neoplasm composed of spindle and epitheloid atypical cells with prominent nucleoli and frequent mitosis. It was sheet disposed and foci of tumoral necrosis were present.

On immunohistochemical assay expressed HMB-45 and vimentin. Patient developed progressive neurologic damage and die at 23th day. Necropsy was performed to reject the existence of cutaneus, mucosal or ocular primary melanoma and the diagnosis of PMMCNS was confirmed.

PMMCNS is a rare neoplasm which requires rejection o peripheral melanoma for its diagnosis so they can be morphologically and immunophenotipically identical. Also, can be associated with melanosis neurocutaneus syndrom, Ota`s nevus and as complication of neurofibromatosis. Differential diagnosis with meningeal melanocytoma must be considered although the later lacks clear evidence of mitosis. necrosis and its well circumscrite.

## P-432

HISTOLOGICAL AND BEHAVIORAL OUTCOMES OF REVERSIBLE GLOBAL CEREBRAL ISCHEMIA IN RATS: PROTECTIVE EFFECT OF γ-HYDROXYBUTYRATE

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**Aims:** To investigate the possible protective effect of gamma-hydroxybutyrate (GHB), a CNS depressant naturally occurring in brain, on reversible global cerebral ischemia in rats.

**Methods:** GHB (300 mg/Kg) was administered 30' before or 3' after bilateral vertebral carotid artery occlusion and twice daily (100 mg/kg) for 10 days to rat groups A and B; group C rats underwent carotid occlusion and saline treatment; control rats were sham operated and treated with saline. Locomotor activity, open field behavior, learning and memory (water maze test) were studied 27 days after surgery. Haematoxylin-Eosin, Feulgen staining, immunohistochemical GFAP and NSE detection (ABC System) were performed on formalin fixed and paraffin embedded sections of rat hippocampus (step-section-technique), 3, 10 and 15 days after ischemia. Morphometrical data were collected using a VIDAS Zeiss or SCATI system.

**Results:** GHB-treated rats (groups A and B) had behavioral performances similar to those of control rats, whereas significant longer latencies ($108.7 \pm 4.1$ vs $62 \pm 11.7$, $p<0.05$ at the end of the 4$^{th}$ day trial) were observed in saline-treated rats (group C). Necrotic area and percent of damaged neurons were also higher in saline-treated rats than in control and GHB-treated rats.

**Conclusions:** These results show that GHB has a significant protective effect both on histological and behavioral consequences of a transient global cerebral ischemia in rats.

## P-433

PARAGANGLIOMA OF CAUDA EQUINA:TWO CASES.

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**AIMS**: We present two cases of paraganglioma of the cauda equina, a slow-growing, mostly benign intradural-extradural tumors, clinically and radiographically indistinguishable from others neoplasms arising in this location.

**METHODS**: Two patients of 29 and 44 years old presented with low back pain and sciatica of several months of evolution. The RMN showed delicately encapsulated intradural masses, sugestive of ependimoma in one case and neurinoma in the other one. The masses were removed, standard histological procedure and immunohistochemical studies were performed.

**RESULTS**: The histological studies showed encapsulated masses moderately cellular composed of uniform cells (chief cells) arranged in large lobules, nests termed "zellballen", surrounded by capillaries disposed throughout the lesion. Sustentacular cells, regularly ocurring but inconspicuous components of paraganglioma, tend to form a flattened, nearly uniform layer. The chief cells exibit immunoreactivity for neuron-enolasa specific, chromogranin and synaptophysin and the sustentacular cells for S-100.

**CONCLUSIONS**: Paraganglioma of cauda equina represents a rare entity. Because they only occur sporadically, they are often not included in the differential diagnosis of mass lesion of the region of cauda equina. Almost all paraganglioma of the cauda equina are well circunscribed masses which can be totally resected and cured. The small percentage that cannot be ressected may recur.

## P-434

MELANOTIC SCHWANNOMA OF THE SPINAL NERVE ROOT.

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**Aims:** Peripheric pigmented tumors of the nervous system that affect craneal nerves or spinal nervous roots are rare entities that often display differential diagnostic problems with melanic malignant neoplasias.

**Methods:** 33-year-old woman presented with sciatic pain irradiated to L5 territory. Computerized Axial Tomography and Magnetic nuclear Resonance showed the presence of a neurinoma located at right L5. Surgery was performed by lumbar laminectomy, achieving the complete removal of the tumor. The patient did not develop additional deficits.

The histologic study revealed the presence of a neoplasia with compactly arranged spindle cells with long oval nuclei oriented with their long axes parallel to one another. The tumor showed zones were the cells were arranged in palisades at either end of a bundle of parallel fibers. Many of these cells contained melanic pigment. It was negative for iron techniques and positive for immunohistochemical stains S100 and HMB-45.

**Conclusions:** Although it is well known the common origin of the Schwann cells and the melanoblasts, the co-existence of both types of cells in the tumor of peripheric nerves is a rare event. The absence of mitosis, necrosis, or any other malignant signs, that would support the diagnostic of melanoma, confirm the good prognosis of this lesion that follows the complete removal of the tumor.

## P-435

**PITUITARY XANTHOHISTIOCYTIC PROLIFERATION RESEMBLING XANTHOMATOUS HYPOPHYSITIS (XH). REPORT OF ONE CASE.**

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**Aims:** XH is a newly described entity of obscure etiology and unknown natural history. We report the clinical and pathological findings for one patient, who underwent a transesphenoidal resection for presumed pituitary adenoma.

**Methods:** The patient was a 69 y.o. man, who presented in October 1997 with a visual deficit, manifested by bitemporal hemianopsia, and a preceding headache of 9 months duration. Preoperative endocrinologic studies were normal. Neuroimaging studies showed a homogeneous suprasellar mass, wich enhanced with contrast, and which compressed the optic chiasm. With the clinical diagnosis of probable pituitary adenoma, the lesion was partially resected in May 1998. A moderate increase in the size of the lesion was observed in the 10 months after the operation.

**Results:** Histologically, the lesion showed a solid pattern of short spindle cells, with clear or granular, slightly eosinophilic cytoplasm and focal xanthomatous change. Mitotic figures were ocasionally observed. There were no necrotic or haemorrhagic foci, or granulomas. Zhiel-Neelsen, Gram, PAS, and methenamine silver stains failed to identify infectious microorganisms. The inmunostains revealed diffuse positive expression of CD68 and α1-antitrypsin, and negative results for chromogranine A and adenohypophysal hormones. A few, residual anterior, pituitary acinar cells were recognized with the ACTH antiserum. The S-100 protein, CD1a, GFAP, CAM 5.2 cytokeratin, and EMA were negative. The ultrastructural study confirmed the histiocytic character of the cells.

**Conclusions:** Some differences between our case and others reported of XH are evident, like the age of the patient, and the enlargement of the lesion after operation. A low-grade, neoplastic proliferation cannot be fully discarded in the present case.

## P-436

NASAL GLIOMA OR NASAL GLIAL HETEROTOPIA
Morphological And Immunohistochemical Study Of Two Cases

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**Introduction:** The term nasal glioma was introduced by Schmidt in 1900 to define a benign neoplastic lesion located in the nasal region formed of nervous tissue mixed with connective tissue. Opposed to this neoplastic concept Scherer in 1940 proposed a dysembryoplastic origin of this lesion. Following the same concept Lantos in 1997 denominates it Nasal Glial Heterotopia. It presents as a congenital benign tumor, composed of heterotopic nervous tissue situated extracranially at the nasal or paranasal area. The lesions are etiopathogenically related to encephaloceles. They present a 19% of recidives although these are frequently related to incomplete surgical excision.

**Cases:** In this communication we present two cases of Nasal Glioma at the nasal root in the subepidermic zone. CAT scan did not demonstrate bony defects or relation to the CNS. The first case corresponds to a child 3 months of age who presented a tumor 2cm in diameter. The histological study showed in the dermis nervous tissue formed by well differentiated, GFAP and S-100 positive, astrocytes forming cordonal structures delimited by connective tissue. There were no neurones. We observed myosin, actin and desmin positive, striated muscle fibers closely related to the nervous tissue. The second case corresponds to a new-born with a CNS malformation consisting of agenesia of the posterior 2/3 of the corpus callosum and multiple heterotopic lesions, the largest situated in the cerebellum and in the supratentorial meninges. There was a 1,5cm tumor at the nasal root. Histology showed a mixed nervous tissue composed of GFAP and S-100 positive, differentiated astrocytes and areas of Enolase positive neurones. In the two cases, Ki-67 and PCNA staining did not demonstrate proliferative activity.

**Conclusions:** The heterogeneous characteristics of the tumors, their association with malformations (case 2) and the absence of proliferation support their dysgenetic origin as heterotopias caused by closure defects of the neural tube in the area of the anterior neural bud. The heterogeneous histological composition (glial or glioneuronal) of these lesions might be caused by apoptotic changes in the heterotopic nervous tissue with loss of the neuronal component, similar to observations at other sites.

## P-437

CANINE ASTROCYTOMA. A COMPARATIVE STUDY.

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**Aims:** As it is in humans, canine astrocytoma is one of the most common tumor of the central nervous system. Of the domesticated animal species, most examples are seen in dogs, and the spectrum that has been described is quite broad. The incidence of intracranial neoplasms is estimated at 14.5 per year per 100,000 canine population as risk. As the request of pet owners for biopsy, surgical removal and therapy of brain tumors increased there is a need to improve our understanding of genetic basis of central nervous system (CNS) tumors. Genetic changes ultimately determine the outcome of the tumor progression.

**Methods:** In this regard, twenty canine astrocytomas were analyzed retroactively from archival tissues available from several veterinary hospitals. Clinical history, neuroimaging, (computerized tomography - CT), morphoimmunophenotyping and genetic alteration of p53 and epithelial growth factor receptors (EGFR) were investigated in these canine astrocytomas.

**Results:** Neuroimaging localized the brain tumors and morphophenotyping helped in classifying the CNS neoplasms. Genetic alterative changes of p53 and EGFR showed a similar pattern of expression as human counterpart. Forty percent of canine astrocytomas exhibited positive immunolabeling for p53 and 35% were positively immunolabeled for EGFR. In contrast with human counterpart, more canine astrocytomas showed an undifferentiated morphological pattern consisting of primitive, "stem cells" proliferation which often did not express any specific phenotypic markers.

**Conclusions:** Results generated from CT, histopathology and genetic alterations of CNS astrocytomas will help to develop a better classification system in veterinary medicine that will reflect the biological behavior of these tumors and therapeutic responses to novel therapies.

## P-438

**THE LYMPHOPLASMACYTE-RICH MENINGIOMA - A POTENTIAL PITFALL IN INTRAOPERATIVE DIAGNOSIS**

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Conspicuous foci of lymphocytes in otherwise typical meningiomas aren't an unusual histological finding. 'Inflammatory' meningiomas with mononuclear infiltrates so dense as to obscur the underlying meningothelial pattern, however, are rare.

We report two young, 17 and 18 year-old female patients with meningiomas, displaying a predominance of lymphocytes and plasmacells, according to the WHO-definition of a lymphoplasmacyte-rich meningioma. The neuroradiological findings of well demarcated lesions approximately 2 cm, respectively 5 cm in diameter were consistent with the diagnosis of a meningioma. Intra-operativelay this diagnosis, however, could not be confirmed.

In the first case, multiple conspicuous follicles of small lymphocytes and plasmacells, some showing germinal centres, were the most remarkable feature. Many plasmacells contained large Russell-bodies.Even a marked mononuclear infiltration around blood vessels existed. Since there were areas of a loosely-textured, myxomatuous stroma displaying small islands of rare meningothelial cells with scant formation of whorls we favoured the diagnosis of a meningioma primarily. In the second case, however, diffuse and only focally nodular plasmacell-lymphocytic masses prevailed in the cryo-slides. Only after paraffin-embedding of the entire biopsy, nests of S-100-positive meningothelial cells, often interspersed by lymphocytes in a peculiar way, could be found. Stains for bacteria,fungi or parasites were negative in both cases. Furthermore, B- and T-cells as well as kappa and lambda light chains could be found immunohistochemically, making a plasmocytoma or an infection improbable, thus establishing the diagnosis 'lymphoplasmacyte-rich meningioma'.

## P-439

**MULTICYSTIC BRAIN METASTATIC CARCINOMA WITH NEUROENDOCRINE DIFFERENTIATION: A CASE REPORT**

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**Introduction:** Cystic metastatic brain lesions without perifocal edema and central necrosis are rare and were described in some cases of pulmonary carcinoma with neuroendocrine differentiation.

**Clinical details:** 11 months before death, 47-year-old male had vertigo. CT imaging appeared normal. After two months patient had transitory hemianopsis and persistent vertigo. MR imaging revealed several cystic lesions (each up to 10 mm in diameter) in the brain, without central necrosis and perifocal edema. Neuroradiological diagnosis was cysticerkosis which was not, however, confirmed by biochemical analysis of serum and CSF. After one month patients neurological signs worsened (left hemiparesis and bradiphrenia). MR imaging showed increased number and size of cystic lesions and in some of them hyperintensive signal after contrast application and stereotactic biopsy was performed. Two weeks after biopsy patient died and autopsy was done.

**Neuropathology:** The stereotactic biopsy yielded 6 needle cores of cerebral tissue infiltrated by anaplastic tumor composed of small cells. Endothelium of the tumorous blood vessels was proliferating. Tumor cells were positive for cytokeratin and neuroendocrine markers (NSE, synaptophysin and chromogranin). On autopsy more than 30 brain metastatic lesions grayish, soft and in some part infiltrative were found, but were not cystic except two lesions. Around principal bronchus of the right lung was white lobular tumor tissue, which did not compress bronchus but infiltrate its wall from the out side. Histology of this tumor was the same as of metastatic cystic lesions.

**Conclusion:** Neurological alterations with initial normal CT imaging and later progression of illness with multicystic brain lesions could guide to metastatic dissemination of pulmonal carcinoma with neuroendocrine differentiation.

## P-440

**CHORDOID MENINGIOMA: STUDY OF 42 CASES.**

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**Introduction:** The term "chordoid meningiomas" was first used to describe a tumor of young patients associated with microcytic anemia and/or dysgammaglobulinemia. Composed of chordoma-like clusters and cell cords in a myxoid matrix, they often featured a prominent lymphoplasmacellular infiltrate.

**Methods and Results:** In our study, the 42 chordoid meningiomas represented 0.5% of all meningiomas operated at Mayo Clinic. The male to female ratio was 1:1, and the age range was 12 to 77 years (mean 47.4). Only 2 (5.2%) occurred in children. The majority (88%) were large and supratentorial; 5 others arose in the posterior fossa (cerebello-pontine angle) or cervical spine. No manifestation of systemic disease was noted. Chordoid elements comprised 10 to 100% of the tumors; 35 were more than 50% chordoid. Their matrix had the characteristics of sulfated acid mucin. Immunoreactivity for vimentin and membranous EMA staining was noted in all cases; focal positivity for S-100 protein and Cam 5.2 was seen in 5% each. MIB-1 labeling indices ranged from 0.4% to 11.4% (mean, 5.2%). Thirty-seven tumors (88%) were classified as typical, 4 as atypical, and 1 as anaplastic. Lymphoplasmacytic infiltrates varied, being moderate in 10 cases (26.3%), mild in 17 (32%), and absent in 15 (39.5%). There was no association between the extent of inflammation and chordoid features. Ultrastructural study of 4 tumors demonstrated typical features of meningioma. In 14 (42%) of the 33 cases with follow-up, one or more recurrences were noted between 1.8 and 16 years (mean, 5.6 years). All but one recurrent tumor had been subtotally resected. In (86%) of recurrent tumors, the primary lesion was more than 50% chordoid in pattern and contained little or no inflammatory infiltrate.

**Conclusion:** In our experience, chordoid meningiomas are tumors of adults, lack sex predilection, are unassociated with systemic manifestations, and uniformly recur when subtotally excised.

## P-441

EXPRESSION OF WT1 BY DESMOPLASTIC SMALL ROUND CELL TUMOR: A COMPARATIVE IMMUNOHISTOCHEMICAL STUDY WITH OTHER SMALL ROUND CELL TUMORS.

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Desmoplastic small round cell tumors (DSRCTs) present a reciprocal chromosomal translocation t(11;22)(p13;12) wich results in a chimeric transcript EWS/WT1 involving Ewing's sarcoma and Wilms' tumor genes.

The **aim** of the study is to evaluate WT1 immunoreactivity in DSRCT and its utility in the differential diagnosis with other histologically similar tumors.

**Methods**: the study included 14 DSRCTs, 21 Ewing/PNET tumors (EW/PNET), 18 neuroblastomas (NB), 18 rhabdomyosarcomas (RMS), 14 nephroblastomas (NPB) and 2 rhabdoid tumors (RT). Clinical data, HES slides and immunohistochemical studies were reviewed. For each cases the indexed diagnosis was confirmed and WT1 immunostaining was performed on paraffin material using the WT(C-19) antibody (Santa Cruz Biotechnology, dilution 1/1000) after heat-antigen retrieval. Foetal kidney served as positive control and a competitive study using blocking peptide was included. The percentage of positive tumor cells was estimated and scored semiquantitatively (0<5%; 1+ : 6-40%; 2+ : 40-70%; 3+>70%).

**Results**:

| WT1 staining | | DSRCT | EW/PNET | NB | RMS | NPB | RT |
|---|---|---|---|---|---|---|---|
| | n | 14 | 21 | 17 | 18 | 14 | 2 |
| N | 0 | 0 | 21 | 17 | 15 | 8 | 2 |
| | 1+ | 0 | 0 | 0 | 2 | 8 | 0 |
| | 2 and 3+ | 14 | 0 | 0 | 1 | 0 | 0 |
| C | 0 | 11 | 21 | 16 | 14 | 16 | 2 |
| | 1+ | 0 | 0 | 0 | 1 | 0 | 0 |
| | 2 and 3+ | 3 | 0 | 1 | 3 | 0 | 0 |

n: number of cases; N: nuclear staining; C: cytoplasmic staining

**Conclusion**: the present study validate WT1 immunoreactivity as an useful marker for DSRCT

## P-442

**SOLID AND CYSTIC PAPILLARY TUMOUR OF THE PANCREAS (FRANTZ'S TUMOUR) IN CHILDHOOD.**

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Solid and cystic papillary tumour of the pancreas (Frantz's tumour) is a rare, nonfunctioning neoplasm usually seen in young women. It is of low grade malignancy and deserves special note among pancreatic malignancies as it is frequently amenable to local resection and has a good long-term survival rate after excision.

We report a case of a cystic papillary tumour of the pancreas in a 14 years-old child. Ultrasound examination showed, at the tail of the pancreas, a round neoformation about 10 cm in diameter with solid echogenicity slightly ipodense so she underwent surgery for distal pancreatectomy and splenectomy.

The specimen was a well circumscribed multilobulated mass measuring cm 17x14, solid, gray to yellowish and showed, on cut surface, a cystic formation contening hematic fluid.

Histologically, the neoplasm was characterized by the presence of pseudopapillae covered by several layers of epithelial cells. The nuclei were ovoid and folded, with indistinct nucleoli and few mitoses.

Immunohistochemically, there was reactivity for alfa-1-antitrypsina, focal positivity for neuron-specific enolase and negativity for the Factor VIII-associated protein, S-100 protein, Chromogranin and Progesterone receptors. Flow citomtry analysis revealed diploid DNA. The patient is now healty without evidence of disease 4 years after surgery.

In summary, papillary cystic tumor of the pancreas is a rare and quite benign tumor occurring predominantly in young woman. Thus, this tumor should be considered one of the differential diagnosis of abdominal mass in adolescent girls.

## P-443

**MULTIFOCAL SMALL VESSELS HYALINOSIS AND DIFFUSE CERERRAL CALCIFICATIONS : A new syndrome ? Report of a pediatric observation.**

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**CASE REPORT:** M Alain, an 11 year old boy, thin, small, with a triangular facies, thinning grey hair, pale and atrophic skin, suffered, since 1 year, 5 drastic gastro-intestinal hemorrages. On the same time, he presented one generalized epileptic crisis. Digestive endoscopies and surgical examinations: diffuse vascular ectasia extending from stomach to terminal ileum; moderate portal hypertension and discrete splenomegaly.Ocular fundi: peripheral ischemia. IMR: huge calcifications in basal ganglia, dentate nuclei and white matter associated with an old left hemispheric ischemic lesion.

**PATHOLOGY:** multiple and successive ileal biopsies showed mucosal vascular ectasia with thickened and hyalinised walls.These lesions were observed in a lesser degree in gastric and skin biopsies.Liver biopsy showed large portal fibrosis without cirrhosis, but vascular abnomalities were not prominent.By electron microscopy ( skin biopsy ): we observed marked thickening of the arteriolar basal membranes, associated with granular osmiophilic deposits.

These events conduced to revisit the child's history : No perinatal and familial antecedents were found. He presented at 3 year old, a right hemiclonic crisis with a persistent hemiparesia. Cerebral calcifications were found and a foetopathy was evoked .Under treatment his clinical status remained stable during 8 years till now.

**DISCUSSION :** This observation seems to be sporadic, but is similar to 3 previously reported familial cases (1).Other cases are in investigation (2).This new familial syndrome is characterized by a severe evolutive and diffuse (digestive, renal, retinal and cerebral) vasculopathy. Hair and skin abnomalities are associated. Pathogeny is unknown. Genetic study is starting.

**(1) Rambaud J.C, Galian A., et al.**: Digestive tract and renal small vessel hyalinosis, idiopathic non arteriosclerotic intracerebral calcifications, retinal ischemic syndrome and phenotypic abnormalities. A new familial syndrome. Gastroenterology1986,90,930-8.

**(2) Tournier-Lasserve E**; INSERM U 25 Paris. Personal communication

## P-444

TESTING THE UTILITY OF THE SYDNEY SYSTEM IN *Helicobacter pylori*-ASSOCIATED GASTRITIS IN CHILDREN.

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Aims: The aim of our study was to establish the usefulness of the Sydney System (SS) grading in *H.pylori*-associated chronic gastritis in biopsies from pediatric patients.

Methods: Fifteen children (mean age: 10.8 years) with histologically-proven *H.pylori*-associated chronic gastritis were evaluated. Classification and grading of gastritis were performed according to the analogue visual scales described in the updated version of SS (Am J Surg Pathol 20 (10): 1161-1181, 1996). The presence of sulfated mucosubstances was assessed by the Alcian Blue-Periodic Acid Schiff (PAS), pH 1.0 method. For purposes of this study a chart for the morphological grading recording was designed.

Results: We evaluated 127 gastric biopsies (9/15 patients underwent one series of biopsies, 3 had two series, and 3 had 3 series). Neutrophilic infiltrates were absent in 27 biopsies, mild in 35, and moderate in 17. In none of the biopsies it was marked. Mononuclear infiltrates were mild in 38 biopsies, moderate in 36 and marked in 5. Density of *H.pylori* was mild in 39 biopsies, moderate in 27, and marked in 2. In 3 post-treatment biopsies from the same patient there were no *H.pylori*. Other 8 biopsies (7 from the body and 1 from the antrum) showed no *H.pylori* although organisms were simultaneously present in other sites of the stomach. Lymphoid follicles were present in 19/79 biopsies. Intestinal metaplasia was not seen in H&E-stained slides. However, the AB-PAS stain revealed isolated positive cells in 8 of 15 patients. None of the gastric biopsies showed mucosal atrophy.

Conclusion: The results demonstrate that the SS for gastritis applies to pediatric patients. However, the number of biopsies recommended in the SS seems in excess for this age group. In our experience, as *H.pylori* gastritis is usually a pangastritis, the antral biopsy will almost always yield a positive result.

## P-445

MALFORMATION IN FIRST TRIMESTER SPONTANEOUS ABORTION EMBRYOS

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**Aims**: The value of fetal autopsy and its contribution to genetic counseling and prenatal diagnosis correlation is well accepted. Morphologic examination to evaluate developmental abnormalities of first trimester embryos is not considered a routine procedure. In order to assess if morphological evaluation as diagnostic procedure is useful for genetic counseling and management of future pregnancies, we reviewed 70 embryos from spontaneous abortion, some of them with prenatal diagnosis of "possibly malformed".

**Method:**Gross examination and photography were realized by dissecting microscope. Embryos were evaluated for staging (Carnegie staging) and looking for developmental defects. Retinal pigment, hand and foot plates, finger and toes rays and free finger and toes were the main staging hallmarks evaluated. Furthermore serial axial histological sections were performed.

**Results:** Developmental defects were found in 29 embryos aged from 6 to 8 developmental weeks. The most frequent were neural tube defects (23 cases), thoraco and abdominoschisis (10 cases), facial (4 cases) and limb (3 cases) defects. One case presented with a diaphragmatic hernia. 40% showed multiple malformations. Chromosomal abnormalities were found in 4 of them but tissue culture failed in others.

**Conclusions:** Routine morphologic examination of embryos from spontaneous abortion can identify developmental stage and defects.

Most frequently observed defects in first trimester aborted embryos are neural tube defects, thoraco or abdominoschisis and facial and limb defects. Some of these defects imply high recurrence risk so morphologic diagnosis can contribute to genetic counseling, preconceptional treatment and can be a guidance in prenatal diagnosis in future pregnancies.

## P-446

**DIAGNOSTIC PITFALLS IN UNCOMMON HEPATOBLASTOMA: CRITERIA FOR DIFFERENTIAL DIAGNOSIS BETWEEN HEPATOBLASTOMA VERSUS MESENCHYMAL HAMARTOMA.**

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**Aims**: The diagnosis of malignant paediatric liver tumors has long been an important part of surgical pathology. However, with improved radiographic and biopsy techniques, the appropriate pathological identification of malignant from benign liver lesions is becoming even more critical. A case of mesenchymal hamartoma (MH) which was overdiagnoses as fetal hepatoblastoma (HB) on biopsy emphasize on the pitfalls of these tumors.

**Case Report:** A 2 months old girl presented with an abdominal mass. US revealed a large cystic and solid hepatic mass, and CT little enhancement after intravenous contrast injection. Serum AFP levels was raised (5000mg/ml). A fetal hepatoblastoma was diagnosed on a liver biopsy. Treatment according to SIOPEL II (first low risk, then high risk) was started, without effect. The child was treated by an atypical hepatectomy. The tumor, 15x13x5.5cm diameter, well-circumscribed by a capsule with a central cyst, 6 cm diameter, filled with clear fluid was observed. Peripheral, tan-white nodules seaparated by bands of brown liver tissue,, and small cysts were noted. Histologically, the tumor was composed of loose myxoid stroma that underwent cystic degeneration, scattered bile ducts, abnormal occluded vessels, and sheets of smaller than normal hepatocytes with a low nucleo-cytoplasmic ratio. No mitosis, no tumor thrombus, no embryonal, macrotrabecular, small-cell, osteoid, chondroid or squamous component of HB were present. Some hepatocytes were labelled by AFP and MIB-1. 19 months after surgery, AFP returned to normal without recurrence.

**Conclusion:** It is critical to identify a new subtype of MH with raised AFP in children before 1 yr. Nine similar observations were collected between 1983 and 1998. The recommandations include to sample different sites of tumors because the diagnosis on biopsy may be difficult, and to consider surgery first, in order to avoid starting useless chemotherapy.

## P-447

**ROLE OF ALLELIC LOSS OF THE APC-GENE IN THE PATHOGENESIS OF SOLID TUMORS IN CHILDREN**

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**Aims:** Mutations and allelic loss of the adenomatous polyposis (APC) gene play an important role in the pathogenesis of many different tumors such as cancers of the colon, the stomach, the pancreas, the prostate and breast. Therefore, we decided to examine the role of allelic loss of the APC-gene in different solid tumors of the childhood.

**Methods:** Seven nephroblastomas, seven Ewing´s sarcomas of the soft tissue, four primitive neuroectodermal tumors (PNET) and the corresponding normal tissue were analyzed. DNA was extracted from formalin-fixed, paraffin-embedded tissue after deparaffination by proteinase K digestion. PCR was performed with primer pairs flanking basepairs 1458 and 4520, containing frequently polymorphic loci followed by digestion with the restriction endonucleases RsaI and DsaI and restriction length analysis.

**Results:** The series of nephroblastoma included two female and five male patients, with an age range between one and thirteen years. Five of the tumors were heterozygous, one showed an allelic loss and one tumor was not informative. The series of Ewing´s sarcoma cases consisted of three female and four male patients with an age range between six and thirty-seven years. Out of four informative cases one showed an allelic loss. None of the two informative cases of PNET (one female and three male patients with an age range between 17 and 67 years) showed an allelic loss.

**Conclusion:** We conclude that an allelic loss of the APC gene does not seem to play a major role in the pathogenesis of nephroblastoma, Ewing`s sarcoma of the soft tissue and PNET in childhood.

## P-448

PULMONARY IMMUNOHISTOLOGICAL ANALYSIS AND INFLAMMATORY CYTOKINE LEVELS IN AN INFANT WHO DIED OF INFECTION WITH *HAEMOPHILUS INFLUENZAE*

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**Aims:** This study was performed to analyze pulmonary immunohistological findings using monoclonal antibodies (MoAb) against human leukocyte as well as the inflammatory cytokine levels in cord blood in an infant who died of infection with *H. influenzae*.

**Methods and Results:** The patient was born by emergent cesarean section due to maternal prerupture of the membrane at 28 weeks of gestation and weighed 1,454 g. The infant died five hours after birth despite intensive care. *H. influenzae* was detected from gastric fluid, pharyngeal swab and tracheal tube in the patient as well as the maternal vaginal discharge. The serotype and biotype were defined as non-typable and type II, respectively. Analysis of DNA in *H.influenzae* using restriction enzyme *Sma I* and *Sal I* revealed the same patterns. White blood cell count was decreased to 2,800 /$\mu$l without elevation of CRP (0.5 mg/dl) or IgM (3 mg/dl) in cord blood. Inflammatory cytokines including IL-1$\beta$, IL-6 and IL-8 were measured by IRMA and they markedly increased to 402 pg/ml, 581,000 pg/ml, 26,000 pg/ml, respectively, although TNF-$\alpha$ was within normal range. Histological analysis revealed marked congestion in the lung with many mononuclear cells in the interstitial tissue. Since these cells were strongly stained by monoclonal anti-leukocyte common antigen antibody, frozen lung tissue was immunostained by several MoAbs against human leukocyte antigens (CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD14, CD15, CD19, CD20, CD25, CD33, CD34, CD36, CD45RO, CD68, CD79a, CD117, MPO, HLA-DR and LCA) using DAKO LSAB 2 Kit (DAKO Co., Carpinteria, USA). Mononuclear cells were stained by MoAbs against multipotential stem cells (CD34 and HLA-DR), immature granulocytes (CD117) and immature monocytes (CD36 and CD68). However, other MoAbs including MoAbs against B cell and T cell did not stain them.

**Conclusions:** Our findings showed that the patient was born at the onset of severe infection with *H. influenzae* by vertical infection. In addition, elevated blood cytokines and very immature infiltrated leukocytes contributed to severe pulmonary dysfunction.

## P-449

ATRIOVENTRICULAR CANAL DEFECT ASSOCIATED WITH TRISOMIES OTHERS THAN TRISOMY 21.

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Atrioventricular canal defect (AVCD) is frequently observed with trisomy 21. AVCD may occur in patients with partial deletion of short arm of chromosome 8, and usually present in patients with heterotaxia syndromes. There are also inherited cases of autosomal dominant trait. The association of AVCD with other chromosomal abnormalities is quite rare. We report three cases of AVCD, associated with trisomy 13, 18 and 22.

Aetiology of atrioventricular canal defect is still obscure. There are evidences, that in those cases with Down syndrome a genetic locus 21q22.2-21q22.3 is responsible for congenital heart disease, which means mainly (about 40%) AVCD. There are also theories, that increased cell adhaesiveness would result the malformation. Common occurrence of 8p deletion with AVCD suggests another specific region of the malformation. Linkage analysis of families with autosomal dominant trait proves, that genetic basis of familial AVCD is different from the 21 chromosome associated cases.

Coincidence of heterotaxia syndrome and AVCD may represent an other specific genetic locus. Occurrence of AVCD with trisomies 13, 18 and 22 suggests another mechanism.

Our observed AVCD cases with trisomies others than trisomy 21 are quite rare. In these karyotypes ventricular septal defect or aortic coarctation are the most common. We suppose that the common genetic cause is a susceptibility for congenital heart disease and a teratogenic effect specifies the malformation.

## P-450

PROGNOSTICALLY RELEVANT SUBGROUPS OF PATIENTS WITH NEUROBLASTOMA ACCORDING TO TUMOR CELL POSITIVITY WITH PROLIFERATING NUCLEAR ANTIGEN

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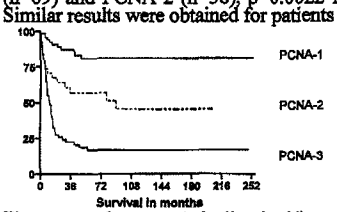
**Aims:** Childhood neuroblastoma (NB) represents a major problem in treatment strategies. The therapy has not influenced its behavior to the extent comparable with other pediatric malignancies over last decades. We made an attempt to use immunohistochemical (IHC) detection of proliferative pool in NBs applying proliferating nuclear antigen (PCNA) in order to find out whether this method may be of value in estimating the prognostic categories of neuroblastomas.

**Methods:** A group of 170 patients with NBs entered on the Children's Tumor Registry in Prague (1974 – 1995) having paraffin blocks available was analyzed for positivity of PCNA. The nuclear positivity of the tumor cells was estimated semiquantitatively and graded into three categories - PCNA1-3, in which PCNA-1 was assigned to tumors with a low proliferation score whereas PCNA-3 represented a high proliferative fraction, and PCNA-2 stood in an intermediate position. The results were statistically evaluated and compared with those obtained for the same group of patients classified according to Joshi's grading system (POG).

**Results:** The graph shows the proportion of survival of all patients with NB irrespectively of the clinical stage in the three categories of PCNA positivity. The results were statistically significant (p=0.0002 for PCNA-1 (n=69) and PCNA-2 (n=38), p=0.0022 for PCNA-2 and PCNA-3 (n=63). Similar results were obtained for patients at clinical stage III and IV.




There was also a statistically significant correlation of the PCNA groups and Joshi's grading system which uses a combination of counting mitotic figures and presence of calcifications.

**Conclusions:** We provide an alternative tool in delineating prognostically valuable subgroups of patients with NB using a semiquantitative estimate of proliferative pool of the tumor mass using PCNA positivity.

## P-451

**EXPRESSION OF MULTIDRUG RESISTANCE GENES *MDR*-1 AND *MRP* IN CHILDHOOD BONE TUMORS**

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**Aims:** Among tumors of childhood and adolescence, bone tumors are subsidiary of primary chemotherapy and subsequent surgery. At this regard, previous knowledge of the response to chemotherapy would be of great interest. The significance of certain factors, such as p-glycoprotein, multidrug resistance-associated protein (MRP) or p53 expression is still under research. In this study we assess the immunohistochemical expression of p-glycoprotein, MRP and p53 in a group of bone tumors of the childhood.

**Methods:** A total of 19 bone tumors (ten osteosarcomas (OS), seven Ewing's tumors (ET), one mesenchymal chondrosarcoma (MC), and one eosinophilic granuloma (EG)) were studied by means of conventional avidin-biotin-peroxidase technique for p-glycoprotein (JSB-1 and C494 antigens), MRP and p53 (DO-7). When fresh material was available, expression of *MRP* and *MDR*-1 genes was tested using reverse transcriptase-polymerase chain reaction (RT-PCR).

**Results:** Immunohistochemical expression of MRP was detected in all of the cases, with the exception of a postchemotherapy specimen of OS. Expression of p-glycoprotein was detected in 6/10 OS and 2/7 ET (using both JSB-1 and C494), and p53 was detected in 7/10 OS, 6/7 ET, EG and MC. Immunohistochemistry and RT-PCR results correlated in 9/11 cases for *MDR*-1 and 4/11 cases for *MRP*.

**Conclusions:** Our study highlights the role of multidrug resistance genes *MDR*-1 and *MRP* in childhood bone tumors and provides evidence for the use of p-glycoprotein immunostaining, that showed a high correlation with the *MDR*-1 mRNA analysis. Further studies are needed to clarify the role of p53, frequently expressed in these tumors.

## P-452

**MATURE TERATOMA OF THE MEDIASTINUM WITH EMBRYONAL RHABDOMYOSARCOMA**

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**Aims:** We report a case of embryonal rhabdomyosarcoma developed in a mature teratoma of the mediastinum.

**Methods:** The patient is a 3 year old boy with a huge mass in the mediastinum and compression of the right lung.

**Results:** The tumor measured 22 x 20 x 18 cm, was well encapsulated and showed cystic and solid areas. Histology showed a mature teratoma in which a sarcomatous proliferation was recognized. In this area rhabdomyoblasts were found and desmin and actina (HHF35) were clearly positive by PAP method. Alfa-feto-protein and carcino-embryonic-antigen were positive in the epithelial surface. Thirteen months later he developed local recurrence of embryonal rhabdomyosarcoma with right pleural efussion. He is at present under chemotherapy for rhabdomyosarcoma.

**Conclusions:** Primary teratomas of the mediastinum are germ cell tumors probably originated from misplaced germ cells. They occurs most often from the anterior mediastinum of men. Examples of benign and malignant cases are well documented in literature. These tumors have been well defined in recent series and classification includes mature and immature teratomas, and teratomas with additional malignant component which may be rhabdomyosarcoma, angiosarcoma, liposarcoma, neurogenic sarcoma and osteosarcoma. Patients with these sarcoma had rapidly fatal outcomes and dead is usually related to compromise of regional structures.

## P-453

PRENATALLY DIAGNOSED UMBILICAL HAEMANGIOMA OF AN OTHERWISE HEALTHY NEWBORN.

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**Introduction**: Tumours of the umbilical cord are quite rare. The commonest is the umbilical haemangioma, which is not even a tumour, but rather a hamartoma. There are only a few reports on it. It is often accompanied by elevated alpha-fetoprotein level in the maternal serum. Localisation is mainly on the placental end of the umbilicus. Complications are in certain cases fetal hydrops, umbilical haemorrhage, thus compression of vessels, or fetal demise.
**Patient:** A 35 years old patient is reported with gestational diabetes and umbilical haemangioma, elevated maternal serum and amniotic fluid AFP level (7.7 MoM and 93μM/ml respectively). The tumour was diagnosed by ultrasonography. Normal flow has been detected, the fetus did not show any sign of hydrops, asphyxia or cirulatory disturbance. Genetic amniocentesis resulted 46 XY, normal male karyotype. The newborn was 3550g, Apgar 8/9, healthy, delivered vaginally. On gross examination he did not show any sign of vascular abnormality.
**Result:** Pathologically the placenta was consistent with gestational age. At the placental end of the umbilical cord a firm, 12x4 cm large, greyish-white, spindle shaped tumour was found. In the Wharton jelly beside fibrotic changes, lobules of cavernous vascular spaces were seen covered with endothelium sometimes near to the umbilical arteries. In some places haemangiomatosus nests with narrow vascular spaces resembled to a capillary haemangioma. Other areas were characterised by well formed vessels. Capillaries were observed also in the wall of umbilical arteries. No inflammation was seen.
**Conclusion:** Our case is unic, since in the uneventful pregnancy a quite large but symptomless umbilical haemangioma was diagnosed at 22nd week of gestation which failed to cause any complication. In case of elevated AFP level beside other etiologic factors the possibility of an umbilical haemangioma has to be also considered.

## P-454

NAEVUS COMEDONICUS
REPORT OF A BILATERAL AND EXTENSIVE CASE

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**Aims:** Naevus comedonicus (NC) is a rare benign lesion, described as an abnormality of the skin and characterized by an unilateral, usually linear distribution of groups of comedones.
We report the case of a 24 years old man who developped numerous 1 to 3 mm, raised, firm comedo-like lesions on the right palm, the posterior surface of the 2nd, 3rd, 4th right fingers, distal forearms, wrists and foots. Past medical and family histories were not remarkable. Lesions had been present since adolescence. Laser treatment was unsatisfactory. Surgical ablation of an involved area of hand's skin with subsequent grafting was made. This case, differing from previous descriptions, had unusual features included bilateral involvement, non linear distribution and extensive lesions on the glabrous and non glabrous skin. The histologic examination suggested the pathogenesis of these lesions.
**Methods:** Multiple sections were stained with Hematoxylin-eosin.
**Results:** Microscopic findings revealed papillomatous epidermal hyperplasia with widely dilated orthokeratotic invaginations. These invaginations had a central lumen wich were plugged by keratinous material. They are lined by squamous stratified epithelium. Rarely, rudimentary hair follicles had been observed. No sebaceous, sweat glands or duct lumen were identified in association with these structures. Sweat glands had a normal distribution and location. There was no suppuration.
**Conclusions:** We were unable to find sweat glands and concluded that lesions correspond to poorly differentiated hair follicles. NC is generally presumed to be a developmental abnormality of the pilosebaceous apparatus wich is unable to produce mature hairs, matrix cells or sebaceous glands but is able to form keratin. According to some authors (Marsden R.A. et al), palmar lesions derived from eccrine ducts may represent another variant of NC.

## P-455

INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) EXPRESSION IS TIGHTLY LINKED TO HYPERPROLIFERATIVE SKIN DISEASES

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Previous studies from our laboratory demonstrated that nitric oxide (NO) is a pleiotropic mediator of keratinocyte growth and differentiation *in vitro*. Low or intermediate levels of NO promote keratinocyte proliferation, whereas high levels of NO arrest cell proliferation and initiate the switch to terminal differentiation. In the present study, the role of NO in keratinocyte growth and differentiation was explored *ex vivo* in several hyperproliferative epidermal diseases. Lesional skin specimens from patients with psoriasis (n=10), seborrheic keratosis (n=5), actinic keratosis (n=5) and basal cell carcinoma (n=5) were stained with monoclonal iNOS and polyclonal ncNOS antibodies to characterize the tissue distribution of NOS protein expression. As control, we used skin specimens from healthy volunteers and isotype control antibodies. Immunostaining revealed high levels of iNOS protein in the proliferating compartment of psoriasis, seborrheic keratosis, actinic keratosis and basal cell carcinoma, but only weak immunoreactivity for ncNOS protein in some suprabasal and occasional basal keratinocytes. No immunostaining for iNOS and ncNOS protein could be detected in epidermal keratinocytes of normal human skin. Collectively, these data suggest that expression of iNOS may represent a general feature of hyperproliferative epidermal diseases. Future studies should show whether iNOS is inappropriately activated in hyperproliferative epidermal diseases or, on the other hand, counterregulated by as yet undefined growth-stimulating signals.

## P-456

A CARBOHYDRATE, SIALYL-TN, IS UP-REGULATED ON HYPERPLASTIC, NON-MALIGNANT SKIN AND MUCOSAL LESIONS.

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**Aims:** Various cell surface carbohydrates are of functional importance in all stages of tumor development. This study investigated the hypothesis that expression of sialyl-Tn (sTn) is mainly present in hyperplastic squamous cell lesions, but not in squamous cell carcinomas.
**Methods:** 50 oral and cutaneous squamous cell carcinomas (SCC), 40 skin keratoakanthomas (KA) and 20 hyperplastic oral lesions (focal epithelial hyperplasias and verrucous hyperplasias) were studied. TKH2 antibody, reacting with sTn, was used in an immunoperoxydase method. The staining pattern of basal and parabasal cells (proliferative compartment) was compared with the expression of Ki-67 in parallell sections by immunoperoxydase staining, and by double immunofluoresence.
**Results:** Normal epithelia and SCCs did not generally express s-Tn in the proliferative compartment. In contrast, KAs, and the other hyperplastic lesions expressed sialyl-Tn on basal/parabasal cells in 85% of the cases ($p<0.001$). Double immunofluoresence showed that Ki-67 and sTn generally were expressed in different cells. Furthermore, a striking up-regulation of STn was observed in morphologically normal epithelial cells adjacent to tumors in approximately 90% of the cases.
**Conclusion:** STn is over-expressed on hyperplastic, non-malignant squamous cell lesions. Double stainig experiments indicate that sTtn is not directly related to cellular proliferation.

## P-457

**HAPTEN AND SOLUBLE PROTEIN POSITIVE PATCH TEST PATHOLOGY IN ATOPIC PATIENTS**

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**Aims:** Contact sensitivity has served as useful model for primary activation of T cells in skin and skin associated lymphoid tissue. Hapten cutaneous immune response involves predominantly T helper (Th) 1 cells. Soluble protein induces mainly Th2 cells characterized by secretion of interleukin (IL) -4,5,10 that promote hyperIgE and eosinophil chemoattraction. The aim of this study is to compare pathological changes among hapten and soluble proteins induced eczema in atopic and non atopic patients in order to demostrate that the immune response is conditioned by the antigen nature.

**Methods:** Twenty six skin biopsies from 22 adult patients were studied using H&E and Giemsa staining. Ten patients were atopics. Samples were obtained from 10 positive Der Pt/Der Fa mix patch test, 8 positive nickel sulphate patch test (atopics and non atopics), 4 clinical contact dermatitis and 4 healthy skin. Blind assessment of qualitative and quantitative pathological characteristics was performed by three independent observers.

**Results:** The histological picture of contact dermatitis and positive hapten/protein patch test was clearly eczematous. Only the eosinophilic component of the inflammatory infiltrate showed significant diferences ($p>0.035$) among nickel sulphate positive patch test (1.7±0.75 e.st. eos x field) and aeroalergen positive patch test (11.7±4.2 e.st.).

**Conclusions:** A shift in cutaneous cytokine profile between both subsets of T helper cells in hapten/protein induced eczema has been described. Our findings support the eosinophilic chemoattrative role of IL-5. The study suggests that the contact immune response is conditioned by the antigen nature.

## P-458

**HIGH LEVELS OF p27 EXPRESSION CAN EXPLAIN THE BENIGN BEHAVIOR OF PROLIFERATING TRICHILEMMAL CYST**

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**Background:** Proliferating Trichilemmal Cyst (PTC) is an uncommon skin adnexal tumor. Even though several well documented PTC instances have undergone carcinomatous transformation , PTC is still considered to be an essentially benign neoplasm. Recent studies have detected a p53 mutation in PTC cells, along with an increased proliferation index.

**Aims:** With the objective of better understanding PTC biological behavior, we have studied its immunoreactivity for p53 protein as well as its expression of ciclin kinase inhibitors (CKIs) such as p21 and p27.

**Methods:** Immunostaining for p53, p21 and p27 was carried out on paraffin embedded samples from 7 PTC cases, 2 cases of trichilemmal cyst and 2 cases of trichilemmal carcinoma.

**Results:** p53 immunoreactivity was observed in all cases, being weak and limited to the basal layer in trichilemmal cysts, variable but mainly basal in PTCs and stronger and involving over 90% of cells in trichilemmal carcinomas. Expression of p21 was variable, ranging from 5 to 50% in PTCs. Finally, p27 immunostaining was strong and suprabasal and affected over 70% of cells in PTCs, was suprabasal and involved 90-100% of cells in trichilemmal cysts, and was weak and affected under 20% of cells in trichilemmal carcinomas.

**Conclusions:** In view of these results, we suggest that a) p53 gene mutation in this group of lesions results in a positive immunohistochemical reaction for p53 protein, and b) the benign biological behavior of PTC may be due, in part, to overexpression of p27, which exerts a cell cycle control function.

## P-459

**THE VALUE OF THE POLYMERASE CHAIN REACTION IN THE DIAGNOSIS OF EARLY MYCOSIS FUNGOIDE**

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**Aims**: To determine the usefullness of antigen receptor genes rearrangement in T cells by polymerase chain reaction (PCR) in the diagnosis of patch or plaque stages of mycosis fungoides (MF).

**Methods:** We analized 32 formalin-fixed and parafin embeded biopsies from different patients with a clinical features of MF. Histologically, all the selected cases were in patch stage. A minimun lymphoid exocytosis was required to asses the diagnosis. According to the lymphocyte epidermotropism each case was graded in mild, moderate or severe. The presence or absence of a likenoid infiltrate in dermis was evaluated. All cases were studied for CD3, CD4 and CD8 expression by immunohistochemistry and the infiltrate was graded into mild, moderate or intense. All cases were analyzed by PCR using primers complementary to the consensus sequences on the TCR-γ gene. The gels were examined without knowing the histological results. Gene rearrangements were acepted only if the band was sharp edged, greater than 1mm in width and within the predicted size range, measured to the nearest 5 base pairs.

**Results:** TCR-γ gene rearrangement in early mycosis fungoides were observed in 18 cases (56%). We found correlation between TCR-γ gene rearrangement and the presence of likenoid infiltrate in dermis and a mild CD8+TC population since 77% and 75% of the cases of each group showed a positive TCR-γ gene rearrangement result. There was not correlation between TCR-γ gene rearrangement and the intensity of the lymphocyte epidermotropism.

**Conclusions:** We find similar results of positive TCR-γ gene rearrangement compared with literature. Study of TCR-γ gene rearrangement using PCR may be usefull in those cases with a not conclusive histological diagnosis of MF by proving the monoclonality of T lymphocyte proliferation. However, its negativity does not exclude a MF diagnosis.

## P-460

STUDY OF INFLAMMATORY INFILTRATE IN TICK BITE CUTANEOUS REACTION.

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**Aims**:Tick is an arthropod of the Ixodes Ricinis complex that can attack any available animal, including man. Tick bite produce local dermal reactions as urticarial papule, and tick bit-induced granuloma. Ticks also convey infectious diseases as Lyme disease (caused by Borrelia burgdorferi). The distinctive cutaneous manifestations of Lyme disease are erythema chronicum migrans and borrelial pseudolymphoma. Our purpose is to determine the phenotype of the inflammatory infiltrate in tick-bite cutaneous reactions.

**Methods**: We review 43 biopsy specimens with histological diagnosis of tick-bite reaction, and we select 20 cases with the clinical confirmation and the date of the bite. We study 4 mm biopsy specimen with B-cell markers (L-26 and LN-2), T-cell markers (UCHL-1 and MT-1), histiocytes (CD-68) and bcl-2.

**Results**: In the early phase, the inflammatory infiltrate is composed by small T lymphocytes and eosinophils. In the full developed reactions we observe T- lymphocytes, B- lymphocytes, eosinphils and histiocytes. If the borreliosis occurs, the infiltrate appears composed by B- lymphocytes and plasma cells, and could be so abundant to produce a borrelial pseudolymphoma.

**Conclusions**: The study of lymphoid infiltrate in tick bite-reactions is useful to differenciate the early reaction (by hypersensitivity) rich in T-cells and the borreliosis lesions with B-cells predominance.

## P-461

**SELECTIVE SENTINEL LYMPH NODE BIOPSY IN MALIGNANT MELANOMA.**

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**Aims**: Sentinel lymph node (SLN) is defined as the first lymph node where an initial metastases can be found, whereas non-sentinel nodes in the same lymphatic group are not affected or only later are affected. Selective SLN biopsy implies that if we do not found metastases in this node we can avoid the conventional lymphadenectomy.

**Methods**: To SLN detection, technetium TC 99 in nanocolloid was injected intradermally around the primary melanoma. One hour after, this substance was found in the SLN. We used preoperative lymphoscintography and intraoperative mapping to confirm the location of the SLN and this was removed.

We prepared tissue blocks of 2 to 4 μm thin that includes a continuous sampling throughout SLN, and they are paraffin- embedded after fixation in formol 10%. From each block we made 3 slides for routine hematoxylin - eosin staining and 2 more for immunohistochemical studies with S-100 and/or HMB-45.

**Results**:

| Location | N° patients | N° SLN | With metast. (%) | Free metast. (%) |
|---|---|---|---|---|
| Trunk | 15 | 61 | 1 | 14 |
| Head/neck | 4 | 19 | 2 | 2 |
| Arms | 7 | 15 | 0 | 7 |
| Legs | 19 | 36 | 3 | 16 |
| Total | 45 | 131 | 6 (13.3) | 39 (86.7) |

**Conclusions:** Combination of preoperative lymphoscintigraphy and intraoperative mapping is a reliable way to identify regional SLN. Further follow-up is needed to assess the outcome of this group of patients for regional and systemic recurrences.

## P-462

EXPRESSION OF CELL-CYCLE REGULATOR $P27^{Kip1}$ IN KAPOSI'S SARCOMA CORRELATES WITH STAGE AND EXTRACUTANEOUS INVOLVEMENT.

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**Background:** Down-regulation of the cell-cycle regulator p27 has been shown in hyperplastic processes as well as in some neoplasms, and is usually associated with aggressive behavior and development of metastases. Kaposi's sarcoma (KS) is an angioproliferative disease which probably begins as a hyperplasia and may evolve into a malignant neoplasm in some patients. It is usually limited to the skin, but in aggressive cases it can disseminate to mucous membranes and internal organs.

**Aims:** To evaluate the potential role of p27 alterations in cutaneous and extracutaneous KS, by using immunohistochemical methods.

**Methods:** Forty-nine cases of KS corresponding to 31 cutaneous (10 macules, 7 plaques and 14 tumors) and 18 extracutaneous biopsy specimens were immunostained using anti-p27 protein mouse monoclonal antibody, clone 1B4 (Novocastra,UK). The percentage of positive cells was evaluated independently by two researchers in at least 500 tumor cells or 20 fields (in macule and plaque stages were the total number of cells was low).

**Results:** The mean percentages of p27 expression were significantly higher in biopsy specimens from skin lesions (78.5±21.1) than in those from extracutaneous locations (43.2±26.2). As for cutaneous lesions, p27 expression was significantly higher in macules (83.8±18.5) and plaques (91.4±6.4) than in tumors (68.3±23.5).

**Conclusions:** Down-regulation of p27 immunohistochemical expression is related to advanced histopathological stage and extracutaneous involvement.

**Acknowledgements:** We thank Dr Matias-Guiu, Dept. of Pathology, Hospital de la Santa Creu i Sant Pau, for providing the paraffin blocks corresponding to six extracutaneous KS cases. This work was supported in part by CYCIT grant SAF 97/0220.

## P-463

**LYMHOMATOID PAPULOSIS TYPE A AND C**

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Lymhomatoid papulosis is defined as a chronic, reccurent, self-healing eruption of papules and small nodules with the histopathological features of a cutaneous T-cell lymphoma. It is considered as a disease with an indolent clinical course. Three main histological subtypes have been described: Type A ('histiocytic' type), Type B (mycosis fungoides-like) and Type C (anaplastic large cell lymphoma-like) The last type is histologically indistinguishable from anaplastic large cell lymphoma.

The present case concerns a 60-year-old woman who presented with a small ulcerated nodule on the upper third of the left thigh, followed by the appearance of a new nodule on the right thigh two years later. The histoligical features were consistent with lymphomatoid papulosis. In particular the first nodule corresponded to lymphomatoid papulosis type A, while the second to type C. The large atypical lymphomatoid cells were positive for $CD_3$ and $CD_{30}$ while monoclonality for TCRγ was confirmed by PCR analysis.

During a three year follow up there was no disease recurrence despite the fact that the patient received no treatment.

**Conclusion**: The histopathological features of lymphomatoid papulosis are variable and it is important to recognize that they can all be observed in one patient at the same or different time intervals and lack prognostic significance.

## P-464

SOLITARY FIBROFOLLICULOMA OF THE SKIN

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**Aim:** Seven cases of solitary fibrofolliculoma have been published to date, the first one in 1984. We report an additional case and include an immunohistochemical study of the cytokeratine profile in the lesion.

**Methods:** An otherwise healthy 76-year-old woman presented to her practitioner with a solitary, 6 mm papule on the volar side of her right forearm. The lesion had been unaltered for several years. It was eventually removed because of recent growth.

**Results:** Histologically the lesion was composed of a central keratin-plugged follicle-like structure .In continuity with the basal epithelial layers of the "follicle", strands of delicate, partly anastomosing epithelial proliferations, focally with " squamous eddies", extended into a mucinous, moderatly hypercellular, richly vascular stroma.

The characteristic slender epithelial proliferations showed a strong positive reaction with cytokeratin 5/6, cytokeratin 14 and cytokeratin 17, whereas cytokeratin 4 was negative.

**Conclusions:** Detailed immunohistochemical examinations of the cytokeratin profile of a solitary fibrofolliculoma, indicate that the proliferative epithelial component of this lesion may originate in the external root sheath.

## P-465

ATYPICAL FIBROXANTHOMA: A COMPARATIVE STUDY OF Ki-67 AND P53 PROTEIN EXPRESSION IN ITS MORPHOLOGICAL VARIANTS

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**Aims**: Atypical Fibroxanthoma (AFX) is regarded as a superficial form of malignant fibrous histiocytoma, which, by virtue of its location, almost invariably pursues a benign course. Some have suggested that AFX has morphological subtypes in a spectrum that varies from a predominant spindle cell pattern with focal pleomorphism to numerous bizarre epithelioid cells. We investigated a serie of AFX to evaluate their pattern of p53 and Ki-67 expression, and to elucidate if these immunostaining could be of help in distinguishing the morphological varieties of AFX.

**Methods**: Fifteen cases of AFX were examined using immunohistochemistry to determine Ki-67 and P53 immunostaining.

**Results**: Seven cases showed a predominant atypical-pleomorphic pattern in haphazard distribution. Three cases showed a more monomorphic spindle-celled fascicular pattern, and one has spindle cells in storiform pattern, with only very focal mild pleomorphism. In four cases there were a mixed pattern with both spindle cells in fascicular distribution and atypical pleomorphic cells. In all cases abnormal mitoses varied from 2 to 20 per 10 hpf, without relationship with the morphological type. The mean of Ki-67 positive cells was from 0.4 to 7.7 per $mm^2$ and the expression of P53 protein was positive in all cases (>50% of cells) also without relation with the morphological variety.

**Conclusions**: The morphological varieties of AFX has not significative differences in its immunohistochemical expression of Ki-67 and P53 protein. This results do not suggest a role of p53 and Ki-67 immunochemistry as a markers of different morphological variants of AFX.

## P-466

FOCAL DERMAL HYPOPLASIA (GOLTZ SYNDROME). PRESENTATION OF TWO CASES.

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**AIMS**: Focal Dermal Hypoplasia (FDH) is a rare X-linked dominant ectomesodermal dysplasia syndrome that may affect several organs such as eyes, bone, teeth, soft tissues and skin, histologically caracterized by hypoplasia or focal absence of dermal collagen. We present two cases in two patients aged 11 and 12 months old. The lesions were located in knee skin and buttock (multiple lesions) and showed erytematous sligthly raised and papillomatous features respectively.

**METHODS**: Surgical specimens were processed for histological and immunohistochemical studies.

**RESULTS**: Microscopic examination of the papillomatous lesion showed prominent fibrovascular papillomas with acanthosis and focal parakeratosis. The collagen fibers of the dermis were markedly attenuated and absent in some areas with replacement by adipose tissue extending upward to the epidermis. Small sub-epidermal bullous clefts and sparse lymphohistiocytic infiltrate around the blood vessels were present. Microscopically the erythematous lesion displayed acanthosis and hyperkeratosis of the epidermis and striking hypoplasia of the connective tissue with apparent herniation of the fat.

**CONCLUSIONS**: The FHD is apparently easily diagnosed but it may be a problem for the pathologist because of its infrequent presentation and the wide variety of clinical expressions of the syndrome. The histogenesis of this genodermatosis is unclear. It has been proposed a defective collagen formation and an abnormality in the growth kinetics of mesodermal tissue fibroblasts as responsible for the connective tissue defects.

## P-467

LIPO-CHONDROID TRICHOFOLLICULOMA. NEW VARIANT OF HAIR FOLLICULAR TUMOR.

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**Introduction:** We describe a peculiar case of hair follicular tumor characterized by the presence of abundant chondroid and lipomatous tissue in the stroma. We don't try in the literature any report about the presence of cartilage and adipose tissue in the pilar tumors. In this case the presence of the patterns of stromal transformation is so peculiar that we could consider the neoplasm as a distinct histologic variety of trichofolliculoma.

**Case report**: The patient, female, 50 years old, presented a painless, slow growing swelling of the superior lip. The lesion was removed. It was formed by a nodule sized 2 x 1 x 1 cm. The whole section was embedded in paraffin. Sections 5 microns thick were stained with Hematoxilyn-Eosin, PAS and with antibodies for panCK, Ck 7, CKs 8-18-19, CK 34 β E 12, EMA, CEA, S 100 protein, GFAP, SMA, CD 34 and tenascin.

The neoplasm was located in deep dermis and presented a central, wide dilated infundibulum closely related to the epidermidis from which branched many epithelial cords. The neoplastic elements were formed by basaliod cells sometimes forming immature hair follicles, by rare small glandular formations and by many keratin cysts. The stroma presented large areas of lipomatous and chondroid differentiation, the latter mainly distributed at the periphery of the lesion.

Focusing on the stromal transformation the immunohistochemistry is characterized by the obvious positivity of the stromal elements for vimentin and by the positivity of the adipose and chondroid cells for S 100 protein. GFAP was positive in the cells located at the periphery of the chondroid areas and in clusters of cells located near the pilar formations. This is a good point to discuss the origin of chondroid tissue in this lesion.

**Conclusions:** The tumor we observed is a benign acquired neoplasm with hair follicular differentiation. The peculiar finding of this lesion is the presence of well differentiated stromal components, lipomatous and chondroid, which has hitherto not been described in the follicle hair tumors to our knowledge. We consider the lesion as an unusual variant of trichofolliculoma with lipo-chondroid differentiation of the stroma. This observation supports the possibility that also tumors with pilar differentiation of the skin could present these types of stromal transformation, as we see in sweat glands tumors.

## P-468

**GLOMERULOID HEMANGIOMA**

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**Aims:** The classification of vascular lesions has been expanded and modified and new entities have recently been described, some of them associated with specific clinicopathologic syndromes. A rare cutaneous vascular proliferation seems to be specific of POEMS syndrome which makes it recognition important.

**Methods:** Surgical specimen was processed for histological and immunohistochemical studies.

**Results:** A 7-years-old boy presented with a raised red papule of 5 mm in the face slightly painful, without other symptoms which was surgically removed. The histopathology examination showed a dermal lesion formed by multiple ectatic vascular structures containing groups of capillary loops resembling renal glomeruli. One layer of flat or hobnailed cells surrounded by pericytes covered the capillaries. These cells were positive with factor VIII, Ulex Europaeus and CD 31 antibodies being negative for actin. Some of these cells contained eosinophilic globules in their cytoplasm which were PAS positive and stained with Kappa and Lambda light chains antibodies.

After the diagnosis of glomeruloid hemangioma was made it was suggested to study the patient because this lesion appears to be specific of POEMS syndrome.

**Conclusion:** It is important to know the clinicopathological features of glomeruloid hemangioma as well as its association with POEMS syndrome because in some cases as the presented case it may be the initial manifestation. As clearly showed in our case, the clinicopathologic features are so specific that allow us to diagnose POEMS syndrome in unusual clinic settings.

## P-469

CUTANEOUS LYMPHOMA: A SERIES OF 92 CASES

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**Aims:** To review the clinico-pathologic features of the cutaneous lymphomas (CLs) diagnosed at our institutions over the past 10 years.

**Methods:** We retrospectively review 92 CLs, subdividing them according to the REAL classification.

**Results:** 39 patients had *primary* CL; 23 cases were of B-cell derivation and 15 cases were of T-cell origin. The phenotype was unknown in one case. The lymphomas were subclassified as marginal zone B-cell (12 cases), diffuse large B-cell (10 cases), anaplastic large cell (4 cases), peripheral T-cell (9 cases), not subclassified (3 cases) and subcutaneous panniculitic T-cell (one case). 45 patients had lymphoma *secondarily* infiltrating the skin; 28 cases were of B-cell derivation and 16 cases were of T-cell origin. The phenotype was unknown in one case. The CLs were subclassified as diffuse large B-cell (21 cases), anaplastic large cell (7 cases), angiocentric (3 cases), angioimmunoblastic T-cell (3 cases), peripheral T-cell (2 cases), mantle cell (2 cases) and one case each of precursor B-lymphoblastic, follicle center, small lymphocytic, high grade B-cell Burkitt-like, marginal zone, anaplastic large cell Hodgkin´s like and not subclassified. The remaining 8 cases were *unclassificable*. Outcome was significantly correlated with type of CL (primary vs secondary; p=0.000007), phenotype (p=0.046) and distribution of skin lesions (localized vs systemic; p=0.030). Multivariate analysis: absence of non-cutaneous lesions 6 months after diagnosis, B-cell phenotype and complete remission were predictors of improved survival (p<0.00001).

**Conclusions:** (1) The REAL classification is adequate for CLs. (2) Absence of non-cutaneous lesions 6 months after diagnosis was the strongest independent marker of favourable prognosis. (3) A B-cell phenotype or the presence of localized lesions may also predict a favourable outcome in CLs.

## P-470

**DNA ploidy topographic profile in primary cutaneous malignant melanoma**

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**Aims:** Melanoma has a reputation as an unpredictable disease. Numerous prognostic factors have been investigated (Breslow, Clark...). However, the prognostic significance of DNA ploidy evaluated by topographic compartments is not available to date.

**Design:** 31 cutaneous melanomas, 0.98-12.86 mm thickness (Av ± SD: 3.54 ± 2.61) were included in this study. DNA ploidy was sistematically evaluated by image cytometry (CAS-200, Becton Dickinson) in two compartments (superficial and deep) each one of 0,75 mm thickness. At least 300 cells by compartment were measured in all cases. DNA ploidy were correlated with disease's outcome.

**Results:** Superficial compartment: All patients with diploid pattern are alive after a mean follow-up of 45,10 months, while 44% of patients with aneuploid pattern have died (P<0,001). Deep compartment: 90% of patients with diploid pattern are alive and 47% of patients with aneuploid pattern have died (P<0,05). Disease-free interval for superficial compartment were 52,41 months (diploid patterns) and 13,69 (aneuploid patterns) (P<0,002); and for deep compartment, 44 months (diploid) and 28,31 (aneuploid) (P 0,282). Survival analysis (Kaplan Meier) for DNA ploidy were P<0,0002 (superficial compartments) and P<0,034 for deep compartments.

**Conclusions:** Statistical results showed more accuracy in superficial component. DNA aneuploidy, mainly "superficial DNA-aneuploidy", is shown to be an negative prognostic factor in malignant melanoma.

## P-471

CIRCUMSCRIBED STORIFORM COLLAGENOMA

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**Aims:** The circumscribed storiform collagenoma also named sclerosant collagenoma or sclerotic fibroma is a rare solitary nodule lesion that occurs in adult of both sexes without an specific topographic localization. Some authors consider this lesion to be a benign neoplasia with histological pattern similar to fibroma of the tendon sheath, while others think of it as an special form of dermatofibroma or fibrosant dermatitis. Moreover, multiple papullar lesions with the same histologic features have been described as the Cowden´s disease (multiple hamartoma).

**Methods:** A 36-year-old with one year evolution of an asymthomatic nodullar lesion in the nasal back . No familial or personal background of clinical interest. Normal physical examination. Initially referred as trichoepithelioma, the biopsy was performed and sent to the Pathology laboratory.

**Results:** We observed the skin that presented a dermal nodule, hypocellular, well circumscribed, formed by hyalinized collagen bundles separed by clefts and arranged in a storiform pattern.

**Conclusions:** In contrast to the multiple nodules found in the Cowden´s disease, the storiform collagenoma can be also presented in the form of solitary nodule, whose simple removal is the effective therapy.

## P-472

ACQUIRED PROGRESSIVE LYMPHANGIOMA

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Acquired progressive lymphangioma (APL) (benign lymphangioendothelioma) is a rare,benign proliferation of lymphatic capillary origin, that has a tendency to appear in childhood and to progress slowly over the years. It particularly involves the extremities, especially the upper limbs, although the anatomical distribution is wide. It presents as a solitary well-defined erithematous macule or plaque that gradually increases in size. Occasional partial spontaneous regression is seen.

We present a case de APL in a 2- years-old girl, the youngest reported patient in the literature,with clinical diagnosis of aplasia cutis,which showed a round lession without hair reaching 1.2 X 1.0 cm in size, located in the scalp.Histologically is characterized by superficial and deep dermal involvement by horizontal, irregular, thin-valled vascular channels lined by a single layer of flat attenuated endothelial cells that are seen dissecting the collagen bundles. The channel apear empty, but occasionally proteinaceus material or red blood are seen.

APL may mimic low-grade angiosarcoma and patch-stage Kaposi`s sarcoma. The former has at least focal cytological atypia and multilayering and the clinical setting is different. In the latter there are ussually multiple lesions, and histologically there is haemosiderin deposition with extravasated erithrocytes and adyacent inflamatory cells, including plasma cells. Distinction from lymphangiomatosis is afforded mainly by the clinical extent of the lesion; histologically this condition shows massive dissection of the dermal collagen. Haemosiderin deposition of uncertain pathogenesis is usually a prominent feature.

Pathologists and dermatologists should be aware of this entity, as surgical treatment may be totally curative when the lesion is limited in size.

## P-473

S100A PROTEIN EXPRESSION REFLECTS THE DEGREE OF ATYPIA IN NEVI WITH ARCHITECTURAL DISORDER

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**Aims**: To test the hypothesis that S100A protein expression in nevi with architectural disorder (NAD) or "dysplastic nevi" can be used in grading the degree of atypia of the lesion.

**Methods**: 123 melanocytic lesions and 15 control tissues were placed into the following categories: NAD-mild, NAD-moderate, NAD-severe, melanoma in situ, invasive melanoma, and other control lesions. The grading was evaluated on multiple, H&E stained, routine sections of the whole lesion before immunostaining. Paraffin sections were pretreated with pepsin (0.25%w/v, pH 2.0) for 5 minutes at 45°C. and then reacted with the monoclonal antibody MAC387, which binds to S100A8, S100A9, and S100A12. Forty-five lesions were also reacted with monoclonal antibody to S100A2. The bound antibodies were localized by reaction with biotinylated horse anti-mouse IgG and a strepavidin-alkaline phosphatase detection system using a red chromogen.

**Results**: MAC387 staining in the keratinocytes overlying melanocytic lesions was found to be strong in 0/13 NAD-mild (0%), 5/24 NAD-moderate (21%), 6/23 NAD-severe (26%), 19/27 melanomas in situ (70%), and 16/24 invasive melanomas (67%). Strong staining was not found over ordinary compound and intradermal nevi unless traumatized, or in normal skin unless ulcerated, inflamed, or over a recent scar. Spitz nevi also had strong staining of keratinocytes. S100A2 did not show as striking differences as MAC387.

**Conclusions**: MAC387 staining of keratinocytes over NAD and melanomas may reflect activation of cell movement in the lesions and consequently the degree of architectural disorder. Many NAD are stable lesions, clinically unchanging, and are negative or show only weak staining with MAC387. NAD that are strongly positive for MAC387 staining should be removed completely.

## P-474

**p53 CODON 72 POLYMORPHISM AND THE DEVELOPMENT OF HUMAN PAPILLOMA VIRUS-ASSOCIATED SKIN CANCER.**

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**Aims :** A recent report[1] suggests that a polymorphism of the p53 tumour suppressor gene that results in the substitution of a proline residue with an arginine residue at position 72 of the p53 protein might act as a risk factor in human papilloma virus (HPV)-associated malignancies. We examined the role this polymorphism might play in the development of cutaneous carcinomas.

**Methods :** Blood samples were taken from 55 skin cancer patients and 114 ethnically matched volunteers. A polymerase chain reaction (PCR)-based assay was used to determine the p53 codon 72 genotype of the skin cancer patients and the control population. 20 viral warts and 23 squamous cell carcinomas (SCC) were also excised from the skin cancer patients and HPV typed by PCR and automated sequencing.

**Results :** 100% of the warts (20/20) and 87% of the SCCs (20/23) were HPV-positive and were shown following sequencing to harbour either common cutaneous HPV types or Epidermodysplasia verruciformis-associated HPV types. The frequencies of p53 codon 72 genotypes were 78% arginine homozygous, 2% proline homozygous and 20% heterozygous among skin cancer patients and 79% arginine homozygous, 3.5% proline homozygous and 17.5% heterozygous among the control population. Statistical analysis showed no significant differences in the distribution of the two p53 isoforms between the skin cancer patients and the control population

**Conclusions :** Our results suggest that there is no correlation between the presence of HPV, the p53 codon 72 arginine polymorphism and the development of skin cancer.

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## P-475

TYPE I PRIMARY MENINGIOMA OF THE SCALP. A CASE REPORT

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Rarely meningiomas can be found in an extracranial site, one of the commonest of which is the subcutis of the scalp. We report the case of a subcutaneous scalp nodule of a 26-year-old male who several months before the present sampling underwent to incomplete excision in the same site without postsurgical analysis.

**Case report:** A solitary, firm, apparently well circumscribed subcutaneous nodule of the scalp was excised. It was covered by normal hairy skin. No apparent connection with underlying bony structures was noted. The clinical diagnosis was of a benign cystic malformation.

**Gross findings:** The specimen consisted of a 2.5x1.5 cm ellipse of the scalp with subcutis deep 1.5 cm housing a nodule of 0.7 cm in its greatest diameter.

**Materials and methods:** After formalin fixation and paraffin embedding, multiple 5 μm thick sections were stained by haematoxylin and eosin and indirect immunoperoxidase technique using a panel of monoclonal antibodies.

**Light microscopy:** Skin and adnexal structures were normal. The tumor was composed of scattered lobules and nests of spindle-shaped to oval cells with well-defined oval basophilic nuclei with dispersed chromatin and a weak eosinophilic cytoplasm often showing indistinct cell borders, intermixed with fibrocollagenous tissue. The lobules and nests of cells occasionally had a whorled feature. On high-power examination some tumor cells were arranged in narrow cords and trabeculae embedded within a fibrous stroma sometimes in intimate association with vessels or fat cells, simulating a pseudoinfiltrative pattern. The tumor cells showed diffuse immunoreactivity for vimentin, EMA and S100 protein, while no CK116, Fact.VIII, S100 protein, desmin, and smooth muscle actin immunostaining was noted.

**Discussion:** Histological and immunohistochemical data demonstrated the meningothelial nature of the tumor cells. According to Lopez *et al.* there are three types of skin meningiomas: type I occurs in the scalp, face or paravertebral region of children and young adults and probably originates from arachnoid cell rests displaced during embryogenesis; type II occurs around sensory organs of the head of adults and is considered an ectopic soft tissue meningioma arising from arachnoid cell rests along nerve sheaths; type III represents a direct extension of an intracranial meningioma. Our case shows many features of type I primary cutaneous meningioma.

## P-476

**OVEREXPRESSION OF APOPTOSIS REGULATORY MOLECULES BCL-2, BCL-X, AND MCL-1 IN ANGIOLYMPHOID HYPERPLASIA WITH EOSINOPHILIA: A COMPARATIVE STUDY WITH PLAQUE-STAGE KAPOSI SARCOMA**

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**Background:** Angiolymphoid hyperplasia with eosinophilia (ALHE) is an angioproliferative process of unclear nature and etiology, although Kaposi sarcoma-associated herpesvirus infection has been implicated in its pathogenesis. Recent investigations have demonstrated that alterations of Bcl-2 family antiapoptotic members may be involved in Kaposi sarcoma (KS) progression. To the best of our knowledge, similar studies have never been performed in ALHE.

**Aims:** To evaluate possible alterations in the immunoreactivity of Bcl-2 family antiapoptotic molecules (Bcl-2, Bcl-x, and Mcl-1) in ALHE, comparing the results with those obtained in plaque-stage KS.

**Methods:** Immunohistochemical expression of Bcl-2, Bcl-x, and Mcl-1 was examined in paraffin embedded samples from 9 cases of ALHE and 10 cases of plaque stage KS. The stains were scored from 0 to 12 according to intensity and percentage of positive cells. Results were statistically studied using analysis of variance and Kruskall-Wallis test.

**Results:** Bcl-2 and Mcl-1 are overexpressed in ALHE and KS, their immunostainings reaching similar scores in both lesions. There is a strong BCl-x overexpression in most cases of ALHE (predominantly in the characteristic hobnail endothelial cells), and a less intense Bcl-x expression in KS cases. The scored values of Bcl-x in ALHE are significantly higher than in KS (HALE: 9±5.14, KS:5.3±4.9, p=0.003).

**Conclusions:** Overexpression of antiapoptotic molecules in ALHE suggests that apoptotic inhibition might play a role in its pathogenesis. The contribution of Bcl-x seems to be especially relevant, since its expression in ALHE is almost twice as high as that in KS.

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## P-477

CUTANEOUS LEISHMANIASIS. IMMUHOHISTOCHEMICAL EVALUATION OF MACROPHAGOS AND LYMPHOCYTES. A COMPARISION WITH LEPROSY CUTANEOUS LESIONS.

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**Aims:** It has been referred that lesional pattern in leishmaniasis is related to the type of immune response. We try to define in this study the type of lymphocytic macrophages involved in cutaneous leishmaniasis, including a group of AIDS patients, in comparision with our previous findings in leprosy.

**Methods:** We have selected 54 skin lesions of leishmaniasis in 54 patients, 11 of them with AIDS. Four lesional patterns were considered: Epithelial: grouped epithelioid macrophages surrounded by lymphocytes; Diffuse: mixed macrophages and lymhocytes; Intermediate pattern and AIDS pattern with very slight lymphocytic component without macrophage activation and with abundant parasites. The immunohistochemical panel included S100 protein, CD68, muramidase, Factor 13a, HLA-DR, CD1a, CD4 and CD8.

**Results:** The immunopathogenic findings are not equivalent comparing leishmaniasis and leprosy. Macrophages without activation do not express protein S100 as happens in lepromatous macrophages. In lesions with higher macrophage activation, no nerve lesion appear and although DR is expressed by macrophages in those lesions with epithelioid pattern, the expression is always lower than in tuberculoid leprosy patients. No cases with DR expression in keratinocytes have been found. Langerhans cells in the epidermis were variable but absence or patent hyperplasia as in lepromatous or tuberculoid leprosy patients has not been found. The proportion and topography of CD4 and CD8 lymphocytes has been considered in each of the four lesional patterns.

**Conclusions:** Our findings support the idea that macrophages express a variable immunophenotype in accordance to the lymphocytic response. However, the macrophage immunophenotype is not equivalent to that found in leprosy.

## P-478

DYSPLASTIC MELANOCYTIC NEVUS (DN). CLINICOPATHOLOGICAL FEATURES.

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**Aims:** It still remains the controversy about nomenclature, nature and biologic behaviour of DN. In this study we try to determine the age of presentation, the most frecuent location and the relationship with other melanocytic lesions of DN. All cases were selected only when showed strict histopathologic diagnostic criteria.

**Methods:** 97 DN in 64 patients were selected. All showed intraepidermic and junctional melanocytic proliferation with architectural disorder, random nuclear atypia and mesenchymal reaction in papillary dermis.In all cases the histopathologic diagnosis was made in 1993 or 1994, and a search of previous biopsies (since 1967) and posterior lesions (until 1999) in these patients with a histopathologic diagnosis of DN or melanoma, was done in the computer of the Pathology Department .

**Results:**The incidence of DN in our series, referred to acquired conventional and congenital nevi, was 1/18 and 1/4 respectively. Most of them were excised in patients between 10 and 50 years of age. No cases were found in the first decade of life. 70% were located in the back. Lesions in the face or extremities were unfrequent. In 7 patients we found 21 previous biopsies of DN. In 17 patients, 39 had posterior biopsies of DN. In 7 patients there were 8 previous melanomas and in 1 we found a posterior diagnosis of melanoma. There was a higher incidence of melanoma in those patients with a larger number of DN.

**Conclusions:** DN, when diagnosed with strict histopathologic criteria, appears as a real entity with specific morphological features, lesional multiplicity and is related to melanoma.

## P-479

**Polypoid melanomas: kinetic and DNA-ploidy markers.**

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**Aims:** Polypoid melanoma (PM) is a variant of cutaneous malignant melanoma (mainly nodular or superficial spreading types) characterized by exophytic growth, highly tumor thickness and poor prognosis.

**Design:** 31 cutaneous malignant melanomas (6 polypoid and 25 non-polypoid ones), 0.98-12.86 mm thickness (Av ± SD: 3.54 ± 2.61) were included in this study. The proliferative parameters included mitotic figures counting and MIB-1 labelling index. DNA ploidy was sistematically evaluated by image cytometry (CAS-200, Becton Dickinson). At least 500 cells per case were measured.

**Results:** All polypoid melanomas were of nodular type (P<0,016). Mitotic figures counting and MIB-1 index in polypoid melanomas were higher than those in non-polypoid melanoma (P<0,05). All PM were aneuploid, while 52% of the non-PM. Survival analysis showed significant differences in overall survival (non-PM 52,2 months and PM 19,2 months, P<0,005) and disease-free interval (non-PM 42,5 months and PM 3,7 months, P<0,000 ). To date, 75% of patients with non-polypoid melanomas are alive, while 83,3% of patients with polypoid melanomas have died (P<0,05).

**Conclusions:** The results suggest that polypoid melanoma has high proliferation rates and is usually aneuploid, two features related with poor clinical outcome.

## P-480

CUTANEOUS PANARTERITIS NODOSA: A CLINICOPATHOLOGICAL EVALUATION OF A CONTROVERSIAL ENTITY

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**Aims:** The objective of our study is to evaluate the evolution of 24 patients affected by cutaneous processes, first diagnosed as cutaneous panarteritis nodosa (PAN), as well as discuss the possible relationship between this disease and other dermatoses.

**Methods:** 24 cases initially diagnosed as cutaneous PAN in the department of Pathology at the "Doce de Octubre" Hospital were studied retrospectively. Biopsy specimens were reviewed in detail, and six cases initially diagnosed as cutaneous PAN were reclasified, basing the final diagnosis in histopathological and clinical features. These patients formed the "false cutaneous PAN group", and were compared with the remaining cases, which fitted the criteria for the diagnosis, forming the "real primary cutaneous PAN group". Clinical features, laboratory results, outcome and response to treatment were evaluated.

**Results:** Cases forming the "false cutaneous PAN group" consisted of two lobular panniculitis, two thromboflebitis, one systemic PAN, and one stasis dermatitis. Clinical and laboratory differences were found between both groups, more reliable in sex distribution, incidence of systemic symptoms (higher in the real PAN group), and the presence of possitivity to antinuclear antibodies (ANA).

**Conclussions:** We conclude that cutaneous PAN is a distinct localized vasculitic process that involves medium-sized vessels in the deep dermis, with a favorable outcome and no visceral involvement. Its diagnosis must be based on clinical and histopathological data. Nodular vasculitis (erythema induratum) may mimic cutaneous PAN in early stages, being differential diagnosis difficult or even imposible. Other differential diagnosis include systemic PAN and thromboflebitis.

## P-481

MICROVESSELS COUNT AND ANGIOGENIC FACTORS IN THICK CUTANEOUS MELANOMA (>3 MM)

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**Aims:** Although the importance of angiogenesis in the process of tumor growth and metastasis has been well established for many human tumors, the prognostic significance of tumor vascularity in cutaneous melanoma is still a controversial issue. Aims of the current study were to evaluate whether angiogenesis, quantified by microvessel counting, and the expression of angiogenic mediators, such as inducible nitric oxide (iNOS) and cyclooxygenase-2 (COX-2), are related to the development of metastases and clinical outcome in patients with thick cutaneous melanoma.

**Methods:** A series of 12 patients with melanoma (>3 mm) who did not experience disease progression after 10 years follow-up and 24 matched controls who underwent recurrence and/or metastases were selected for the study. Tumor microvessels were stained with anti-CD31 antibody, and their number was recorded in the one field (x400) out of five examined fields with the highest number of microvessels, after selection of the areas with the greatest vascular density ("hot spots"), excluding the areas of regression. iNOS and COX-2 expression was semiquantitatively evaluated (0; ≤20%; >20% of positive cells).

**Results:** Mean microvessel count was 13.83 (range 5-31) for cases who did not undergo progression and 20.91 (range 6-40) for the metastasizing melanomas, the difference being statistically significant ($p$=0.04, Mann-Whitney U test). iNOS staining was observed in 9 out of 12 (75%) long-term surviving cases and in 16 out of 24 (66.7%) cases who underwent progression, with no statistically significant differences between the two groups. COX-2 was diffusely expressed in all cases investigated, independently from the clinical outcome. No significant correlation was found between microvessels count, iNOS and COX-2 expression and other clinicopathologic features, including age, sex, histotype, level, regression, mitotic count, ulceration, type of invasive front.

**Conclusions:** Our data indicate that tumor vascularity has a prognostic role in thick cutaneous melanoma whereas iNOS and COX-2 expression is not useful in predicting survival.

## P-482

CLUSTER ANALYSIS OF BENIGN PIGMENTED SKIN LESIONS

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Pigmented skin lesions represent one of the challenging fields of pathology. So far many morphometric studies, assessing mainly nuclear features have been undertaken with variable results. The distribution of cells in a lesion in also a potentially valuable parameter. Cluster analysis can be used to describe the spatial distribution of objects. In our study we investigated the distribution of nevocytic cells in different benign pigmented skin lesions using cluster analysis. **Methods.** Samples of 30 pigmented skin lesions, previously diagnosed as compund, intradermal or dysplastic nevi respectively, were included in the study. Using interactive, semiautomatic image analysis system (VAMS, Zagreb) and a custom made cluster analysis program. **Results.** Cluster analysis showed a non-random distribution in all type of analysed lesions. Due to group overlapping the single type of lesions could not be separated according to their clustering pattern. **Conclusion.** As the clustering pattern of benign skin lesions shows a non-Poisson distribution, a further investigation of this problem by more sophisticated expert systems seems to be indicated.

## P-483

**ACQUIRED PROGRESSIVE LYMPHANGIOMA.**

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**Aims:** Acquired Progressive Lymphangioma is a rare vascular proliferative lesion. This lesion may mimic angiosarcoma and patch-stage Kaposi´s sarcoma.

**Methods:** We describe a 55-year-old patient with an erythematous lesion in right thigt which slowly developed over a period of some years.

**Results:**

Gross findings: a flat gray-yellowish lesion which measured 15x10 mm.

Microscopic findings: the lesion showed thin-walled interconnecting vascular channels throughout the dermis and subcutis, arranged horizontally. The vessels were lined by a single layer of plump endothelial cells, dissecting the dermal collagen bundles. Immunohistochemical studies disclosed no factor VIII-related antigen on endothelial cells. Basement membrane and intracitoplasmic Weibel-Palade bodies have been absent by electron microscopy.

**Conclusion:** Acquired Progressive Lymphangioma is a benign vascular proliferation, characterized histologically by dermal vascular channels and a " dissection of collagen " appearance. Patch-stage Kaposi´s sarcoma may be impossible to distinguish from acquired progressive lymphangioma. Pathologists and dermatologists shoud be aware of this entity, as early surgical treatment may be totally curative when the lesion is limited in size.

## P-484

MORPHOGENESIS OF NORMOTROPHIC, HYPERTROPHIC, AND KELOID SCARS IN CHILDREN

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**Aims**: to study the patho- and morphogenesis, differential diagnosis of normotrophic (NS), hypertrohpic (HS), keloid (KS), and embryonic scars (ES).

**Methods**: operative skin scar biopsies of 82 children aged 6 months to 14 years and the time of the scar existence ranging from 6 months to 5 years were studied using histological, histochemical, electron-microscopic techniques, morphometry, and score assessment of 38 morphological characteristics. The scars were caused by burns, injuries, and operations.

**Results**: dermal fibroblasts (Fb) ensuring complete regeneration of the skin without scar formation are primarily involved in the healing of superficial skin defects. As for deep full-thickness skin wounds, nonspecific Fb forming NS or HS with longitudinal and parallel orientation of collagen fibers (CF) relative to the skin surface and partial regeneration of elastic fibers (EF) play a leading part in the healing process. HS differ from NS in the predominance of hyperplastic processes (permanent proliferation and metabolic activity of Fb and myofibroblasts, the synthesis of collagen), intensified vascularization, retarded moturation, the absence of involution, vasculitis, lymphomacrophageal infiltration, and epidermic dystrophy. KS significantly differ from HS in nodular structure, spherical and arcate fiber orientation, the presence of atypical and giant Fb, the absence of EF, multicentric growth, and cyclic remodelation.

**Conclusions**: NS occur when autoregulation of connective tissue growth is retained, HS develop in dysregulaton and prolonged hyperplasia, and KS result from local dysplasia similar to pseudoneoplastic fibromatoses.

## P-485

APC AND C-NEU IN MALIGNANT AND BENIGN MELANOCYTIC LESIONS

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This work was supported by the Upper Austrian Cancer Society

**Aims:** This immunohistochemical study is analysing the expression and distribution of APC and c-neu gene product in malignant and benign melanocytic tumours. Our results confirms in vitro studies, in which both genes are shown to play an important role in melanoma development.

**Methodes:** 20 paraffin slides of patients suffering from malignant melanomas were evaluated on haematoxylin and eosin sections using 10 benign naevi as a control group. The avidin-biotin method was employed for immunostaining, using monoclonal mouse antibodies against APC and c-neu products. Assessment of immunostaining was performed by using normal keratinocytes of the same sample as an internal control. The intensity and distribution of APC and c-neu expression was reported and correlated with morphological and clinical features.

**Results:** APC immunohistochemistry exhibit a reduced expression in malignant melanomas (many of the tumour cells were completely negative), whereas benign naevi show a clear cytoplasmic staining. C-neu was overexpressed in all melanomas compared with weak immunoreactivity in benign naevi. There was no correlation in APC and c-neu immunohistochemical staining and tumour location, Clark's level, growth pattern and depth of invasion.

**Conclusion:** As APC and c-neu are interacting with ß-catenin, loss of APC in malignant melanomas may lead to increased cell proliferation by increasing intracytoplasmatic ß-catenin levels. The overexpression of c-neu may cause a disruption of the E-cadherin-ß-catenin complex, resulting in an invasive tumour phenotype. Our morphological investigations support in vitro studies showing that both genes are important in melanoma development.

## P-486

**p16 protein expression as an important prognostic factor in primary cutaneous malignant melanoma.**

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The role of p16 mutations in inherited susceptibility to familial cutaneous malignant melanoma (CMM) is clear, but the p16 involvement in sporadic CMM must be clarified.

The expression of p16 protein has been reported to be altered in a substantial number of advanced melanoma cases (Reed et al 1995, Straume et al 1997) but the prognostic role of p16 alterations has not been focused upon in survival studies.

In the present immunohistochemical study, the expression of p16 and its prognostic relevance has been examined in 165 primary CMM: 25 tumours Clark I and II, 78 Clark III, 62 Clark IV and V; 42 tumours Breslow <0.75, 38 Breslow 0.75-1.5 and 85 Breslow >1.5.

p16 expression was positive in 54% of the primary CMM studied.

A 5-year recurrence-free survival was significantly more frequent in the p16 positive primary CMM compared with the p16 negative (93% vs 38%), (p<0.0001), with a sensibility of 89% and specificity of 74%.

In multivariate analysis, lack of p16 staining significantly increased the predictive power of the Clark and Breslow classification (p<0.0001).

Our present results indicate that loss of p16 expression is significantly associated with increased probability of recurrence of the disease.

Thus, p16 immunochemistry is an easy technique, our results could suggest its incorporation in the routine prognostic evaluation of primary CMM patients.

References:
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Straume O and Akslen LA. Alterations and prognostic significance of p16 and p53 protein expression in subgroups of cutaneous melanoma. Int J Cancer (Pred Oncol), 74, 535-539 (1997).

## P-487

**LOSS OF NUCLEAR EXPRESSION OF p16 PROTEIN CORRELATES WITH INCREASED TUMOR CELL PROLIFERATION AND POOR PATIENT OUTCOME IN PATIENTS WITH VERTICAL GROWTH PHASE MELANOMA**

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**Aims and methods:** The *CDKN2a (p16$^{INK4a}$)* cell cycle inhibitory gene has been associated with development of familial melanoma. Additionally, recent studies have demonstrated that p16-alterations occur frequently in sporadic melanomas. To investigate whether differences in expression were associated with tumor cell proliferation, tumor progression and patient survival, we examined the immunohistochemical staining of p16 protein, and compared the results with Ki-67 expression, angiogenesis, clinico-pathologic variables and survival data in 202 sporadic vertical growth phase melanomas.

**Results:** 45% of the cases showed absent or minimal nuclear staining for the p16 protein product. These cases were significantly associated with high proliferative activity, assessed by the expression of Ki-67 (Mann-Whitney test, p< 0.0001). No significant associations were present with tumor thickness, Clark's level of invasion, anatomic site, microvessel density, or p53-protein expression. In univariate survival analysis, absent nuclear p16 expression significantly predicted poor patient outcome (log rank, p=0.003), with 44% and 67% estimated 10-years survival for cases with absent or present p16-expression, respectively. In multivariate survival analysis, p16-expression was an independent prognostic factor (p=0.005, hazard ratio 2.3), along with Clark's level of invasion, anatomic site, p53-expression, microvessel density and proliferative rate by Ki-67 expression.

**Conclusions:** Our results indicate that loss of nuclear p16 expression is a frequent event in vertical growth phase melanomas, and that altered p16 plays an important role in the regulation of proliferation and progression of this tumor.

## P-488

ECTOPIC EXPRESSION OF αIIbβ3 INTEGRIN IN HUMAN MELANOMA

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**Aims:** Literature data indicated that rodent tumors can express thrombocyte integrin αIIbβ3 ectopically. Experimental studies also shown that this ectopic integrin may play a crutial role in tumor dissemination as adhesion and signaling molecule. Therefore we have postulated that human melanoma may also express this ectopic thrombocyte integrin.

**Methods**: Expression of the αIIb chain in human melanoma cell lines (7) was studied at genetic as well as protein level using RT-PCR, Western blotting and immunocytochemistry. In human melanoma samples (30) αIIb and av expressions were studied using double labeling immunohistochemistry and confocal microscopy .

**Results**: All the human melanoma cell lines studied, expressed αIIbβ3 to various extent both at genetic as well as protein level. On the contrary to the constitutive homogenous expression of the αv chain in human melanoma samples, the expression of the αIIb chain was much more unique and was detectable only in a smaller proportion of the tumor cell population. Beside thrombocytes and melanoma cells no other cell type stained positive for αIIb.

**Conclusion**: Our experimental and pathology data indicate that the megakaryocytic cell line-specific αIIbβ3 integrin can be expressed ectopically in human melanoma cell lines and human skin primary melanomas. On the contrary to the constitutive expression of the αvβ3 integrin in melanoma, the ectopic αIIbβ3 complex is restricted to individual tumors and to cell populations in the individual tumor. Based on our experimental data we suggest that the ectopic αIIbβ3 integrin expression may have prognostic significance in human melanoma.

# P-489

## COMPLICATIONS AND CAUSES OF DEATH IN PSORIATIC ARTHRITIS

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Psoriasis is complicated by *psoriatic arthritis (PsA)* in 5% of the cases.
**Objective**: The *basic* and *accompanying diseases*, the major *complications* and *causes of death* were determined and histologically confirmed in a randomized autopsy population of 12 in-patients (female 6, average age: 66.5 years; male 6, average age of 62.8 years at death) with *(PsA)*, who died at the National Institute of Rheumatology between 1968 and 1998.
**Methods:** The tissue specimens were fixed in 8% formaldehyde at pH 7.6 and embedded in paraffin. Serial sections were cut and stained with haematoxylin-eosin, Ziehl-Neelsen stain, PAS reaction, and Congo red according to Romhányi. The (AA) amyloid was determined and characterized histochemically.
**Results:** Mortality in PsA is summarized in table I.

| n | Basic disease | Complication(s) | Cause of death | Associated |
|---|---|---|---|---|
| 1. | Atherosclerosis | Pericarditis | Heart failure | PsA |
| 2. | Prostatic hypertrophy | Operative complication | Pulmonary embolism | PsA |
| 3. | Hypertension | Pulmonary embolism | Circulatory failure | PsA |
| 4. | Atherosclerosis | Purulent bronchitis | Circulatory failure | PsA |
| 5. | Tuberculosis | Pulm.art.arrosion-(AA) | Haemorrhage | PsA |
| 6. | Atherosclerosis | (AA) amyloidosis | Myocardial necrosis | PsA |
| 7. | PsA-Synovectomy | Operative complication | Pulmonary embolism | |
| 8. | Hypertension | Atherosclerosis | Myocardial necrosis | PsA |
| 9. | Atherosclerosis | Myocardial fibrosis | Heart failure | PsA |
| 10. | Cirrhosis of liver | Ascites | Bronchopneumonia | PsA |
| 11. | Hypertension | Atherosclerosis | Heart failure | PsA |
| 12. | Atherosclerosis | Hypertension | Pulmonary edema | PsA |

**Discussion**: In one case the cause of death was thrombembolism as a postoperative complication of synovectomy. In the remaining 11 cases some other basic disease (atherosclerosis and/or hypertension in 8, tuberculosis in 1, postoperative thrombembolism in 1, cirrhosis of the liver in 1 patient) led to death. Our results are similar to the mortality data of the literature. Systemic secondary AA amyloidosis is a rare complication of PsA: in one of our cases it was probably caused by tuberculosis, and in one case it appeared to be related to PsA itself.

# P-490

## Significance of a the small subtelomeric area of chromosome 1 (1p36.3) in the progression of malignant melanoma.

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**Introduction**: In melanomas two growth forms determine the clinical outcome. Melanomas with radial growth can be cured by simple excision. Tumors who achieved or primarily show vertical growth are associated with metastasis. Studies indicate that these tumor forms are associated with specific chromosomal aberrations. In nodular melanomas (NM)a deletion of 1p36 was found in an augmented percentage while superficial spreading melanomas (SSM) did not show this aberration.
**Aims**: to study the occurrence of this deletion in the progression of malignant melanomas and to evaluate the extension in order to confine the loss of possible relevant DNA material more precisely.
**Methods**: 7 melanoma cell lines (MC), 32 primary tumors (PT)(16 SSMs and 16 NMs) and 32 metastatic tumors (MT) where included in this study. Fluorescence in situ hybridization was carried out with a repetitive telomeric DNA probe D1Z2 in 1p36, eight YAC DNA probes hybridizing to 1p36, 1p32, 1p31 and 1p21, and the centromeric probe D1Z1 as internal control.
**Results**: All MCs, 91% of the MTs and 63% of the NMs but none of the SSMs showed a deletion in D1Z2. With YAC probes, the most frequent deletions were found in 1p36 in all MCs, in 13% of NM and in 44% of MT. They were never detected in SSM. The region 1p31 was concerned rather equally in NM, SSM and MT.
**Conclusion**: In our study the deletion in 1p36 occurred in nearly all MTs, but was mostly confined to a rather small area near the locus D1Z2. SSMs with vertical growth did not show this deletion while MTs derived from these tumors showed the loss of DNA material in 1p36. The results underline the importance of this part of 1p36 in the pathogenesis of malignant melanoma. Furthermore the augmented accumulation of this deletion point toward genetic changes during tumor progression which might be of significance for the acquisition of a metastatic potential.

# P-491

## CLINICOPATHOLOGIC STUDY OF 7 SOFT-TISSUE MYXOMAS APROPOS OF A CASE OF RECURRENT JUXTA-ARTICULAR MYXOMA WITH AGGRESSIVE FEATURES.

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**Aims:** To compare clinical presentation, histological features, tumor size and recurrences of both juxta-articular (JAM) and intramuscular (IMM) myxomas. Moreover, we report a case of a huge JAM of the knee that recurred a year after surgery mimicking a malignant neoplasm.
**Methods:** Formalin fixed, paraffin embedded tissue from all the cases diagnosed as soft-tissue myxomas in our institution, were examined by histochemical and immunohistochemical methods. Cytological and ultrastuctural studies were performed in the cases with suitable material. Clinical information and follow-up were obtained from medical reports.
**Results:** We have reviewed 7 cases of soft-tissue myxomas (4 IMMs and 3 JAMs). The background was myxoid and hypocellular, with fusiform and stellate cells without atypia or mitoses. Immunohistochemically, cells were only positive for vimentin. Blood vessels were very scanty and lipoblasts absent. Ultrastructural studies revealed myofibroblastic differentiation.
Our series shows differences between JAM and IMM. JAMs tend to locate in the vicinity of large joints associated with a story of degenerative arthritis. Histologically, JAMs show cystic changes with focal areas of hemorrhage and infiltrative growth pattern. Although benign, 2 of the 3 JAMs from our series locally recurred after surgery simulating a malignant tumor, specially a case which recurred one year after excision with a radiologically aggressive pattern, measuring 20cm. On the contrary, IMMs located in large muscles are histologically uniform, with no recurrences.
**Conclusions:** We report the clinicopathological features of 7 cases of soft-tissue myxomas emphasizing the different follow-up between JAM and IMM. We also describe a peculiar 20cm JAM which represent the larger reported case. Knowledge of microscopic findings as well as clinical potential aggressiveness of this entity is very important in order to differentiate these neoplasms from tumors with mixomatous change, which would imply different treatment and prognosis.

# P-492

## INTIMAL SARCOMAS OF THE PULMONARY ARTERY

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**Aims:** The characterization of clinical, histopathological, immunohistochemical and genetical features of the rare type of primary tumors of the pulmonary artery.
**Methods:** Clinical data were obtained on the course of eight patients diagnosed with a sarcoma of the pulmonary artery between 1994-1998. Routine histopathological examination of four resected lungs, one end-arterectomy specimen and three biopsies were performed. Immunohistochemistry and comparative genomic hybridisation (CGH) were done.
**Results:** Of the eight patients 4 were male and 4 female; median age 41 years. The predominant clinical presentation was dyspnoe (7 out of 8) and febrile pulmonary disease (4 of 8). Clinical or pathological signs of embolic lung disease were present in all cases. One patient died post-operatively, four patients died of disease at 9 to 30 months after primary presentation, three patient were alive at 8 to 25 months follow up. The histopathological examination of the submitted material showed at least partially myxoid, spindle cell sarcomas in all cases. Metastatic disease was histologically confirmed in three cases (lung, pleura, skull). Immunohistochemically vimentin was the strongest expressed marker. Some degree of positivity was observed for alpha-actin, CD68, p53 and bcl2. The proliferation index Ki-67 was between 5% and 80%. 6 of 7 examined cases were positive for mdm2.In the CGH analysis in 6 of 8 cases (75%) gains and amplification of the 12q12-15 region were found. Other, less consistent alterations were losses on 3p, 3q, 4q, 9p, 11q, 13q, Xp and Xq as well as gains on 5p, 7p, 17p and 17q.
**Conclusion:** The intimal sarcomas of the pulmonary artery are a group of tumors with unfavorable prognosis and myofibroblastic differentiation. High percentage of cases shows a consistent genetic alteration (12q12-15 amplification) implicating mdm2/p53 pathway as a possible mechanism in the tumor pathogenesis.

## P-493

FINE-NEEDLE ASPIRATION OF SOFT TISSUE TUMORS. DIAGNOSTIC ACCCURACY.

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**Aims:** To assess the diagnostic accuracy of fine-needle aspiration (FNA) in the diagnosis of neoplasms of soft tissue tumors and the adequacy of this technique in determining the origin of these tumors.

**Material and methods:** 152 fine needle aspirates of soft tissue tumors were obtained as initial diagnostic procedure between January 1996 an December 1998 in our center. Twenty seven were excluded due to inadequate histologic follow up. They were fixed in 95% ethanol and stained with hematoxylin-eosin. Fine-needle aspiration and histological diagnosis were correlated, the definitive diagnosis being the one provided by the surgical specimen.

Results: A benign diagnosis was provided in 40 patients. It was confirmed in 38 patients by histological examination. A malignant diagnosis was correctly rendered in 81 patients as confirmed by the histological examination. Only the diagnosis in four patients rendered a suspicious malignant lesion, this diagnosis was confirmed by histological examination in three patients. 88 patients were diagnosed as having a soft tissue tumor and 37 as carriers of a secondary lesion depending of bone or other tissues (metastasis). The sensitivity of our method was 97.6 %, the specifity 97.1 %, the positive predictive value 98.8 %, the negative predictive value 95 % and the efficacy 97.5 %.

**Conclusions:** FNA is useful in identifying the nature of a soft tissue tumor. A correct diagnosis of a benign neoplasm avoids unnecessary surgery and permits the clinician to establish and adequate follow up of the patients. It permits to differentiate among primary or secondary lesions avoiding unnecessary treatment in the case of metastastic tumors.

## P-494

**LEIOMYOSARCOMA OF THE OROPHARINX AND NEUROGENIC TUMORS IN A PATIENT WITH TURNER'S SYNDROME**

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**Aims:** A higher incidence of gonadal and extragonadal neoplasms (mainly neurogenic tumors) is a well known phenomenon in gonadal disgenesis. Few example of soft tissue sarcomas are also reported but at our knowledge no leiomyosarcoma has been described yet. On the other hand, leiomyosarcoma is a rare tumor in children and adolescents.Up to date only few cases are reported in soft tissue. The visceral ones are often related to HIV infection or immunosoppressive drugs and arose in the hepatobiliary, gastrointestinal, and tracheopulmonary system.

**Methods:** Our patient was diagnosed to be affected by Turner's syndrome few months after birth. She underwent to thoracotomy because of a mediastinal mass when she was 4-year-old. The histological diagnosis was "imperfect ganglioneuroblastoma" ("well differentiated stroma-rich neuroblastoma, sec. Shimada). After she was treated with radiotherapy, she was well and free of disease for years. At the age of 22, she was referred to our hospital for a huge tumor, more than 10 cm. in largest diameter, involving the whole palatum and the oropharinx.

**Results:** An incisional biopsy yelded to the diagnosis of epithelioid leiomyosarcoma G3, SMA MSA and desmin positive. At the same time, two cutaneous nodules (left and right thigh) of 1.5 cm. in largest diameter and a subcutaneous lesion of 2 cm. (in the right axilla) were excised and proved to be giant cell collagenomas and neurinoma. Because of the site and size of the sarcoma, the patient was treated by chemotherapy but after two cycles she developed regional lynphnodes metastases. Serologic tests were irrelevant; in particular HIV and EBV were negative.

**Conclusions:** This case is the first reported leiomyosarcoma in a patient with Turner's syndrome. Also the site of involvement (palatum and oropharinx) is very unusual for the already rare leiomyosarcomas in the young age.

## P-495

MALIGNANT AND RECURRING GLOMUS TUMOR: A CASE REPORT

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**Aims:** A 19 - year old male presented with a bifocal soft tissue lesion of the posterior face of the left tigh and on the dorsal face of the left foot that was excised and interpreted as a glomangioma . Nine years later, he developped in the same tigh region a larger recurrence involving the left sciatica nerve. The nervous resection measured 18cm and contained numerous tumoral nodules varied in size with a maximum of 6 cm and exhibited rare necrosis.

**Methods**: Immunohistochemistry was performed with an automaton (techmate 500, DAKO ) using the DAKO ChemMate detection kit.

**Results:** This tumor with a nodular pattern dissociated the nerve. The tumor was constituted of round to polygonal tumor cells with a relatively uniform appearance of round to ovoid and regular nuclei, with a slightly eosinophilic cytoplasm. In some areas, the nuclei were pleomorphic and vacuolar. Tumoral cells formed solid sheets interrupted by vessels surrounded by spindle cells. Mitotic index was about one per HPF Immunohistochemically, vimentin was strongly positive.SMA and actin were positive mainly in the perivascular area and desmin, S-100 protein chromogranin A were completely negative. The endothelial marker CD 34 prominently presented the outline of numerous vessels but was negative in the tumor cells.

**Conclusion:** Malignant criteria are not unanimously accepted in the literature. According to Edwin w. Gould and al.( cancer 65:310-318, 1990) the following classification is proposed: locally infiltrative glomus tumor, glomangiosarcoma arising in a benign tumor and the *de novo* glomangiosarcoma. This case was considered to be a glomangiosarcoma arising in a benign tumor.

## P-496

**INTRACAPSULAR AND PARARTICULAR CHONDROMA: REPORT OF TWO CASES AND REVIEW OF THE LITERATURE.**

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AIMS: Intracapsular and pararticular chondroma is a rare tumor having been reported only 22 cases in world literature. We report two new cases of intracapsular and pararticular chondroma of large joints studying its clinical, radiological and pathological features and review the previous cases in order to point out the main clinicopathological features and differential diagnoses of this entity.

METHODS: Routine pathological examination was performed on the masses obtained by surgical resection.

RESULTS: The tumors measured 7x6,5x4 and 4x3,5x2 cm. respectively. Both of them were in continuity with the capsule of the knee, in case 1 localized in the medial aspect, and in case 2 below the rotulian tendon. Radiographs revealed large calcified, soft-tissue lesions. Histologic examination was similar in both tumors: the center of the lesion consisted of areas of mature trabecular bone surrounded by a peripheral collar of hyaline cartilage.

CONCLUSSIONS: These rare benign tumours arise from the capsule or the pararticular connective tissue of a large joint (mainly the knee) which suffers cartilaginous metaplasia and subsequent ossification. The cases of this presentation fit all the features described previously. These tumours should be beard in mind in the differential diagnosis of radiologically calcified soft-tissue lesions located about the joints such as: tumoral calcinosis, calcified synovial sarcomas, synovial chondromatosis and periosteal chondromas.

## P-497

DEDDIFERENTIATED LIPOSARCOMAS. THREE CASES.

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**Aims:** Dedifferentiated liposarcoma is an uncommon tumor containing distinct areas of well-differentiated liposarcoma and non-lipogenic high grade sarcoma. We describe the pathological features of three cases ocurring in our hospital with different pattern of dedifferentiation.

**Methods:** Tissue from all cases was formalin fixed and paraffin embedded. Sections of each block were stained with hematoxylin and eosin, and sections of dedifferentiated areas were also immunohistochemically studied. Dedifferentiation was defined histologically as a region devoid of lipogenic differentiation occupying at least a low-power microscopic field.

**Results:** All cases were situated in retroperitoneum. The patients were 51, 52 and 68 years old. Surgical resection was the treatment in two cases. In the remaining case, a biopsy was performed. The masses ranged from 15 to 24 cm. All cases showed areas of well-differentiated liposarcoma and non-lipogenic areas of different types:

Case 1: Fibrosarcoma.

Case 2: Neural-like patern associated with metaplastic bone formation.

Case 3: Malignant fibrous histiocytoma.

**Conclusions:** Liposarcoma are one of the commonest sarcomas of adult life and are classified into: well-differentiated, myxoid and pleomorphic types. Dedifferentiated liposarcoma is a variant of well-differentiated type situated principally in retroperitoneum. The dedifferentiated component may show a wide variety af appearances, but most commonly resemble malignant fibrous histiocytoma and fibrosarcoma. Differential diagnosis is not difficult when the well-differentiated component is identified. It is a useful rule to regarde high grade retroperitoneal sarcomas as potential dedifferentiated liposarcomas.

## P-498

**TELOMERASE ACTIVITY IN SOFT TISSUE LESIONS. CORRELATION WITH HISTOLOGY, GRADE, AND OUTCOME**

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**Aims:** This study examined the potential utility of telomerase activity (TA) detection in a series of benign and malignant soft tissue lesions. Results were correlated to histology, grade, cellular proliferation and clinical outcome.

**Methods:** 59 frozen samples from 15 benign lesions and 44 soft tissue sarcomas (STS) were examined. Histologic typing was established using the 1994 WHO classification of soft tissue tumors. Histologic grade was established using the updated version of the FNCLCC system. TA was determined using the telomerase repeat amplification protocole (TRAP) assay. Analysis of the expression of the human telomerase reverse transcriptase (hTERT) was carried out by RT-PCR. Immunoexpression of MIB-1 was studied on consecutive frozen sections.

**Results:** TA was undetectable in benign lesions including lipoma (4), hemangioma (3), desmoid tumor (5), cellular schwannoma (1), ossifying myositis (1), and nodular tenosynovitis (1) whereas it was observed in 17 of 44 (38.6%) STS. None of grade 1, 5 of 12 (41.6%) grade 2, and 7 of 17 (41.2%) grade 3 STS showed TA. Leiomyosarcomas (1/10) and storiform/pleomorphic malignant fibrous histiocytomas (1/7) rarely expressed TA, irrespective of their grade. One of 6 local recurrences and 5 of 9 STS metastases showed TA. hTERT mRNA was detected in 18 (42%) of 43 STS including 13 of 16 (81.2%) telomerase-positive and 5 of 27 (18.5%) telomerase-negative cases. None of the benign lesions expressed hTERT mRNA. The MIB-1 labelling index assessed in 41 STS varied between 1% and 70% with 83% of the cases showing less than 15% immunostained nuclei.

**Conclusions:** The presence of TA in soft tissue lesions is synonymous with malignancy but TA is not a reliable marker of STS aggressiveness. TA cannot be used as a reliable method in making the distinction between reactive/benign and malignant (especially low-grade) lesions. TA seems to be histology dependant, seldomly expressed in leiomyosarcomas and storiform/pleomorphic MFH. No significant relationship was observed between MIB-1 labelling and TA.

## P-499

**Hyperthermic Isolated Limb Perfusion with TNF-α and Melphalan in Extensive Soft Tissue Sarcoma: A Histological Study of the Effect on the Tumor.**

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**Background, Materials and Methods:** The specimens of 27 high grade extensive soft tissue sarcomas (STS) and 3 recurrent desmoid tumors of the extremities, after local treatment with hyperthermic isolated limb perfusion (HILP) using TNF-α and Melphalan, were evaluated for the type and extent of tumor necrosis and other histological local tissue changes. Limb preservation was the objective in this selected group of advanced STS's, candidates for amputation or mutilating surgery otherwise. The tumoral masses were obtained 6-8 weeks after HILP, during the definitive surgical resection of the residual tumor according to protocol. **Results:** Typical histological changes were: cystic hemorragic necrosis in the center of the remaining tumor with pericystic extensive fibrosisSome nonspecific changes were noted in the soft tissue around the mass. In 8 cases more than 90% necrosis was achieved. In 14 cases the percent of necrosis was between 60% and 90% (including 4 cases of 80-90%). In 8 cases less than 60% necrosis was obtained. No correlation was found between these histological responses and: the anatomical location of the tumor ; whether the tumor was primary or recurrent; the type of previous treatments (systemic chemotherapy; radiotherapy) and it's size. Some correlation was found with: the histological type of tumor;and with proximal or distal location in the limb. **Conclusions:** This is the first serial histological description of the effect of high dose TNF-α and Melphalan administered via HILP on the tumoral masses of limb STS. The small number of specimens and especially the variability of tumors precludes definitive conclusions from the observed correlations. Larger numbers and more homogen .ity of the histologic types are needed in future series.

## P-500

**HEMANGIOENDOTHELIOMA OF THE TONSIL.**

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**Aims:** a case of a distinctive vascular neoplasm of the tonsil is described, showing overlapping histologic features of different vascular tumors: Spindle H., Kaposiform H, Retiform H..

**Methods:** a 33 years old woman was admitted to INT Pascale, because of a four.months enlargement of the left tonsil on which a diagnosis of Kaposi's sarcoma had been made, elsewhere. Physical examination, laboratory tests, including HIV test and a Total Body T.C. were normal. For conventional light microscopy, routine methods together with PAS and reticulin stains were performed; for immunoistochemical studies, CD31, CD34, FVIII and actin were used.

**Results:** the tumor showed a peculiar lobular architecture with solid areas composed of predominant spindle cells arranged in short fascicles or creating slit-like and sieve-like spaces, often containing RBC, reminiscent of those seen in Kaposi's sarcoma. Within some lobules, plumper, epithelioid tumor cells, with cytoplasmic vacuoles were identified. Slight cellular atypia was focally present and mitotic figures were about 5/10 HPF. At the periphery of the lesion, thin-walled cavernous spaces and an angiomatous area composed of branching channels were observed, with a hemangioma-like and retiform hemangioendothelioma-like appearance. Capillaries and cavernous blood vessels were positive for all the endothelial markers used, whereas spindle cells only stained for CD34 and CD31. KSHV 8 was negative by PCR and in situ hybridization.

**Conclusions:** primary hemangioendothelioma of the lymphoreticular system is a rare lesion, reported under different, often descriptive, terms. We report the first case of hemangioendothelioma occurring in the tonsil of an adult.

## P-501

ANALYSIS OF P53 AND MDM2 PROTEINS IN MALIGNANT FIBROUS HISTIOCYTOMA IN ABSENCE OF GENE ALTERATION: PROGNOSTIC SIGNIFICANCE.

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**Aims:** TP53 and MDM2 genes and their protein expression were evaluated in frozen and paraffin-embedded tissue from 27 patients with malignant fibrous histiocytoma to elucidate the relationship between them, their implication in tumor progression mechanisms and their possible diagnostic-prognostic value.

**Methods:** SSCP (single strand conformation polymorphism) analysis and direct sequencing of polymerase chain reaction (PCR)-amplified DNA were used to study TP53 mutations. Amplification of the MDM2 gene was analyzed by Southern blot. Immunohistochemical and western blot techniques were used to determine nuclear accumulation of p53 and mdm2 proteins.

**Results:** We detected two TP53 mutations: a point mutation and a 63-pair of bases duplication. Amplification of MDM2 gene was observed in two tumors, one of them also carrying the TP53 point mutation. We observed overexpression of p53 protein in 11 cases and 13 cases with mdm2 protein expression. We confirmed coexpression of mdm2 and p53 proteins in 7 cases without TP53 and MDM2 gene alteration. Statistical analysis showed no linear correlation between isolated p53 protein expression and local recurrence, distant metastasis or survival. On the contrary, survival was significantly reduced in those cases with p53 and mdm2 protein coexpression or isolated mdm2 protein overexpression.

**Conclusions:** Simultaneous coexpression of p53 and mdm2 proteins in MFH is significantly correlated with survival in absence of gene alteration, in contrast to the lack of statistical correlation with survival of p53 protein expression.

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## P-502

MESENTERIC AND RETROPERITONEAL CYSTS.

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Mesenteric and retroperitoneal cyst are rare lesions with absence of characteristic clinical findings which makes diagnosis difficult. We submit a review of our files from 1981 to 1999. Selection criteria excludes those cysts related to retroperitoneal organs (pancreas, kidney, adrenal) , inflammatory or infectious origin. With these criteria we have found nine cases (eight of them including clinical data).

Under an histological point of view we have classificated them attending to their cell lining, in mesothelial ( two cases), lymphangiomas ( four cases) and epithelial (three cases).

Of the nine patients six were male and three female, being a little more frequent in males when they are located in mesentery. They ranged in age from eleven to seventy one years old with the most common presenting symptoms being abdominal distension and/or chronic pain ( one patient with acute abdominal pain ). The cysts ranged in size from 3x2x1 cm to 14x12x8 cm with a mean of 8x6x2 cm.

There was only one recurrence due to a partial excision, without other complications or recurrences in the rest of the patients in whom complete excision were performed.

## P-503

CONTRIBUTION OF MOLECULAR METHODS IN THE DIAGNOSIS OF RHABDOMYOSARCOMAS IN CORRELATION WITH HISTOPATHOLOGICAL AND IMUNOHISTOCHEMICAL DIAGNOSIS

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**Aims:** The aim of the study was to identify specific genomic sequences and rearrangements in rhabdomyosarcomas (RMSs) using RT-PCR to improve the diagnostic possibilities and to detect the Minimal residual disease (MRD) in RMSs disseminated to the bone marrow and residual rhabdomyoblasts in tissues after the treatment, and to compare the molecular and histological diagnosis.

**Methods:** RT-PCR, Southern blot and hybridization on membranes were used to determine the presence of rhabdomyoblasts in tissues and to discriminate the alveolar subtype. We used mRNA for MyoD1 for separating the RMSs from other "small round cells" tumors. For the differential diagnosis between alveolar and embryonal forms we utilised the detection of t(2;13)(q35-37;q14) and t(1;13)(p36.1;q14), typically present in 95% of alveolar RMSs. The morphological diagnosis of RMS was supported by immunohistochemical (IHC) investigation of muscle specific proteins (actins, desmin, MyoD1).

**Results:** We amplified MyoD1 transcript in 11 of 13 RMS samples. Six samples revealed translocations t(2;13) or t(1;13) which established the molecular diagnosis of alveolar RMS. The results of molecular analysis corresponded well with the histology and IHC findings in a majority of cases. In one case the molecular diagnosis revealing the translocation didn't correspond with the morphological findings of embryonal RMS and though the morphology did not show features typical of the alveolar form we reclassified the tumor as alveolar on the molecular basis. MRD was identified using the molecular analysis in two cases.

**Conclusions:** We determined successfully the diagnosis of RMS with the RT-PCR method and we also identified alveolar variant of this tumor. Considering, that the two morphological forms of RMS have different behavior, prognosis, and treatment, a determination of an accurate diagnosis is crucial. The molecular approach in diagnosis of RMSs should therefore become an integral part of diagnostic investigations. An open question remains whether we may rely on the specificity of the alveolar translocation detected by the molecular approach and change the diagnosis of a typical case with embryonal morphology into alveolar for the clinical use. Establishing MRD seems indispensable because of its sensitivity superior to any other method.

The study was supported by a Grant IGA MZ CR NM6-3

## P-504

**FIBROHISTIOCYTES ARE STROMAL COMPONENTS IN MALIGNANT FIBROUS HISTIOCYTOMA**

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**Aims:** Immunohistochemical demonstration of the fibrohistiocytic phenotypic marker Factor XIIIa (FXIIIa) in the neoplastic component of malignant fibrous histiocytoma (MFH).

**Methods:** Automated digital image analysis of nuclear characteristics of FXIIIa-positive and FXIIIa-negative cells in 6 cases of pleomorphic MFH using immunofluorescence and hematoxylin nuclear staining combined.

Direct comparison of p53 nuclear staining and FXIIIa cytoplasmic staining by a double immunoenzymatic technique in eleven p53-positive and p21-negative cases of MFH.

**Results:** Significant differences ($p<0.05$) were calculated in mean nuclear area, min. feret, and nuclear form factor between FXIIIa-positive and FXIIIa-negative cells. Differences in nuclear perimeter were not significant between them.

The p53-positive tumor cells were invariably negative for FXIIIa. The FXIIIa-positive cells were consistently negative for p53.

**Conclusions:** The FXIIIa-negative and FXIIIa-positive components in pleomorphic MFH show significantly different nuclear parameters. These findings indicate that they represent two distinct cell populations. The cell population with larger and more irregular nuclei corresponds to FXIIIa-negative neoplastic cells.The p53-positive and p21-negative cells can be considered to belong to the neoplastic cell population in MFH. The neoplastic cells were invariably negative for FXIIIa. Consequently Factor XIIIa is a marker of stromal fibrohistiocytes and cannot be regarded as a differentiation marker in MFH.

## P-505

SYNDECAN-1 EXPRESSION IN DIFFERENT SOFT TISSUE TUMOURS

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**Aims** The purpose of this study was to test pyogenic granulomas, Kaposi's sarcomas, epithelioid sarcomas, synovial sarcomas, alveolar soft parts sarcomas, fibrosarcomas, protuberant dermatofibrosarcomas and fibromatoses with a monoclonal anti-syndecan-1 antibody to examine its reactivity.
**Methods** We selected benign and malignant soft tissue tumours which were previously categorized on the basis of light microscopic and immunohistochemical studies. Formalin fixed paraffin-embedded sections were used and high temperature antigen retrieval was done by a pressure cooker for syndecan-1 immunohistochemistry, and standard ABC technique was employed.
**Results** Anti-syndecan-1 antibody stains the cellular membrane of epithelioid sarcomas and epithelial elements of synovial sarcomas. Gastrointestinal stromal tumours, alveolar soft tissue sarcomas, three of five malignant epithelioid schwannomas and two of five fibromatosis showed intracytoplasmatic reaction while pyogenic granuloma, Kaposi's sarcoma, fibrosarcoma and dermatofibrosarcoma protuberans did not react with syndecan-1.
**Conclusions** It seems that the membrane positivity of syndecan-1 suggests true epithelial differentiation among soft tissue sarcomas. The intracytoplasmatic staining with syndecan-1 in soft tissue sarcomas suggest the potential of histogenetically wide range of tumours to gain epithelioid morphology. Therefore the heterogeneity in the immunohistochemically detected appearance of syndecan-1 in various soft tissue tumours is not simply due to the heterogeneity of the tumour types.

## P-506

**EBV LATENT MEMBRANE PROTEIN (LMP-1) ONCOGENIC MUTATIONS IN EBV-ASSOCIATED SMOOTH MUSCLE NEOPLASIAS.**

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**Aims:** Epstein-Barr virus (EBV)-associated smooth muscle neoplasias (EBVN) have been characterized in recent years. Although their incidence is low they are known to be constantly associated to immunosupression status Their prognosis remains uncertain. Little is known concerning the actual role of EBV in their pathogenesis. We have investigated the presence of oncogenic mutations in EBV latent membrane protein 1 (LMP-1) in EBVN in an effort to clarify this point.
**Methods:** In the last 5 years 3 EBVN occurred in our institutions. Two of them were serendipitous findings as solitary nodules in a grafted heart (a 48-y-old male) and as a splenic nodule in a seropositive 33-y-old HIV female. The third was a relapsing painful hypodermic nodule in a 7-y-old HIV-seropositive boy. In all the cases, we investigated using PCR EBV subtypes (EBNA-2) and the existence of oncogenic deletions in the C-terminal part of the LMP-1 gene.
**Results:** All the cases were initially confirmed to be EBER1 (EBV encoded RNA) positive by in situ hybridization. We found only subtype 1 EBV in all the cases. In two of them (cardiac, splenic), an specific 30 bp oncogenic deletion in the C-terminal part of the LMP1 gene, similar to that reported to be present in certain lymphomas such as Hodgkin's disease could be demonstrated.
**Conclusions:** An specific oncogenic LMP-1 gene deletion known to be present in certain lymphomas appears to be a frequent finding in EBVN. Although the series size is limited, these findings favor and active role for EBV in the genesis of these tumors. Further studies are clearly needed to understand details of the viral latency status in comparatione with other EBV-associated neoplasias.

## P-507

**CORRELATION BETWEEN OCCURRENCE AND DISTRIBUTION OF BROWN ADIPOSE TISSUE AND NUTRITIONAL STATUS IN ADULT HUMAN**

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**Aims:** The aim of our work was to investigate correlation between occurrence and distribution of brown adipose tissue (BAT) in adult human organism and its nutritional status.
**Methods:** Samples of adipose tissue from 107 consecutive autopsies from nine areas of the body were fixed in formalin, processed and embedded in paraffin. Sections were stained with hematoxylin-eosin. Nutritional status of individuals was determined by the Quetelet index. Cases were divided into three groups: hypotrophic, eutrophic and obese.
**Results:** The greatest amount of BAT was found in the group of eutrophic patients (74%), the smallest amount was remarked in the hypotrophic group (48%). The occurrence of BAT in obese patients (64%) was the same as the average value. As to distribution in obese patients was BAT located predominantly in periadrenal region, whereas in eutrophic and hypotrophic patients the predominance of the periadrenal region was less pronounced.
**Conclusions:** An interesting conclusion can be made based on our results, namely that the occurrence of BAT in obese patients does not significantly differ from average occurrence of BAT in our population, but the amount of BAT is the lowest from the three nutritional categories.

## P-508

**EXTRARENAL RHABDOID TUMOUR (ERRT); STUDY OF 3 CASES WITH A MESENCHYMAL, EPITHELIAL AND MIXED PHENOTYPE**

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ERRT are an heterogeneous group of rare neoplasms which have only some characteristics in common with renal rhabdoid tumour, not unanimously recognized as a distinct entity, with a variable biologic behaviour, and still to define in relation to histogenesis and/or cellular differentiation. In the literature are also reported: mesenchymal neoplasms with rhabdoid features, pseudo-rhabdoid sarcomas, epithelial tumours with rhabdoid characteristics, and carcinomas with a rhabdoid phenotype.
We collected three cases of ERRT: a 19 year old woman with a left epitrochlear mass (case 1); a 63 year old woman with a superficial (dermal) plaque of the left lower leg (case 2); a 57 year old man with a small ileal nodule and a subsequent mass in the right quadricipital skeletal muscle (case 3).
Several growth patterns were present: pseudoalveolar structures, nests and cords, solid nonstructured areas, dispersed cells in a myxoid matrix; a rhabdoid cytotype (i.e. large cytoplasmic globule) was prevalent and ranged from 50% to 90% of the cellular proliferation. Immunophenotipically all three cases were reactive for cytokeratins (C), vimentin (V) and E.M.A., and negative for many other differentiative markers. Ultrastructurally, all cases had cells with a globular-filamentous-structure (GFS), from abortive to extremely voluminous, pure (only intermediate filaments) or mixed (filaments intermixed with other cytoplasmic organelles).
Our observations indicate that ERRT can be considered as an autonomic neoplastic histotype if strict criteria are applied for its identification: presence of a pseudoalveolar growth pattern; prevalence of a rhabdoid cytotype; unique and constant immunophenotype (C, V and E.M.A.); presence of GFS in the majority of the proliferating cells.
In particular, case 1 could represent an ERRT with a mesenchymal (cytoskeletal) phenotype, case 2 an ERRT with an epithelial phenotype and case 3 an ERRT with a mixed epithelial-mesenchymal phenotype.

## P-509

A CASE OF HYALINIZING SPINDLE CELL TUMOR WITH GIANT ROSETTES: MORPHOLOGIC AND INMUNOHISTOCHEMISTRY STUDY.

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Aim: A case of hyalinizing spindle cell tumor with giant rosettes in the right lower extremity in a 20 years old woman. In this case the lesion showed classic morphology, as strong calcification of the collagen rosettes.

MATERIAL AND METHODS: The tumoral mass messured 30x40mm, with a smooth surface. The lesion was encapsulated by a fibrous tissue. The cut surface of the tumor had a greyish and white colour with a central area of necrosis. Focally cystic and myxoid areas were observed. A paraffin embeded blocks and E/E, PAS, trichrome and reticulin stains were employed. In addition immunohistochemical stains such as vimentin, keratin, AE1, AE3, EMA, Desmin, smooth muscle actin, sarcomeric actin, S-100 protein, CD34, bcl-2 and CD68 were employed.

RESULTS: Microscopically the tumor showed a spindle cell pattern with irregular hyaline changes between among neoplastic cells and a vast myxoid degeneration in central areas. The tumor cells were arranged forming collagen rosettes with central calcification and isolated calcifications. The tumoral cells were positive with a diffuse cytoplasmic pattern, for vimentin and the actin was also positive in cells inside of the capsule and collagen bands too. This image suggest a relationship between positive actin cells and the origin of these structures. The other immunohistochemical stains performed were negative, except for bcl-2 protein.

The basic aim is:

1- To compare the morphology and the immunohistochemistry of this peculiar case with other previous reported cases,

2- To compare this tumor with other uncommon fibroblastic soft tissue neoplasias, specially with the "Solitary fibrous tumor", "Myofibroblastoma" and other related entities.

3- Speculation about the origin of rosettes structures.

## P-510

IMMUNOPHENOTYPIC HETEROGENITY OF GASTRO-INTESTINAL STROMAL TUMORS

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**Aims**: The histogenesis of GISTs is still unclear. Recent studies postulated that GISTs might originate from the interstitial cells of Cajal (ICCs) because of their frequent coexpression of CD34 and c-kit. In this study we examine the immunophenotypic heterogeneity of GISTs.

**Methods**: Samples of 92 tumors diagnosed between 1983 to 1996 as GISTs by the established standards were available. Immunophenotyping was done using antibodies to SMA, S-100, NSE, chromogranin A, synaptophysin, PGP 9.5, CD34 and c-kit. The staining intensity was graded as negative, weak, intermediate and strong.

**Results**: 50% (46/92) of GISTs were found to be positive for SMA and 54% (50/92) expressed PGP 9.5. CD 34-positivity was seen in 76% (79/92) whereas c-kit-expression was found in only 47 % (43/92). One case only showed weak expression of chromogranin A. 1/92 cases showed no immunoreactivity at all, 6/92 were immunoreactive only for c-kit and CD 34, and 5/92 expressed CD 34 alone. Expression of SMA, S 100 and the neuroendocrine markers was faint to intermediate in the great majority of cases. 34 of 41 tumors with weak to intermediate positivity for SMA additionally reacted with at least one neuroendocrine marker. The 5 evaluated cases with strong SMA positivity and no c-kit expression were reclassified as leiomyomas. From two cases with strong S-100-positivity one had to be reclassified as Schwannoma.

**Conclusions**: GISTs represent a phenotypically heterogeneous group of tumors. They either show a pluridirectional pattern of differentiation with weak expression of SMA and different neuroendocrine markers or are less differentiated tumors positive only for CD 34 and/or c-kit. Detection of c-kit in only half of the cases challenges the hypothesis that all GISTs derive from ICCs.

## P-511

**MORPHOMETRICAL AND IMMUNOHISTOCHEMICAL COMPARATIVE STUDY OF EWINGS SARCOMA, PERIPHERAL NEUROECTODERMAL TUMOR AND NEUROBLASTOMA**

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The differential diagnosis of Ewing's sarcomas,peripheral neuroectodermal tumors (PNET) and neuroblastomas by light microscopic analysis may often be very difficult. Histochemical and immunohistochemical stainings could be very useful and the cytogenetic studies can make the differentiation possible.

**The aim** of this study is to find out if a morphometric method could be helpful in a differential diagnosis among these three entities.

**Material and methods**: A comparative study of the morphometric values of the nuclei of 20 Ewing's sarcomas, 12 neuroblastomas and 10 PNETs was made. The longer diameter of five hundred nuclei of each case, was measured by image analyzing system LUCIA-M, Nikon, in paraffin embedded hemalaun eosin stained samples.

Histochemical stainings with PAS and reticulin and immunohistochemical stainings with NSE, chrogranin, synaptophysin, neurofilament and desmin were made using avidin-biotin immunoperoxidase technique.

**Results and conclusion**: All of the Ewing's sarcomas , 10 of the neuroblastomas and 2 of the PNETs were PAS positive and reticulin negative or had only a few reticulin fibers surrounding large groups of cells.

Immunoreactivity with NSE showed 15 cases of Ewing's sarcoma, 8 cases of PNET and 11 cases of neuroblastoma. Immunireactivity with NF showed 11 cases of neuroblastoma and 4 cases of PNET. Ten cases of neuroblastoma were chromogranin positive and 7 cases of PNET were synaptophysin positive. Two cases of PNET had a weak reactivity with desmin stains in a very few cells. Only one case of neuroblastoma was negative for all stains.

The morphometric analysis showed that there was a significant statistical difference between the dimensions of the nuclei of Ewing's sarcomas and neuroblastomas and between the nuclei of PNET and neuroblastoma. There was no statistical difference between the nuclei of Ewing's sarcomas and PNET.

Morphometric analysis could be useful in differentiating neuroblastoma from Ewing's sarcoma and PNET, but we also recommend using of former mentioned immunostains adding HBA 71, and beta-2-microglobulin for establishing accurate diagnosis.

## P-512

NON HODGKIN'S LYMPHOMA IN SOLID ORGAN ALLOGRAFT RECIPIENTS

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**Aims**: the objective was to analyze non-Hodgkin's lymphomas among 1570 solid organ transplanted recipients in Juan Canalejo Hospital between 1980 to 1998.

**Methods**: seven patients, two cardiac, three renal and two hepatic transplanted patients presented non-Hodgkin's lymphoma. Parameters evaluated were: immunosupression therapy, time since transplant to diagnosis, histopathological features, immunohistochemistry and molecular events by PCR (heavy chain rearrangement and hypervariable amplification to know the exact origin of neoplastic cells, donor versus recipient), Epstein Barr (EBV) association and finally the clinical course.

**Results**: the great majority of lymphomas appeared in the first year postransplant, with extranodal disease. Diffuse large B cell lymphoma with extensive necrosis (lymphoproliferative monomorphus postransplant syndrome) was the most striking pathological pattern. Ig H rearrangement and EBV association were demonstrated. A single case presented a donor cell origin with the best clinical course of all cases.

**Conclusions**: the frequency of non-Hodgkin's lymphoma at our Hospital was 0,5%; a similar frequency in the rest of Europe has been reported. Non-Hodgkin's lymphoma was more frequent among cardiac recipients; a possible explanation is higher immunosuppression therapy; lymphomas are more frequent in the first year postransplant and with extranodal disease. Polymorphic lymphoproliferative syndrome was not present in our cases. Histopathological features were similar to diffuse large B cell lymphoma of REAL classification, associated to EBV, so in this way, identical to VIH lymphomas. The donor origin of lymphoma has prognostic value with better clinical course than the rest of lymphomas.

## P-513

**HUMAN HERPES VIRUS 8 (HHV-8) IS CONSTANTLY PRESENT IN POST-TRANSPLANT KAPOSI'S SARCOMA.**

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**Aims:** HHV-8 was originally isolated in Kaposi's Sarcoma (KS) and later, in rare and peculiar lymphoproliferative disorders. Recently, discussion has been raised about its pathogenic role in multiple myeloma. Although there is a strong association with sporadic and HIV-related KS less information is available concerning HHV-8 presence in post-transplantation KS (PT-KS), an issue possibly related with viral transmission from the grafts. Our aim has been to investigate the prevalence of HHV-8 infection in our own PT-KS series.

**Methods:** We collected 8 PT-KS from our institutions corresponding to patients in which bone marrow (n=1), liver (n=1) and renal transplantation's (n=6) were previously performed. DNA was extracted in all the cases from paraffin-embedded material from cutaneous (n=5), gingival (n=2) and lymph node (n=1) biopsies. A nested PCR assay designed for the HHV-8 ORF 26 was performed according to Cathomas G. et al (J Clin Pathol 1996, 49:631-3). In this particular assay a 233-bp band is routinely obtained. Appropriate samples were also used as controls.

**Results:** In all the cases, a positive result was obtained.

**Conclusions:** At least in our PT-KS series, we have been able to constantly demonstrate the presence of HHV-8 DNA by a PCR assay. Although limited, these results parallel to a large extent previous data obtained in KS arising in different clinical settings reinforcing a pathogenic role of HHV-8 in KS.

## P-514

**DEFINITION OF THE SEPSIS RISK IN TRANSPLANT PATIENTS BY POLYMORPHONUCLEAR LEUKOCYTE FUNCTION TESTS**

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**Aims:** Infections are a major complication in the postsurgical phase after organ transplantation. A method was worked out to define the infection risk and to forecast severe infections.

**Methods:** Starting after surgery, the following polymorphonuclear leukocyte (PMN) functions were measured twice a week in 41 patients: Blood levels of PMN elastase by the Merck 1.12589 kit. PMN migration by a whole-blood membrane filter assay (Egger et al. 1994, Inflammation 18: 427-441); the percental share of PMNs migrating from the blood into a filter upon FMLP stimulation was the significant parameter.

**Results:** 23 patients had an uneventful recovery, 11 suffered from non-septic infections, and 7 developed sepsis. Postsurgical values of elastase above 100 µg/L that were followed by a drop of migrating PMNs below 12% in their blood total predicted sepsis with a sensitivity of 85.7% up to 15 days before the clinical manifestation, and discriminated sepsis from an uneventful recovery with a specificity of 95.6%. Non-septic infections occupied a mid-position.

**Conclusions:** PMN functional tests are a tool to forecast severe infections in transplant patients and may offer the facility to prevent infections by starting the antimicrobial therapy in the preclinical, latent phase.

## P-515

MYOCITE AND LYMPHOCYTE APOPTOSIS VERSUS EBV GENOME IN ENDOMYOCARDIAL BIOPSIES FROM HEART TRANSPLANT RECIPIENTS SHOWING ACUTE REJECTION OR QUILTY EFFECT

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Aims: Does exist any correlation between EBV genome, cellular apoptosis(APP) and lymphocitic infiltration in endomyocardial biopsy (EMB) from Heart Transplant Recipients(HTR) ?.

Methods: 60 EMBs showing mild(n35) to moderate (n25) acute rejection (AR) and 30 EMBs showing Quilty effect (Qe)were selected from a series of 54 HTRs (45 men and 9 females). Multiple paraffin sections from each EMB were submitted to an In situ Hybridisation procedure using a DNA probe for EBV(Eber) and TUNEL for APP : EMBs showing AR were selected at the start of the AR episode before institution of adjuvant specific therapy, to avoid influence of lympholitic therapy on results.

Results: Focal to multifocal myocardial cell apoptosis was observed in 10 of 25 moderate rejections (40%) and never observed in mild rejection (0 of 35). Lymphocitic apoptosis was not observed in AR but was demonstrated focally in Qe (12 of 30:40%) independently of subtype (A or B). EBV-DNA positive lymphocytes were never observed in AR but could be shown in 10 of 30 Qe(33%).Only in 5 EMB with Qe ,APP and EBV-DNA were observed in same lesions(16% of Qe) affecting different lymphocytes .

Conclusions: Myocardial cell apoptosis is a good indicator of moderate AR in EMBs from HTR. EBV-DNA does not have any significant role in AR ,but can be present in Quilty effect according to previous studies. No correlation has been found between EBV-DNA and lymphocyte APP in Quilty effect. Persistence of EBV-DNA in lymphocitic lesions of the transplanted heart does not seem to bear any significant consequence to the graft.

## P-516

HISTOLOGIC APPEARANCE OF PERIPROSTHETIC TISSUE ON REVISON TOTAL HIP ARTHROPLASTY (THA.)

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We examined 47 THA revision patients who were treated between 1993—1997 in our Hospital. Clinically significant pain was found after arthroplasty and radiologically appearance of focal osteolysis can appear until after three or more years. Revision operations were performed on average 3-5 years after the arthroplasty and at the same time samples of periprosthetic tissue were taken. All the specimens were studied histologically. The age of patients ranged from 30 to 81 years; there were 25 men and 22 women. According to the predominating characteristic tissue or cell types we have divided these lesions into three groups: aggressive (gigant cell) granulomatous lesions 18 cases; acute inflammatory lesions 10 cases; incomplete (chronic) inflammatory repair 19 cases.

Histopathology of the granuloms showed collagen deposition, vascular granulation tissue and large number of histiocytes of various size and gigant cells, locally eosinophilic necrotic debris, containing microscopic particles of acrylic cement debris and/or metal debris. Metal appeared as fine, grey-black, weakly bire frigent granules in the cytoplasm of macrophages. Sometimes macrophages and foreign-body gigant cells are contain microscopic cement particles. There was also necrotic bone whith evedence of osteclastic bone resorption or flecks of newly formed bone. Histopathologically their appearances were all similar. The granulomatous lesions were sometimes already large and/or multifocal..

Acute inflammatory lesions histologically there was a neutrophilic infiltrate of various intensity between fibrous tissue. Its main characteristics are the exudation of fluid (edema) and the emigration of neutrophils, often associated with similar size focal abscesses and microorganisms. An abscess is as a focal accumulation of neurotrophils in a space created by liquefactive necrosis of the native cells of periprosthetic tissue. It may expand as a result of the progressive necrosis of surrounded cells (lytic lesions associated with solutio et phagocytosis after infection THA).

In incomplete chronic inflammatory repair at the same time healing by scarring and different vascular granulation tissue, aggregates of mononuclear cells admixed spindle cell (fibroblasts) proliferation and areas amorphous debris (cement and/or metallic debris, old blood, fibrin depletion, islands necrosis – usually surrounded by single foreigen body type gigant cells and histiocytes) were found. These chronic reactions of tissue take place as the attempt of macroorganism to disintegrate the unacceptable substans – peristens infection, nondegradable but potentially toxic(?) substances (cement, metallic or polyethylene).

**In summary**: histology of loosening lytic lesions associated with focal osteolysis, fracture around the prosthetic stem. Our findings indicate that lysis is caused by foreigen body reaction to particular methylmethacrylate. Small amounts of particular methylmetacrylate appear to stimulate endosteal erosio and localised osteolysis.

## P-517

**CHOLANGIOLITIS IN LIVER TRANSPLANTS IN PATIENTS PCR POSITIVE FOR CMV.**

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Aims: Cholestasis is very often the first sign of an eminent acute cellular rejection. However, the characteristic signs of infiltration by lymphocytes into the walls of the interlobular ducts and the vessel wall are lacking. These patients presented with temperature and raised ALT levels. A positive PCR reaction for CMV was recently detected in the serum. These liverbiopsies were examined for CMV infection.

Methods : we investigated 15 PCR CMV positive patients from whom 30 biopsies were taken. Light microscopy excluded acute cellular rejection.

Immunohistochemical examination of the liver samples with application of the antibody IEA-CMV raised against the CMV induced protein in the immediate early phase was carried out.

Results : the liver biopsies show merely aspecific alterations characterised by a slight infiltration of neutrophils and lymphocytes in the portal tracts without any affinity for the bile ducts or the vessels. Foci of neutrophils are present intralobularly in the livercell plates around the intercellular canaliculi in the periportal, midzonal and centrolobular areas. The immunohistochemical staining for CMV is negative.

Conclusion : a discrepancy is found between the angiocholangitis in the liver, the negative immunohistochemical staining for CMV and the PCR positive tests for CMV.

One should be aware of this diagnosis in conditions of a rejection-like clinical picture without proper histological signs of rejection. Under these circumstances, evaluation of the serological CMV is necessary since the attitude in this clinical setting is completely different.

## P-518

**Persistent centrilobular liver cell damage and venular fibrosis in chronic liver allograft rejection**

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**Aims:** Histopathologic analysis of chronic centrilobular liver allograft rejection and correlation with graft function and outcome.

**Methods:** We investigated follow up biopsies of 10 allografts from 8 patients with chronic rejection. A morphometric study of hepatic venules and an immunohistochemical analysis (CD31, CD34, α-actin and collagen type IV) of the centrilobular parenchyma and mesenchyma was performed comparing chronic rejection (10 grafts) with acute rejection (10 grafts) and normal liver tissue (5 cases).

**Results:** A marked fibrous thickening of hepatic venules was observed in 3 grafts of 2 patients with severe chronic rejection. Venular fibrosis was combined with centrilobular peliosis-like sinus dilatation and persistent centrilobular necrosis. In these cases, chronic rejection was leading to graft failure.

**Conclusions:** Acellular concentric fibrous thickening of hepatic venules is a rare observation in chronic rejection and has not been described in detail. This feature is associated with a poor prognosis. It has to be distinguished from centrilobular fibrosis due to outflow obstruction and venoocclusive disease after azathioprin-therapy.

## P-519

CHRONIC REJECTION IN LIVING RELATED LIVER TRANSPLANTATION

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**Aims:** The incidence, clinicopathologic features and outcome of chronic rejection (CR), which consists of ductopenic (DP) and occlusive arteriopathic (OA) rejection histopathologically, were studied in living related liver transplantation (LRLT).

**Methods:** Reviewed were liver allograft biopsies and explants at retransplantation or autopsy from 384 patients, mainly pediatric, who underwent orthotopic LRLT under Tacrolimus immunosuppression at Kyoto University Hospital between June 1990 and August 1998.

**Results:** CR developed in 10 patients (2.6%). In 4 of these with histology showing mainly OA features, CR started between 31 and 75 (median: 49) days posttransplant (dpt) and grafts failed between 81 and 157 (median: 132) dpt. In the remaining 6 with histology showing mainly DP features, CR started between 54 and 395 (median: 115) dpt and grafts failed between 89 and 455 (median: 269) dpt. The clinical course of the former patients was rapidly progressive. On the other hand, the clinical course of the latter was less aggressive, and, if CR was diagnosed early by biopsy, retransplantation was often successful.

**Conclusions:** The incidence of CR may be lower in LRLT than in cadaveric liver transplantation. CR mainly with OA features develops earlier after LRLT and shows a worse clinical course than CR mainly with DP features.

## P-520

DONOR DERIVED EPSTEIN-BARR VIRUS (EBV) ASSOCIATED POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISORDER (PTLD) IN A SECOND RENAL ALLOGRAFT

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Post-transplantation lymphoproliferative disorders are clinically, morphologically, and on the molecular level a heterogeneous group of lesions seriously complicating the outcome of transplantation. They usually originate from the recipient. We report on a case in which a 54-years old male with polycystic kidney disease receiving a second transplant from a female one year after transplantation developed a solid tumor in the renal graft.

Histology showed a polymorphic lymphoproliferative disorder with a wide spectrum of lymphoid cells including Reed-Sternberg like cells.

Immunohistochemically the majority of neoplastic cells were B-lymphocytes (CD20-positive) showing strong reaction for CD30. Latent EBV was found in the tumor cells by immunohistochemistry (LMP) and in situ hybridization (EBER). The patient had a significantly elevated EBV-antibody titre at graftectomy, but his status prior to transplantation was unknown. In situ hybridization with a Y-chromosome "specific" probe, furthermore, demonstrated the tumor cells to be of a female genotype.

Conclusively this is a case of EBV-associated PTLD derived from the donor presumably due to the heavy immunosuppression necessary to control rejection.

## P-521

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Andersen, C.B.*, Thomsen, O.F.*, Ladefoged, J.**
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Conclusively this is a case of EBV-associated PTLD derived from the donor presumably due to the heavy immunosuppression necessary to control rejection.

## P-522

**The Incidence of Prostatic Neoplasia in Dimbouri, Cameron.**

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**Introduction:** Prostate cancer appears to be more aggressive and also occurs at an earlier age in men of African descent living in the USA. Despite the apparent aggressive behavior of this disease in men of African descent, only few studies of the prevalence of prostatic carcinoma have been done in indigenous Africans. Herein we report the results of a clinical screening program for the prevalence of prostatic neoplasia in Dibombari, a rural district of Cameron, Central Africa.
**Methods:** Men 40 years or older were recruited in this study. Ten ml aliquot of blood was taken, a digital rectal examination (DRE) was performed. Individuals with an abnormal DRE and or elevated prostate specific antigen (PSA) were invited for prostate biopsy.
**Results:** Digital rectal examination was performed on a total of 141 men. 62/141 men had enlarged but benign appearing glands. In 35/141 the glands were hard, irregular and nodular. Of the 119 men with PSA results, 12 had PSA levels of >4 ng/ml. 47 men who had abnormal DRE or increased serum PSA levels were invited for biopsy. Only 24 of the 47 men participated in the study. The most common lesion was benign prostatic hyperplasia (8/24), followed by low-grade prostatic intra-epithelial neoplasia (6/24), cancer of prostate (5/24) and high-grade prostatic intra-epithelial neoplasia (2/24).
**Conclusions:** Our study shows that carcinoma of the prostate is not an uncommon disease in Bantu as previously thought. The reasons for the reported low incidence of prostatic carcinoma in sub-Saharan Africa are many, including, lack of any previous population based epidemiological studies and a dearth of access to organized health care in the area. Further studies are required to understand the epidemiology of prostatic neoplasia in the sub-Saharan Africa. These studies may help us comprehend the genetic basis for the aggressive behavior of prostatic carcinoma in men of African descent.

## P-523

**MAST CELLS ARE AUGMENTED IN PERIPROSTATIC VEIN THROMBOSIS AND DISPLAY A FIBRINOLYTIC PHENOTYPE**

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**Aims:** A number of recent data suggest that mast cells (MC) and their products are involved in the pathophysiology of thrombosis. In the present study, we have evaluated the number, distribution, and phenotype of MC in patients with unilateral periprostatic vein thrombosis (PVT) (n=7). The contralateral non-thrombotic venous plexus served as control (CO).
**Methods:** MC were examined by Giemsa staining and by immunohistochemistry using antibodies against tryptase, chymase, c-kit, tissue-type plasminogen activator (tPA), urokinase (uPA), urokinase receptor (uPAR), and plasminogen activator inhibitors (PAI-1, PAI-2).
**Results:** We found an increase in the number of tryptase-positive MC in PVT compared with CO (PVT: 14.5 $\pm$ 2.4 versus CO: 5.2 $\pm$ 0.7 MC/mm$^2$, $p<0.05$). The majority of these MC appeared to accumulate in the adventitia of the thrombosed veins. In both PVT and CO, MC reacted with mAb to c-kit, tryptase, and chymase. MC also stained positive for tPA and urokinase receptor, but did not express detectable uPA, PAI-1, or PAI-2.
**Conclusions:** Together, our results show that MC increase in number in PVT and express a profibrinolytic phenotype. We hypothesize that MC and mast cell-derived profibrinolytic molecules play a role in the pathophysiology of PVT.

## P-524

**TESTICULAR PAPILLARY SEROUS CARCINOMA OF OVARIAN TYPE**

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**Aims:** To report the 8th case in the literature and establish the differential diagnosis with papillary mesothelioma of the tunica vaginalis testis (PMT).
**Methods:** Immunohistochemical differential study of a serous papillary carcinoma of the testis (SPCT) using a panel of various antibodies characteristic of both SPCT and PMT.
**Results:** A 50 year old male with hydrocele. Tumour was an incidental finding during surgery. Orchidectomy was performed. And the patient treated with chemotherapy, is alive and well after 14 months.The SPCT had a typical appearance of branching papillae associated with numerous psammoma bodies. The tumour invaded the underlying tunica albuginea but did not involve the testis. No vascular invasion was seen. Immunohistochemically, the neoplastic epithelium was positive for CAM5.2, CEA, BerEP4, EMA, LeuM1, S100, cytokeratin 7 and OC125 but was negative for Vimentin, HBME-1, thrombomodulin, calretinin and N and E-cadherins. Its main location in the testiculoepididymal groove may reflect its origin from müllerian remnants (appendix testis). Female-type neoplasms of surface epithelium derivatives can occur in males. The majority of the epithelial tumours of ovarian in the testis are serous. The main clinicopathological interest of this lesion lies in its differential diagnosis with PMT, which exhibits a far more aggressive behaviour. In the 7 reported serous carcinomas, follow-up has been usually short, but in 2 cases recurred or metastasized, conversely, PMT invariably behaves aggressively. The differential features between SPCTand malignant PMT are: 1) older age of presentation in PMT (average of 54 years verus 31); 2) history of exposure to asbestos in PMT –absent here- ; 3) more frequent intitial symptom of hydrocele in PMT; 4) very rare parenchymal invasion in SPCT but frequent in PMT; 5) microscopically, presence of psammoma bodies and absence of solid patterns in SPCT versus biphasic patterns (epithelial and sarcomatous) in PMT; 6) immunohistochemically, frequent positivity of "specific" PMT markers such as Vimentin, HMBE1, thrombomodulin, calretinin and N-cadherin in PMT and positivity of "epithelial" markers such as BerEP4, EMA, LeuM1,S100, cytokeratin 7 and OC125 in SPCT.
**Conclusions.** Immunohistochemical phenotype together with typical morphology and location differentiates SPCT from PMT

## P-525

IMMUNOHISTOCHEMICAL CORRELES OF CHROMOGRANIN A, p53 NUCLEAR PROTEIN, bcl-2 EXPRESSION AND MIB1/KI67 PROLIFERATION INDICES IN LOW AND HIGH GLEASON HISTOLOGICAL SCORE OF PROSTATE CANCER

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**Aims:** Are Chromogranin A, p53 nuclear protein, bcl-2 apoptosis protein and MIB1/KI67 markers correlated to Gleason histological score (GHS) in localized prostate cancer (PCa) specimens of patients undergoing radical prostatectomy?

**Methods:** Chromogranin A, p53, bcl-2 and MIB1/KI67 were immunohistochemically detected (Avidin, Biotin Complex System) on formalin fixed and paraffin embedded archival sections of . 20 PCa (average age = 66.8 y): 10 cases with GHS<6 and 10 cases with GHS>7.

**Results:** Positive detection of Chromogranin A, p53, bcl-2 and MIB1/Ki67 were higher in GHS>7 (16.6%, 66.7%, 83.3% and 83,3%, respectively) than in GHS<6 (0%, 25%, 50% and 12.5%, respectively) PCa cases. Cytological examination of the markers (x1000) also showed a higher expression in GHS>7 than GHS<6 cases: p53 (35.9 out of 15.2%), bcl-2 (92.5 out of 36.4%) and MIB1/KI67 (52.3 out of 32.6%). Chromogranin A were found randomly expressed only in GHS>7 tumor cells.

**Conclusions:** The results show a very good correlation between the detection of p53 nuclear, bcl-2 apoptosis and MIB1/KI67 proliferating cell-cycle markers and the Gleason histological score. Therefore, the p53, bcl-2 and MIB1/KI67 immunohistochemical indices appear to be reliable predictive factors of disease progression in prostate carcinoma patients after radical prostatectomy.

## P-526

**HISTOLOGICAL PATTERNS IN TESTICULAR BIOPSIES FROM PATIENTS WITH TOTAL NON-OBSTRUCTIVE AZOOSPERMIA**

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AIMS: To establish the histological patterns on testicular biopsies obtained from azoospermic men in order to evaluate maturation of spermatids for fertilization techniques.

METHODS: Biopsies from 108 azoospermic men, 46 patients underwent bilateral biopsies and 62 bilateral..

Number of tubules, the degree of maturation when existent, maturation arrest or absence of germinal cells in the Sertoli-cell-only syndrome were studied. Percentage of tubules and average postmeiotic cells per tubule were also recorded in cases with tubules with complete maturation.

FSH serum levels were known in all cases.

RESULTS: The mean tubular count was 78 per tissue section in unilateral biopsies, 12% showed Sertoli-cell-only syndrome, 21% maturation arrest 2% tubular hyalinization and 65% complete maturation of spermatids.

In the bilateral biopsies the mean count was 91 tubules per section, Sertoli-cell-only syndrome was bilateral in 26% of patients and unilateral in 2%. Maturation arrest was bilateral in 15% of these patients and unilateral in 7%. Complete tubular hyalinization was diffuse in both testes in 9% of cases and in one testicle in 4% of cases. We found complete maturation in biopsies of both testicles in 33% of cases and in one in 4% of cases.

The mean spermatic count was 12 spermatids per tubule; 44% of cases with complete maturation showed spermatids in more than 75% of tubules.

CONCLUSIONS: We found spermatid maturation in 65% of cases with unilateral testicular biopsies and in 37% of cases with bilateral biopsies from azoospermic men randomly distributed. This fact makes these men susceptible to be included in fertility assisted methods such as spermatid microinjection.

## P-527

TRANSFORMING GROWTH FACTOR-β1 AND SMADS IN HUMAN KIDNEY NEOPLASIA

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**Aims**: To investigate the role of TGF-β1, TGF-βRI, TGF-βRII, SMAD-2 and SMAD-4 proteins in human kidney neoplasia.

**Methods**:The expression of TGF-β1, -RI, -RII, SMAD-2 and SMAD-4 was examined by immunohistochemistry in peritumoral normal and tumoral tissue of 53 paraffin-embedded primary kidney tumors: 1 tubulo-papillary adenoma, 2 oncocytomas, 32 clear cells carcinomas, 5 collecting duct tumors and 13 urothelial carcinoma of kidney pelvis.

**Results**: TGF-β1, -RI, -RII were more strongly expressed in neoplastic tissue than in normal peritumoral renal tissue (tubules and glomerules) adjacent to tumoral areas (96.22%, 79.25% and 75.41% vs 88.37%, 69.76% and 62.69%), whereas SMAD-2 and SMAD-4 were more strongly expressed in perineoplastic normal tissue than neoplastic tissue (23.25% and 30.23% vs 15.09% and 7.54%).Collecting duct carcinomas conteined higher TGF-β1, -RI and -RII levels than renal clear cell and pelvic urothelial carcinoma. TGF-β RII immunohistochemical scores (H-scores) were significantly lower in clear cell and pelvic urothelial carcinoma than collecting duct carcinoma($P<0.01$). Clear cell and collecting duct carcinoma lacked SMAD-4 expression, whereas all histological subtype poorly expressed SMAD-2. TGF-β1, -βRI, -βRII and SMAD-4 histological scores correlated neither with histological grade of malignancy nor with TNM clinical stage; SMAD-2 protein levels were significantly lower in grade 3 than in grade 1 tumours. In kidney neoplasia, TGF-βRI correlated significantly with -βRII, but not with TGF-β1; SMAD-2 with SMAD-4; and SMAD-2, but not SMAD-4 correlated with TGF-βRI and -βRII H-scores. In perineoplastic normal tissue SMAD-2 and SMAD-4 correlated with TGF-βRII, but not with TGF-βRI H-scores.

**Conclusions:** These findings suggest an altered TGF-β / TGF-β receptors/SMAD signaling patway in kidney neoplasia. The low TGF-βRII immunoreactivity and the loss of SMAD-2 and SMAD-4 expression we noted in renal cell carcinoma could inactivate the TGF-β1 response, thus allowing unconstrained cell proliferation and progression of renal neoplasia.

## P-528

**IMMUNOHISTOCHEMICAL EXPRESSION OF WAF1/P21 PROTEIN IN BLADDER CANCER. PROGNOSTIC SIGNIFICANCE AND RELATION TO CLINICOPATHOLOGICAL PARAMETERS.**

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**Aims:** To investigate the prognostic value and interrelations between p21 expression and various parameters in bladder cancer.

**Methods:** Paraffin sections from 130 patients with transitional cell carcinomas (TCCs) [mean follow-up 36 months] were stained immunohistochemically with antibodies to p21 (4D10), p53 (DO1), PCNA (PC10) and Ki 67 (MIB 1).

**Results:** P21 positivity was displayed in 61% of cases and in one third of them was accompanied by p53 accumulation. P21 expression was statistically related to advanced T category. No association was shown between p21 and p53 or proliferation rate. The combined phenotype p21(+)/p53(-), representing the p53 -dependent pathway, predominated among superficial, slowly proliferating tumours.P21 labelling index emerged by multivariate analysis as the single independent indicator of shortened survival in superficial tumors. Moreover, p21 positivity constituted an adverse prognostic discriminator in the subgroup of p53-negative tumours, as pointed out by post-relapse survival analysis. The combined p21/p53 phenotype was also seen to be an independent prognostic factor, by univariate and multivariate analysis.

**Conclusions:** Our results indicate that p21 activation occurs early in tumourigenesis, appears associated with invasiveness and is capable of cell cycle control in TCCs mostly through p53-dependent pathways. Finally, p21 expression may be a major player in determining clinical outcome in bladder cancer alone or in combination with p53, irrespective of other clinicopathological parameters.

## P-529

**SCLEROSING ADENOSIS OF PROSTATE.**

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**Aims:** Description of clinicopathological and immunohistochemical features of sclerosing adenosis (SA) of prostate.

**Methods:** Revision of 11 cases with diagnosis of SA from the files of F. Puigvert between 1993-98. Immunohistochemical antibodies used were CK-903, muscle-specific actin (MSA), S-100 protein and PSA.

**Results:** 8 cases were obtained by transurethral resection and 3 by prostatic adenomectomy. Patients mean age was 74 years. Symptoms were obstructive (O) in 7, irritative (I) in 1 and O+I in 3. Mean follow-up were 16.6 months (5-30 m). Presurgery serum PSA was available in 7 patients and levels ranged from 2.3 to 23.9 ng/mL. None of the patients had PIN; prostatic carcinoma coexisted only in one (PSA pre 3.4). After surgery, all but one (PSA pre/after 8.9/6.6) patients had normal values. 7 cases had one single focus of SA, ranging from 2 to 7 mm; the rest had 2 to 5 foci (range 2-12 mm). In 3 cases the glandular component clearly overgrowth the stromal component. The former, had a predominantly microacinar pattern in 7 cases, being the rest tubular and/or cordonal. Six cases (54%) showed mild atipia and in 2 (18%) was moderate. Nucleoli were small in 4 (36%) and larger in 2 (18%). One case presented mild atipia and large nucleoli; eosinophilic crystalloids were seen in 3 (27%); mucin was seen in 2 (18%). Immunohistochemistry revealed presence of basal cells CK-903 positive, and/or myoepithelial differentiation (MSA, S-100 protein, positive in basal cells and some stromal cells) in all the cases. PSA was positive within cuboidal cells of the glands.

**Conclusions:** Recognition of SA is important to avoid confusion with adenocarcinoma. Presence of basal cells and/or myoepithelial differentiation excludes invasive carcinoma. There appears to be no direct relationship between SA and prostatic carcinoma.

## P-530

**TOXOPLASMOSIS OF THE BLADDER: CASE REPORT OF THE A EXCEPTIONAL LOCATION IN AN ACQUIRED IMMUNODEFICIENCY SYNDROME PATIENT**

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**Aims:** In the urogenital tract, toxoplasm is an exceptional finding, although they have been observed in the testicular and prostate parenchyma. By contrast, bladder involvement is rare, with only five cases reported.

**Methods:** An autopsy, only including thorax and abdomen, was carried out. The samples were processed in a routine way and they were included in paraffin. The histologic sections were stained with H-E, PSA and giemsa and they were immunolabeled with antibodies anti-toxoplasma.

**Results:** Gross examination revealed a bladder with diffuse wall thickening. The vesical mucosa contained purplish-blue plaques. Histologic examination revealed vesical toxoplasmosis, No other organs were affected. The autopsy did not include CNS. Immunolabeling with antitoxoplasmic antibodies confirmed the presence of trophozoites of toxoplasma gondii that they had developed in a cyst and pseudo cysts.

**Conclusions:** Toxoplasma gondii is a intracellular parasite which is responsible for an asymptomatic or minimally symptomatic lymphadenopathy syndrome. Toxoplasmosis commonly affects the central nervous system. Genitourinary involvement is exceptional. In AIDS, vesical involvement has been the subject of only rare histologic descriptions, and could be asymptomatic or masked by the presence of neurologic, respiratory, or gastrointestinal symptoms. Because of it, vesical lesions are certainly underestimated.

## P-531

**EXPRESSION OF p53, p21 AND bcl-2 IN CARCINOMA, PIN AND NORMAL PROSTATIC TISSUES**

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**Aims**: To study the expression of p53, p21 and bcl-2 in normal, premalignant and malignant prostate tissue.

**Methods:** 24 carcinomas, 15 high-grade PIN and 23 normal tissue cases were inmunohistochemically stained for p53, p21 and bcl-2. Cases were graded as positive or negative depending on the number of nuclei stained (>20 % for p53, >10 % for p21) and the existence of cytoplasmic bcl-2.

**Results:** Results are summarized in the following table:

| | Total | p53 + (%) | p21 + (%) | bcl-2 +(%) |
|---|---|---|---|---|
| Normal | 23 | 2 (9 %) | 2 (9 %) | * |
| PIN | 15 | 2 (13 %) | 3 (20 %) | 9 (60 %) |
| Carcinoma | 24 | 11 (46 %) | 8 (33 %) | 4 (17 %) |

* bcl-2 was only positive in atrophic glands and basal cells

p53 and p21 were more frequently positive in carcinoma than in PIN and normal tissue, whereas bcl-2 positivity was frequently observed in PIN and atrophic glands. p53 and p21 were coincidentally positive in 4 tumors (17%).

**Conclusions:** We conclude that expression of p21 can be independent of that of p53 in prostate carcinoma and they are more frequent in the invasive stage of prostatic neoplasia. Contrarily, bcl-2 expression is more frequently seen in preinvasive lesions and could have a role in the early stage of neoplastic transformation.

## P-532

LYMPHOEPITHELIOMA OF BLADDER ARISING IN A DIVERTICULUM.

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Lymphoepithelioma, a term used to designated a typical nasopharyngeal neoplasm, have also been reported in other locations (lung, stomach, salivary glands, cervix, bladder...), although in these organs the relationship of EBV has not been established.

**Case report:** A 77-year-old man presented with gross hematuria in May 1998. Cystoscopy showed a 4x3 cm solid sessile tumor arising in a large diverticulum (5x4 cm). The remainder of the bladder mucosa appeared normal. The tumor was resected by partial cystectomy, and pathological evaluation revealed invasive lymphoepithelioma, with two positive pelvic nodes. The patient did not receive any chemotherapy. Our case has insufficient follow up (8.5 months) to be able to evaluate the prognostic significance. However, a preliminary assesment showed no evidence of residual disease.

**Histological findings:** Neoplasm was characterized by sheets of undifferentiated malignant cells arranged in syncitia, with round vesicular nuclei, frequent mitoses and large nucleoli, intermingled with a prominent lymphoid reaction. Tumor cells were strongly positive for cytokeratin (AE1-AE3) but negative for leucocyte common antigen. The adjacent mucosa showed a dense chronic inflammation with no evidence of urothelial dysplasia. No signs of transitional neoplasm were found in any sections.

**Discussion:** Lymphoepithelioma of the bladder is a rare histological type in its pure form, yet more arising in a vesical diverticulum. Two importants reasons torecognize this variant are: tomake the distinctionfrom lymphoma, and think about the apparent favorable outcome, based on the reported sensitivity to chemotherapy and, therefore, the possibility of salvaging bladder function.

## P-533

INTERSTITIAL CYSTITIS: NEW CONSIDERATIONS

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**Introduction:** Interstitial cystitis was described by Hunner in 1915 as a "rare type of bladder ulcer". Almost a century later, it continues to be an infrequent entity of unknown origin, with predominance of female patients (9:1), diagnosis requiring the absence of urinary pathogens, although its association to collagen disorders[i] and to bladder cancer[ii] point towards some type of altered immunity in these patients or to a auto-immune origin of the disease.

**Cases:** 4 patients (2 male, 2 female) presenting chronic irritative bladder symptoms resistant to standard treatments and with repeated negative urine cultures. One male patient had been treated on 7 occasions for superficial transitional cell carcinoma of the bladder and had received intravesical BCG treatment. There was no concomitant pathology. After 2 years free of disease he presented severe cystitis with reduced bladder capacity that needed cysto-prostatectomy. Histology: Chronic ulcerative and interstitial cystitis with marked parietal fibrosis; no neoplastic lesions. The second male patient showed the typical symptoms of the disease; history showed intolerance to acetyl-salicylic acid and benign prostatic hyperplasia (BPH). One female patient suffered steroid-treated rheumatoid arthritis, the second patient had penicillin allergy. In these 3 cases, histology was non ulcerative interstitial cystitis.

**Conclusions:** 1) Histology in our 4 cases confirms the existence of 2 types of interstitial cystitis (ulcerative and non ulcerative) as observed by other authors[iii] but may also represent 2 stages in the evolution of the process. 2) There may be a higher incidence for males than previously thought. This higher incidence may be masked by lower urinary symptoms related to BPH. 3) The association to tumours, BCG treatment, medicamentous allergy and auto-immune disease supports the hypothesis of a immune disorder in the origin of the interstitial cystitis.

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[i] Mana F, et al.: The association of bronquiolitis obliterans organizing pneumonia, systemic lupus erytematosus, and Hunner's cystitis. Chest. 1993; 104 (2):642-4.
[ii] Hamm M, et al.: Chronic interstitial cystitis (Hunner) associated verrucous carcinoma of the urinary bladder. Urologe A. 1997; 36(1):50-3.
[iii] Koziol JA, et al.: Discrimination between the ulcerous and the nonulcerous forms of interstitial cystitis by non-invasive findings. J Urol. 1996; 155(1):87-90.

## P-534

**SYNCHRONOUS OCCURENCE OF URINARY BLADDER CARSINOSARCOMA AND TESTICULAR SEMINOMA**

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Carcinosarcoma is a rare malignant neoplasm of urinary bladder. Coexistence of this tumor with a primary tumor of another organ is a rare phenomenon. We report a case of carcinosarcoma with simultaneous testicular seminoma. To best of our knowledge, there is only one case reported in the literature with the same tumor combination.

Sixty-year-old male presented with painless gross hematuria and a left testicular mass of 5 years duration. A polypoid mass measuring 5x6 cm was detected in the left lateral vesical wall. After transurethral resection patient was treated by left inguinal orchiectomy, radical cystoprostatectomy and pelvic lymphadenectomy.

Both biopsy and surgical resection of bladder revealed a tumor deeply infiltrating muscular tissue with histologic features of transitional, squamous and small cell carcinoma and spindle cell sarcoma. Epithelial component was present as small discrete foci within the sarcomatous areas. There was no transition between two components. Areas of carcinoma in situ and papillary transitional cell carcinoma were not detected in the surrounding mucosa.

Immunohistochemistry showed positivity with epithelial membrane antigen and cytokeratin for the carcinomatous component. Sarcomatous component revealed immunoreactivity only with vimentin. Epithelial markers, desmin, S-100, neuron specific enolase and chromogranin were negative.

Orchiectomy specimen showed a classical seminoma with vascular and tunica albuginea invasion. Lymph nodes did not show metastasis.

At 12 months follow up. patient is free of disease.

Subsequent occurence of bladder malignancy as a sequela of radiotheraphy given for testis tumor has previously been reported. However, in our case both tumors were diagnosed at the same time and there was no history of previous radiotheraphy for any reason.

With this report we aimed to add another case to the relevant literature on this rare matter.

## P-535

Prostatic Adenocarcinoma: Prognostic value of Apoptotic, Proliferative and Hormonal Profile, in Prostatic Needle Biopsies.

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The aim of our study was to investigate immunohistochemically the prognostic significance of the above parameter using Ki-67, P-53, Cerb-2, and Bcl-2 antibodies and Estrogen and Progesteron Receptors expression in a series of 30 patients with prostatic Ca. with correlation with additional pathologic parameters ( percentage of involvement of each prostatic tissue core and the number of tissues cores involved by carcinoma) and PSA serum titers.

Fifteen biopsies had a score (Gleason System) of less than 5, and 15 biopsies showed a score of more than 5.

Immunohistochemistry studies were performed.

The primary antibodies used were - Ki-67, P-53, C-erbB-2, Bcl-2, Estrogen and Progesteron receptors.

The P-53 and Ki-67 were higher in the group with the Gleason score less than 5. Bcl-2 and Estrogen receptors were higher in the group with the Gleason score more than 5.

C-erbB-2 was negative in all of our cases.

There was a positive correlation between serum PSA levels and BCL-2 expression in our study group.

Our study appears to indicate that tumors which significantly overexpress BCL-2 and Estrogen receptors were of a moderately or poorly differentiated type, while well differentiated tumor tended to overexpress P-53 and Ki-67.

## P-536

**THE TRANSITIONAL CELL CARCINOMAS OF THE BLADDER RELATED TO EXPOSURE OF PESTICIDES ARE LESS AGRESIVE.**

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**Aims:** The transitional cell carcinoma (TCC) includes two basic groups: superficial and muscle-invasive tumors, each one with its own natural history and, probably as well, with different risk factors implicated in the origin. TCC are more frequent in industrial countries. Nevertheless, TCC has a great prevalence in our area (Málaga, south of Spain) where tourism, service sector and agriculture are the main sources of income.

We have carried out an epidemiological study with the following objectives: 1) Analysis to see if people exposed to pesticides (PE) have an increase risk of developing TCC in comparison to non-exposed (PNE). 2) Histopathological comparison (grade and microstage) of TCC between PE and PNE groups.

**Methods:** We made a case-control study, including 96 cases of TCC and a control group of 96 cases from the hospital patients. Histopathologically, TCC was divided into low and high grade, and the U.I.C.C. scheme was used for microstage. The data was statistically analyzed by logistic regression method.

**Results:** The PE population showed an increased risk for TCC, as much with low grade as the high grade, although more frequently for the low grade TCC (OR=2.6, IC=1.3-5.2). In relation to the stage, PE cases presented a major risk for superficial TCC (OR=2.3, IC=1.2-4.4). The density of cellular inflammation associated to TCC was different in PE and PNE cases, higher in the former (OR=4.5, IC=1.8-11.1).

**Conclusions:** The subjects exposed to pesticides have a greater risk of developing TCC of the bladder, with higher frequency of low grade, lower level of wall invasion and more cellular inflammation than the TCC in unexposed patients.

## P-537

METANEPHRIC ADENOMA OF THE KIDNEY
Case Report with Immunohistochemical Analysis

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We describe an additional case of an uncommon tubular-acinar and papillary neoplasm of the kidney.
A right renal tumor was identified by abdominal computerized tomography in a 49 years-old Caucasian female with flank pain .
Macroscopic examination of the resected kidney revealed a well-circumscribed, gray-yellow, firm tumor, 3,5 cm in diameter, located in the lower pole.
Microscopically, the tumor exhibited a predominant tubular-acinar pattern with very scanty stroma. Occasional branching elongated tubules, papillary and glomeruloid formations were present but blastema was absent. Tumor cells were uniformly small and bland in appearance, showing immunoreactivity for Vimentin, Leu 7 and Cytokeratin 18.
Clinical follow up (74 months) has revealed no evidence of recurrence.
This tumor appears to be benign with no malignant potential, best classified as metanephric adenoma, because of its embryonic architectural, cytological appearance and immunohistochemical profile. That can be recognized by its very characteristic pathological features, and its main importance is related to differentiate it from epithelial Wilm's tumor or renal cell carcinoma.

## P-538

IMMUNOHISTOCHEMICAL STUDY OF DNA TOPOISOMERASE II-A AND KI-67 ANTIGEN IN TRANSITIONAL CELL BLADDER CARCINOMA.

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Topoisomerase II-α controls the topology of DNA by cleaving one DNA strand, passing the other DNA strand through the transient gap and rejoining the two strands. Expression of this enzyme has been demonstrated to increase rapidly at the end of the S to G2/M phase and decrease after the completion of mitosis.
Aims: To investigate the immunohistochemical expression of topo II-α in relation to Ki-67 expression; to correlate them with clinicopathological data.
Methods: Formalin fixed paraffin embedded tissue from 55 cases of bladder carcinomas, that were retrieved from our files between Oct 1996 to Febr 1998, was stained by immunohistochemistry for topo II-α and Ki-67.
Results: Topo II-α nuclear staining was observed in almost all the cases of bladder carcinomas. The mean value of labeling index for topo II-α in grade I+II was 16,46% and that of grade III was 49,28% (P<0,0002). The mean value of labeling index for topo II-α in superficial tumous was 17,43% and that of invasive was 42,14% (P<0,01). The mean value of Ki67 (52,14%) in grade III was higher in relation to the mean value of Ki67 (10,76%) in grade I+II (P<0,00001). The mean value of Ki67 (51%) in invasive tumours was higher in relation to the mean value of Ki67 (13,29%) in the superficial tumours (P<0,00001). We found that the expression of the enzyme correlates well with Ki67, a known proliferation marker, with a correlation coefficient of 0,365 (P<0,05)
Conclusions: Higher levels of topoII-α expression were strongly related to higher tumour grade and stage. We suggest that those tumours with high topo II-α indices would most likely be the ones responsive to chemotherapeutic protocols involving topo II-α targeted drugs, and those tumours with low topo II indices would be relatively resistant. Given the limited numbers of patients and the short duration of follow-up we could not show whether topo II-α expression has a prognostic value in bladder carcinoma.

## P-539

EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE IN PROSTATE ADENOCARCINOMA

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**Aims**: The aim of this study was to evaluate the imuno-expression of Inducible Nitric Oxide Synthase (iNOS) in prostate adenocarcinoma, in order to understand if it can help in the diagnosis and prognosis

**Methods**: Sextant prostate biopsies from 50 cases of prostate adeno-carcinoma and prostatic nodules of 25 cases of benign hyperplasia were studied. In addition to HE stain, immunoexpression of iNOS was done in paraffin embedded sections and evaluated in non-neoplastic epithelium; high grade PIN1, adenocarcinoma and also in the the stromal tissue

**Results**: Biopsies with carcinoma: Immuno-reactivity for iNOS was observed in all prostates with adenocarcinoma. The positivity was usually strong in the cytoplasm of malignant cells and basal cells. Immuno-reactivity was also found in the cytoplasm of a small number (25% of cases) of non-malignant glandular cells, although much lighter than in the tumour. Positive cells were yet seen in the cytoplasm of spindle cells and macrophages of the stroma.
Prostate nodules without carcinoma: No clear immuno-reactivity was found in the glandular, basal or stroma cells of prostate with benign hyperplasia. Only a very light "back-ground like" reactivity was detected in a small number of cases in which doubts persist about if it was real or artefact.

**Conclusions:** Prostate tissue with adenocarcinoma has a high iNOS content, which doesn't occur in benign tissue. So, iNOS expression can help in the diagnosis of adenocarcinoma, namely in the cases in which differentiation from small acinar benign aggregates is difficult. The biological significance of this event must be studied better

## P-540

**P-105 ANTIGEN EXPRESSION AND DNA CONTENT BY MEANS OF FLOW CYTOMETRY IN RENAL CELL CARCINOMAS.**

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**Aims**: Prediction of Renal Cell Carcinoma (RCC) is difficult besides stage and histopathological parameters.The utility of DNA ploidy and S-phase fraction determined by flow cytometry have been reported with contradictory results. The aim of our study was determine the relationship of DNA ploidy and S-phase fraction and the expression of the proliferative nuclear antigen p-105 by of flow cytometry in RCC.
**Methods**:106 paraffin embedded samples from patients with RCC were used. Nuclear suspensions were prepared using a modified Hedley's method. Nuclear sediment was resuspended with an antimouse p-105 monoclonal antibody and a goat antimouse immunoglobulin IgM-FITC. To study cell cycle Propidium iodide was used as fluorochrome. Level of fluoresecence was determined with an EPICS Profile II cytometer (Coulter). DNA cell cycle histograms were analyzed with Multicycle software (Phoenix).For statistical analysis were used the Chi-square test and the Kaplan-Meier method to calculate survival differences.
**Results:**Ploidy correlated with stage (p=0.001), nuclear grade (p=0,003) and tumoral size (p<0.0001) but did not correlate with survival . Proliferative index and S-phase correlated with tumoral size (p=0.0001) and survival (p=0.002). The expression of the nuclear antigen p-105 had a median value of 69,61% but didn't correlate neither with classic prognostic factors nor with survival.
**Conclusions**: S-phase fractions seems to be good a prognostic factor for RCC but not ploidy nor p-105 expression.

## P-541

**Poorly differentiated bladder carcinoma with choriocarcinomatous features in a patient with renal cell carcinoma**

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**Aims:** A poorly differentiated urothelial carcinoma of the bladder, with choriocarcinomatous features, co-existing with a renal cell carcinoma in a 63-year old man is reported and the possible histogenesis discussed.

**Methods:** The patient presented with haematuria, elevated serum PSA (17 µg/ml), and atypical cells in the urine. A 3 cm atypical, easily bleeding bladder tumour was found and resected transurethrally. A 6 cm renal tumour was also detected and nephrectomy performed. Biopsies of the prostate were benign. Other organs, including the testes, appeared clinically and radiologically normal.

**Results:**. The bladder tumour was composed of pleomorphic malignant cells, growing mainly in a solid manner with focal necrosis and haemorrhage. In resected material depth of invasion could not be assessed. Some more uniform cells expressed cytokeratin 20. Scattered multinucleated giant cells showed intense reactivity for ßHCG. The renal tumour was a renal cell carcinoma, nuclear grade 2, showing no choriocarcinomatous elements.

**Conclusion:** The findings suggest choriocarcinomatous metaplasia in urothelial carcinoma. Evolution of choriocarcinoma from urothelial carcinoma has been described with rapid transformation into aggressive HCG-secreting tumour and progressive metastatic disease. The concept of "choriocarcinoma mimicry " suggests the origin from a somatic cell during the process of malignant transformation of the uroepithelium. According to the "germ cell rest" theory extragonadal choriocarcinoma may develop from germinal cell remnants in the urogenital ridge. In our patient no testicular neoplasm or teratomatous component of the bladder tumour was detected. Primary choriocarcinoma of the bladder has been reported and a germ cell rest origin of bladder tumour in our patient should also be considered in view of co-existing other abnormalities such as renal carcinoma.

## P-542

**INTRATUBULAR GERM CELL NEOPLASIA IN MALIGNANT TUMORS OF TESTIS**

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**Aims:** To study the histopathological and immunohistochemical features of the intratubular germ cell neoplasia (ITGCN) in the testicular tissue adjacent to malignant testicular tumors.

**Methods:** Out of 62 germ cell tumors, in 57 the adjacent testicular tissue was present and studied for ITGCN. The ITGCN was subclassified as (1) unclassified, (2) intratubular seminoma and (3) intratubular embryonal carcinoma. PAS (Periodic-acid Schiff), PLAP (Placental-like Alkaline Phosphatase) and NSE (Neuron Specific Enolase) stains were used on the tumor and on the adjacent testicular tissue. The subtypes of the ITGCN were correlated to the tumor by means of the staining results. The stains were graded as 1 when 5-50%, as 2 when > 50% of cells were positive. The thickness of the basement membrane of the tubuli was studied and graded as 1 when < 0.3mm and 2 > 0.3mm.

**Results:** The patients were divided into a group of adults (14 to 60 years) and children (1 to 13 years). In the first group ITGCN was found in 51 (96.2%) out of 53 patients. The PAS stain was positive in 98% of ITGCN and in 100% of the tumor tissue; the PLAP stain was positive in 80.4% of ITGCN and in 88.2% of the tumor tissue; and NSE stain was positive in 47.1% of ITGCN and in 86.3% of the tumor tissue. In all cases of ITGCN the basement membrane was thickened (grade 1 in 47% and grade 2 in 53%).

**Conclusions:** The ITGCN is a common feature in the adjacent testicular tissue and there was a high correlation in the PAS and PLAP stains between the main tumor and the ITGCN; the NSE stain was positive in only about a half of cases of ITGCN as compared to the main tumor. The findings of ITGCN are important for diagnostic purposes in nontumorous cases when biopsies are performed in young patients for infertility and other causes of testicular atrophy.

## P-543

**THE EVALUATION OF THE NUCLEOLAR ORGANIZER REGION (AGNOR), Ki-67 AND PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) IN PRIMARY SQUAMOUS CELL BLADDER CARCINOMA**

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**Aim:** The purpose of this study was to determine comparatively the expression of Ki-67, PCNA and the number of NOR's in primary bladder squamous cell carcinomas and to investigate how they correlate with the tumor grade, stage and local recurrence.

**Methods:** Twenty tumors from 15 men and 5 women with a mean age of 57.2 ± 19.9 (Range 20-90) years were examined. Tissue sections from these patients were stained with Ki-67, PCNA and AgNOR. Ki-67 and PCNA stained slides were assessed quantitatively .2000 cells were counted in the most stained areas in a blinded fashion using a 1500 magnification. For AgNOR 200 nuclei were assessed under 1500 magnification and the mean number of AgNORs per nucleus was calculated for each specimen.

**Results:** A highly significant correlation between grade and clinical stage was observed ($x^2$=18.3 $p<0.001$). The average of Ki-67 PCNA and AgNOR index of all cases were 57.7 ± 19, 67.2 ± 18.6 and 9.45 ± 4. The statistical studies showed that proliferation indices of tumors are significantly related with grade ($p<0.001$) and stage ($p<0.001$). The higher the stage and grade the higher the proliferation index was established in all cases. Tumors with local recurrence had shown high proliferation indices with three cell biological parameters than tumors without local recurrence ($p<0.001$).

**Conclusion:** These findings suggested proliferation indices measured by Ki-67, PCNA and AgNOR in primary bladder squamous cell carcinoma are correlated to the grade, stage and local recurrence. It was also shown that a good linear correlation exits between PCNA, Ki-67 and AgNOR count. In conclusion Ki-67 and PCNA and AgNOR gives the most accurate cell kinetic information for more immediate application as diagnostic and prognostic tools and may also have an impact on the treatment of patients with squamous bladder carcinomas.

## P-544

**RELATİON BETWEEN APOPTOSİS REGULATOR PROTEİNS (BCL 2 AND P53) AND GLEASON SCORE İN ADENOCARCİNOMA OF THE PROSTATE**

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**Purpose:** Cellular proliferation and programmed cell death (apoptosis) are associated with tumor growth in general, and prostate cancer growth in particular.

The aim of this study was to examine and regulate the expression of the apoptosis regulating genes bcl 2 and p53 and Gleason score in adenocarcinoma of the prostate.

**Methods:** Using immunohistochemistry, we studied bcl 2 and p53 expression in 12 case of low grade (Gleason score 2-5), 12 case of intermediate grade (Gleason score 6-7) and 8 case of high grade (Gleason score 8-10) adenocarcinoma of the prostate. In this study we evaluated only core needle biopsy specimens of prostate cancer.

**Results:** Overexpression of bcl 2 was noted in 3 of 32 patients (9.32 %). One of them was high grade; Others were intermediate. There was no staining with bcl 2 in low grade group. Expression of p53 was noted in 3 of patients (9.32 %). One of them was low grade; others were high grade. There was no staining with p53 in intermediate grade. Statistically, there was no relation between elevated levels of apoptosis regulator proteins (p53 and bcl 2) and Gleason score.

**Conclusion:** The present data suggest that there is no significant relation between p53 and bcl 2 expressions and Gleason score in adenocarcinoma of the prostate.

## P-545

HUMAN CRIPTORCHIDISM: A NATURAL MODEL FOR THE STUDY OF ANDROGEN RECEPTOR AND TESTOSTERONE PARACRINE REGULATION IN THE TESTIS

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**Aims.** We have examined the androgen receptor (AR) immunoexpression in the cryptorchid human testis, an ideal model to investigate possible paracrine interactions occuring during spermatogenesis. In the same sample, distinct histotypic phenotypes such as tubules expressing hypospermatogenesis (HYPO), tubules containing only Sertoli cells exhibiting a pubertal morphology (SCO), and hypoplastic tubules containing immature and dysgenic Sertoli cells (IMMA) may all be detected.

**Methods.** Biotin-streptavidin immunoperoxidase was employed to resolve the distribution of AR and testosterone (T) in 32 cryptorchid testes from postpubertal patients ranging in ages between 17 to 29 years. All testes were localised either within the abdomen proper or in the inguinal canal. Rabbit antisera to T (Biogenex) and mouse monoclonal anti-AR (DAKO) were used at 1:200 and 1:400, respectively.

**Results.** In HYPO tubules, the majority of Sertoli cell nuclei exhibited a robust AR immunostaining, but negative nuclei were also present. In SCO tubules, Sertoli cell nuclei were also AR positive, although the intensity of staining was clearly less. However, AR negative nuclei were infrequent ($p < 0.005$). In IMMA tubules, Sertoli cell nuclear AR immunostaining intensity was less than in the other two types of tubules, and the number of AR negative Sertoli cell nuclei was greater ($p < 0.01$). In Leydig cells associated with both HYPO and SCO tubules T staining was present, but absent from those related to IMMA tubules.

**Conclusions.** The present results leads us to suggest that positive AR staining in Sertoli cell nuclei is irrespective of germ cell presence, but is related to the content of T in nearby Leydig cells.

## P-546

GRANULAR CELL TUMOUR OF THE URINARY BLADDER. REPORT OF A CASE WITH HETEROGENEOUS IMMUNOPHENOTYPE.

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Granular cell tumour (GCT) is a relative rare disease, that may be found anywhere in the body; however, it occurs rarely in the urinary bladder. To our knowledge only 7 cases of this location has been reported, one of them with features of malignancy.

We present a case of a 54-year-old white woman with microhaematuria. At cystoscopy, a bladder tumour sessile and solid was observed behind the trigone. Transurethral resection of the lesion was done and no recurrence was evident one year after surgery. Removed material weighed 2,5 g. and consisted of irregular, soft or elastic fragments of tissue from 0.2 to 1.5 cm in diameters. Microscopic examination revealed a proliferating growth of cells with relatively large cytoplasm containing fine, eosinophilic PAS positive granules, and interspersed with a fine stroma. Nuclei were round and regular and no pleomorphism, nucleoli or frequent mitosis were evident. Among the solid sheets of granular cells there were some groups of cells with large vacuolated cytoplasm and oval nucleus, that resembled histiocytes, very similar to proliferating cells at first sight.

Immunohistochemical staining of proliferating cells was strongly reactive with S-100, vimentin (membrane staining), NSE, CD68 and alpha-1-antitrypsin, weakly positive to cathepsin D and neurofilament, and negative to EMA, keratins (AE1-AE3), muscle specific actin, desmin, myoglobin, lysozyme and CD31. The accompanying histyocite-like cells were also positives to CD68, alpha-1-antitrypsin and vimentin (weakly), but negatives to S-100, NSE and neurofilament. However, a strong to moderate reactivity with cathepsin D, CD31 and lysozyme was observed.

Histogenesis of GCT is subject to controversy. Immunohistochemical and ultrastructural evidence supports a Schwann cell origin for the majority of GCT; however, some conventional GCT express histiocytic, mesenchymal, endotelial, epithelial or myogenic markers indicating other possible histogenesis. In our case the evidence of histiocytic markers (CD68 and alpha-1-antitrypsin), as the presence of groups of histiocytic cells between the granular proliferating cells supports this origin, but positivity to other markers suggest also schwann cell or mesenquimal origin.

## P-547

TESTICULAR PLASMACYTOMA: A REPORT OF 3 CASES, INCLUDING ONE WITHOUT FURTHER MYELOMA MULTIPLE DEVELOPMENT.

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**Aims:** Our purpose with this study is to evaluate the clinicopathological implications of testicular involvement by plasma-cell neoplasms.

**Methods:** Formalin fixed, paraffin embedded tissue were examined by histochemical methods and immunohistochemically. Clinical information and follow-up were obtained from medical reports and the patients.

**Results:** We report 3 cases of testicular plasmacytoma. All of them had similar histological features, with immunohistochemical tumor cell expression of monotypic cytoplasmic immunoglobulin.

A 53-year-old man with a multiple myeloma (MM) diagnosed in the course of an admission for an ulcerous process suffered, one year later, involvement of the left testis despite proper treatment.

The other two reported cases were men aged 60 and 76 with testicular enlargement. One was misdiagnosed as malignant lymphoma and submited to further classification to our consultants, who made the diagnosis of plasmacytoma. Six months later he developed systemic disease. The last case was an incidental finding in a surgical specimen of bilateral orchiectomy for treatment of a prostatic carcinoma. Two years later the patient is alive and free of disease.

**Conclusions:** 1. Plasma-cell infiltration of the testis could either be primary or developed in the context of a MM.

2. Testicular involvement by plasma cells can be the first manifestation of a MM.

3. One of our cases is the ninth reported in the literature that only affects the testis, without progression to MM after 24 months of follow up.

## P-548

IDENTIFICATION OF TWO ARCHITECTURAL PATTERNS ("NODULAR/SOLID" *versus* "INFILTRATIVE") IN MUSCLE-INVASIVE TRANSITIONAL CELL CARCINOMAS OF THE URINARY BLADDER: PATHOBIOLOGIC AND PROGNOSTIC IMPLICATIONS.

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**Aims:** Architectural patterns most frequently identified in transitional cell carcinomas (TCCs) of the urinary bladder ("papilar" *versus* "nodular/solid") are associated with different biologic potential and prognostic significance in superficial tumors, but not in invasive ones. This work describes two different architectural patterns ("nodular/solid" *versus* "infiltrative") in muscle-invasive TCCs of the urinary bladder, and studies the pathobiologic and clinical implications of these division.

**Design**: Seventy-two cases of muscle-invasive TCCs of the urinary bladder, with a mean follow-up of 32 months, were studied in order to evaluate the histologic grade (low *versus* high), DNA ploidy (measured by static cytometry), mitotic counting (MC) and MIB-1 index. Moreover, assessing mainly the deep topographic compartments, every tumor was classified, according to the predominant architectural pattern observed, as: a) "nodular/solid" (macronodules or diffuse sheets of neoplastic cells, with scarce stroma between them, that effaced muscular fibers), or b) "infiltrative" (rows and micronodules of neoplastic cells, setted in an abundant stroma, leaving intact, at least partially, the normal histologic structures of the bladder wall).

**Results**: Tumors classified as "nodular/solid" revealed significant higher proliferative activity (both measured by mitotic counting and MIB-1 immunohistochemical expression) than those classified as "infiltrative". Also, "nodular/solid"category was significantly associated with aneuploidy and high grade status, while "infiltrative" category was so with diploid and low grade ones. Finally, "nodular/solid" tumors showed a mean survival of 37 months; and "infiltrative" ones, of 20 months.

| Architectural pattern | MC (%) Av +/- SD | MIB-1 (%) Av +/- SD | DNA Ploidy | Histologic Grade | Mean survival (months) |
|---|---|---|---|---|---|
| nodular/solid | 0.66 +/- 0.38 | 20.65 +/- 4.94 | aneuploid | high | 37 |
| infiltrative | 0.39 +/- 0.24 | 14.94 +/- 4.28 | diploid | low | 20 |

**Conclusions**: Identification of an infiltrative architectural pattern in muscle-invasive TCCs of the urinary bladder seem to be linked to a more uniformly agressive clinical course.

## P-549

IMUNOEXPRESSION OF MUC 1 IN PROSTATE ADENOCARCINOMA

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**Aims**: The aim of this work was to study the imuno-expression of MUC 1 during the process of neoplastic transformation of prostate, trying to evaluate if it can help in the differential diagnosis with non neoplastic small acinar glands.

**Methods**: Sextant prostate biopsies from 50 patients were studied. In addition to HE stain, immuno-expression of MUC1 was done in paraffin embedded sections and evaluated in non-neoplastic epithelium; high grade PIN1 and adenocarcinoma.

**Results**: In non-neoplastic glands a light immuno-reactivity of MUC1 was found only in luminal membrane of glandular cells; the cytoplasm was negative. In the basal cells a light immuno-reactivity was found also in the cytoplasm of a small number of cells

In high grade PIN immuno-reactivity for MUC1 was found in luminal membrane and also in the cytoplasm of about an half of the epithelial cells. This cytoplasm positivity was light and homogeneous

In the adenocarcinoma, a strong immuno-reactivity was found in the cytoplasm of most of the neoplastic cells (more than 80%). This positivity was granular and heterogeneous. Aggregates of small tubular glands expressed the same type of immuno-reactivity when malignant, but not when non-neoplastic.

**Conclusions**: In the prostate, cytoplasm MUC 1 immuno-expression was found only in adenocarcinomas and can be looked as an additional method for the differential diagnosis with non-neoplastic small acinar aggregates. The biological meaning of this phenomena still persist unknown.

## P-550

RIBOSOME-LAMELLA COMPLEX AND LARGE LUCENT LYSOSOMES IN THE NON-INVOLVED UROTHELIUM OF A PATIENT WITH TRANSITIONAL CELL CARCINOMA

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**Aims:** The detection of Ribosome-Lamella Complex (RLC) and Large Lucent Lysosomes (LLLs) are described for the first time in the urothelium surrounding the tumor in a male patient with transitional cell carcinoma (TCC) of the urinary bladder. The finding was incidental, during a study of the non-involved urothelium of TCC bearing patients.
**Methods:** The tumor was resected transurethrically and it was grade II TCC. Specimens from the non-involved but surrounding the tumor urothelium were obtained and routinely processed for ultrastructural study. The patient did not have a history of previous neoplasia or lysosomal storage disease.
**Results:** Granulo-filamentous bodies presented with a hollow cylindrical structure composed of ribosome-studded spirals and concentric lamellae (RLC) were detected in few urothelial cells of the surface layer. Moreover, large lucent lysosomes were also noted in adjacent urothelial cells. Most of them were characterized by flocculent reticulated material lying in an electron lucent matrix. They also contained ribosomes, vesicles and granules. Some LLLs, however, were less organized, more lucent and did not contain granules.
**Conclusions:** The significance of RLC and LLLs - which have mainly been detected in hairy cell leukemia and lysosomal storage disease correspondingly - is obscure. We could speculate, however, that they might be preneoplastic in nature or they might be considered as a result of the underlying disease, further strengthening the suggestion of the initiation of the urothelium as a whole.

## P-551

MIB-1 EVALUATION IN $G_2$ BLADDER UTOTHELIAL CARCINOMAS.

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Aim: The recent WHO/ISUP (1998) urothelial lesions classification forces pathologists to formulate a more rigorous differential diagnosis between high and low histological grade.
Methods: 95 cases of $G_2$ superficial bladder urothelial carcinoma ($Ta$-$T_1$) were morphologically and immunohistochemically evaluated by grading and MIB-1 proliferation index. Moreover similar evaluations were performed in 86 $G_1$ and $G_3$ cancers as positive controls. 5 years follow up was available in 93 out of 181 cases.
Results: In $G_2$ carcinomas we observed 2 distinct groups: the first with high proliferation index (mean 50.6 ±s.d. 12) with a biological clinical behaviour (more than 3 recurrencies in 5 years) similar to $G_3$ patients (mean 55.7 ± s.d. 15). The second group showed low proliferation index (mean 27.6 ± s.d. 7) with a biological and clinical behaviour just a little worst (less than 3 recurrencies in 5 years) than to $G_1$ patients (mean 14.1 ± s.d. 6).
Conclusions: The use of MIB-1 proliferation index may be helpfull in grading urothelial bladder carcinomas reducing most of cases of doubtful cancer histological grade.

## P-552

**LYMPHOEPITHELIOMA-LIKE CARCINOMA OF URETHER**

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**Aims.-** Lymphoepithelioma is a term used for calling a poorly differenciated nasopharyngeal carcinoma, with a distinctive intense lymphoid reaction. Occasionally, tumors with similar microscopical findings have been described in a variety of other sites, under the designation of Lymphoepithelioma-like carcinoma (LLC). A few cases of LLC have been reported in the bladder, but the occurrence of this neoplasm in urether is very rare. It is necessary the pathological recognition of these tumors –specially in sites other than the ussually described –as the present case- due to their therapeutic implications.
**Methods.-** A 58 year old male presented with an episode of gross hematuria. Cystoscopic examination revealed a normal bladder, but an intravenous pyelogram and a CT scan showed a 4,5 solid mass in the left pelvic urether. A radical nephroureterectomy was carried out. Multiple histological sections were seen and an additional immunohistochemical study was made, using the ABC method and including the following monoclonal markers: pan-Cytokeratin (AE1 & AE3), LCA, pan-T (CD 43) & pan-Bcell (MB 2),VIM, S-100 and NSE.
**Results.-**Macroscopically an 4,5 cm. ulcerative-infiltrating mass in the left pelvic urether was detected, penetrating through the entire wall.The tumor cells showed a syncitial growth pattern of undifferentiated cells, with pleomorphic nuclei and numerous mitoses, associated with a dense lymphoid infiltrate. Neoplastic cells were highly immunoreactive for AE3 and the accompanying lymphocytes were positive for CD43 and MB2.
**Conclusions.-** Although very rarely, the urether must be keep in mind as a site where the LLC can be placed. It is imperative the recognizing of this tumor, for making the distintion, chiefly with lymphoma, poorly differentiated transitional cell carcinoma and chronic cystitis because of their different prognostical and therapeuthical approachment.

# P-553

## CORRELATION BETWEEN CYTOKERATIN 19 ( CK19), p-53, AND GLEASON SCORE IN PROSTATIC CANCER.

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**Aims:** Immunohistochemical studies have shown that expression of CK19 in human prostate is heterogeneous, staining basal and luminal cells in normal and benign hyperplastic tissues and predominantly luminal cells in prostatic cancer. We correlated the expression of CK19, p-53, Gleason's score and outcome, in prostatic biopsies of patients diagnosed with cancer and receiving hormonal treatment.

**Methods**: Forty cases of prostate cancer (20 needle biopsies and 20 transurethral resections) were histologically reviewed for Gleason score, and stained by immunohistochemistry with CK19 and p-53.

**Results:** Thirty one cases (77.5%) showed positive stain with CK19 in the luminal cells. There was a significant correlation between CK19 and Gleason grade with strong staining in carcinomas Gleason grade 1 to 3 and decrease staining in grades 4 to 6. Nine cases were negative Thirty nine cases (97.5 %) were negative for p-53 oncogene expression The only case that stained positive for p-53 expression had already metastasized to the urinary bladder.

**Conclusions:** We conclude that expression of CK19 may be an indicator of good prognosis since it correlates well with low Gleason scores. P-53 may not play an important role in the development of prostate cancer, but it might be a good marker for aggressive behavior.

# P-554

## MORTALITY DUE TO BENIGN AND MALIGNANT TUMOURS IN RHEUMATOID ARTHRITIS

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**Aim:** Malignant lymphomas may be associated with rheumatoid arthritis (RA) and may be linked to immunsuppressive treatment of RA. *Mortality* due to *benign (bTu) and malignant tumours (mTu)* were studied in a randomized autopsy population of 234 hospitalized patients with *RA* (according to the criteria of ACR) to determine the prevalence of neoplasms, the types of tumours (the risk of lymphoproliferative disorders), the mortality due to benign and malignant tumours, and the possible link of immunosuppressive treatment to neoplasia. *Paraneoplastic syndromes with rheumatoid complaints* could be excluded by the onset and duration of RA and tumours.

**Methods**: The tissue specimens were fixed in 8% formaldehyde solution at pH 7.6 and embedded in paraffin. The tumours were diagnosed histologically and identified by streptavidin-biotin-complex/horseradish peroxidase immunohisto-chemical reactions.

**Results**: Six benign (2.6%), and twenty-six malignant tumours (11.1%) were found in 32 (13.7%) of 234 cases. One benign (0.4%) and twelve malignant (5.1%) tumours led to death in 13 (40.6 rel%) of 32 cases. Only two cases of malignant lymphoproliferative disorder (one malignant Hodgkin's lymphoma, and one multiple myeloma) were confirmed histologically. Neoplasms were recognized clinically in 11 of 13 cases (84.6 rel%). None of these patients received immunosuppressive treatment. The patients received steroids and non-steroidal anti-inflammatory drugs.

| Basic disease | Complication(s) | Cause of death | Associated |
|---|---|---|---|
| Ependymoma | Vertebral fracture - (AA) | Pulmonary embolism | RA |
| Ca. of thyroid gland | Arrosion of laryngeal artery | Massive haemorrhage | RA |
| Ca. of gallbladder | Metastases | Cachexia | RA |
| Gastric Ca. | Metastases | Cachexia | RA |
| Bronchoalveolar Ca. | Cerebral metastasis | Cachexia | RA |
| Bronchial Ca. | Metastases | Cachexia | RA |
| Bronchial Ca. | Metastases | Cachexia | RA |
| Ca. of breast | Metastases | Cachexia | RA |
| Ca. of prostate | Metastases | Bronchopneumonia | RA |
| Renal cell Ca. | Metastases | Uraemia | RA |
| Malignant synovioma | Metastases | Cachexia, Sepsis | RA |
| Hodgkin's disease | Metastases | Cachexia | RA |
| Multiple myeloma | Metastases | Cachexia | RA |

**Conclusion**: ***Our data do not support the assumption of a high risk of malignant lymphomas associated with RA treated with immunosuppressive therapy.***

# P-555

## CONFERENCE ON HISTORY OF MEDICINE.

## AUTHENTICITY OF DISSECTION IN ARABO-ISLAMIC MEDICINE

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**Aims :** Our aim was to look for anatomic studies and practice of dissection in Arabo-Islamic Medicine.

**Methods :** We studied original books of Arabo-Islamic physicians and we compared anatomic data to previous medicine in previous civilisations and to actual's. We elaborated two thesis of medicine respectively on the anatomy of the upper lim and of the brain in Ibn Sina (Avicenna) medicine.

**Results :** Severals anatomic details showed that Arabo-Islamic physicians practised anatomy and dissection. Aboul Abbas Al Majoussi and Ibn Sina (Avicenna) knew fetopathology. Ibn Sina was the first to describe sesamoid bones in phalyngeal joints. Abdellatif AL Baghdadi described the mandible as only one bone in disagreement with Galenus and Avicenna.

Ibn Nafis discovered the Cardio-pulmonary blood circulation in the thirteenth centry, four centuries before Miguel Seritus and William Harvey.

**Conclusion :** Our studies showed that Arabo-Islamic physicians practised anatomy and dissection and added their own anatomic criteria and even their discoveries to the Human medical knowledge.

# P-556

## ENHANCING THE EDUCATIONAL VALUE OF THE AUTOPSY IN HISTOPATHOLOGY TRAINING

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**Aims:** The aim of this undertaking was to maximise the educational value of autopsy gross specimen review, and to develop macrophotographic skills.

**Methods:** A weekly autopsy conference for all resident and staff pathologists was re-established in September 1997. Selected organs are retained fresh or fixed by the resident and staff pathologist rotating on autopsy that week. All significant gross lesions are photographed by prosectors or by pathology technicians using an SLR or digital camera. Material is presented with a summary of the clinical details. Contributions and questions are encouraged.

**Results:** From September 1997 to March 1999, each resident performed an average of 35 autopsies (mean: 8- 49). A total of 243 cases were discussed at weekly autopsy conferences, an increase in exposure to autopsy pathology of 400%-500%. The main benefits of selected organ review relate to i) gross diagnostic skills, applicable to autopsy and surgical specimens, ii) case presentation, iii) clinicopathological correlation, particularly in cardiac, pulmonary and gastrointestinal pathology, iv) formulation of autopsy reports, and v) an awareness of specific procedural complications, and of disease trends in the community. Alternative dissecting techniques are suggested and carried out. Controversial or puzzling gross lesions are clarified in microscopic sections reviewed at a weekly microscopic conference. Finally, photographic skills are acquired and digital images are made available for many different purposes.

**Conclusions:** Our experience re-emphasises the incalculable educational value of the autopsy in histopathology training.

## P-557

### CLINICAL AUTOPSY. AN ASSESSMENT OF THE ACCURACY OF CLINICAL DIAGNOSES.

Authors: R Bernat*, F Riu*, C Guallar**, M Vadillo**, R Pujol**, M Gorriz***. Pathology* Internal Medicine**, and Intensive Care Unit***. Ciutat Sanitària i Universitària de Bellvitge. Barcelona . University of Rovira i Virgili. Spain. *Aim of the study.* To determine which factors influence the performance of autopsies and the discrepancy between clinical and autopsic diagnoses using the autopsy as "gold standard". *Methods.* A 3-year, transversal study was conducted in a tertiary hospital of Barcelona (Services of Internal Medicine, Intensive Care Unit and Emergency), analyzing 209 correlative clinical autopsies who had previous consensus on their clinical diagnoses. Clinical diagnosis were daily collected in a consensus meeting of all staff members before the autopsy performance. Subsequently, when autopsy was performed, clinical and autopsic diagnosis were compared. All discrepancies were discussed and analyzed by clinicians and pathologists. Autopsy results were used as a reference or "gold standard". According the degree of discrepancy between clinical and autopsy diagnoses, this was classified in: ***Minor Discrepancy*** when the diagnostic deficiency should not reasonably have been expected to impact on survival; and ***Major Discrepancy*** when misdiagnosis hindered optimal clinical management and, consequently, had an adverse impact on the prognosis of the patient. Possible factors influencing performance of autopsies and discrepancy were analyzed. *Results.* Clinical autopsy was performed in 217 (11,6%) out of 1864 patients. By Services autopsy rate was: 4% in Emergency, 15,1% in Internal Medicine and 23% in Intensive Care. The autopsy rate was lower in elderly patients (OR: 0.97;95%CI:0.97-0.98) and in those dying in the Emergency Room (OR:0.19; 95%CI:0.13-0.28). Of the 217 autopsied patients 8 were excluded, and 209 were fully studied. In 47% of cases the autopsy gave non-suspected clinical diagnoses. In the main diagnosis, the overall discrepancy rate between clinical and autopsic diagnoses was found in 17.7% of cases, major discrepancy in 11.5%. Independent factors associated with an increase of this discrepancy were to die in the Internal Medicine service (OR:2.95;95%CI:1.33-6.48) and old age (OR:1.04, 95%CI:1.01-1.07). *Conclusions.* The results reported in this study suggest that nowadays autopsy checks the accuracy of clinical diagnosis and, as a consequence, offers valuable information to clinicians for the improvement of their practice. Comunication between clinicians and pathologists is essential to optimize autopsy results.

## P-558

### MALIGNANT MESOTHELIOMA OF THE PLEURA IN AN AREA AT HIGH RISK, NORTHEASTERN ITALY

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**Aims:** To delineate the principal features of malignant pleural mesothelioma in the Trieste-Monfalcone area, northeastern Italy, a narrow coastal strip (total population about 300,000) with large shipyards.

**Methods:** A series of 485 cases diagnosed during the period 1968-98 were reviewed. Necropsy findings were available in 417 cases. Occupational histories were obtained from the patients or from their relatives by personal interviews. Routine lung sections were examined for asbestos bodies in 412 cases. In 108 cases isolation and quantitation of asbestos bodies were performed after chemical digestion of lung tissue.

**Results:** The series included 431 men and 54 women, aged between 32 and 93 years. A majority of the patients (66%) had worked in shipbuilding. In the remaining cases, maritime trades and various nonshipbuilding industries were the most frequent occupations.The time intervals elapsed between first exposure to asbestos and death, ranged from 14 to 75 years (mean 48.6; median 51.0). Asbestos bodies were observed on routine histological sections in 67.5% of the cases. After isolation, asbestos bodies ranged between 20 and about 10 millions/g dried tissue.

**Conclusions:** Severe exposure to asbestos occurred in the Trieste-Monfalcone area until the 1980s. The long latency periods observed in our study suggest that in this area mesothelioma incidence will remain high in the next decades.

## P-559

### INTRAOCULAR BIOCOMPATIBILITY OF A FERROFLUID USING SILICONE AND PHYSIOLOGIC SERUN LIKE CARRIER MEDIUM.

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INTRODUCTION: the use of ferrofluids in the medical area is very extensive because of the possibility to be positioned in specific zones by using a magnetic field. Our aim is to value the biocompatibility of a ferrofluid in an intraocular aplication comparing two different carrier media: silicone and physiologic serum, and evaluate, at a histologic level the inflamatory response produced when is inyected in the anterior ocular chamber of rabbits.

METHODS: 24 ocular globes obtained from 12 albino rabbits were studied both by optic and electron microscopy. Left eyes were used as control injectivy in 6 of them only physiology serum and in the other 6 silicone. Right eyes were injected with ferrofluid; in 6 of them were used silicone like carrier medium and, in the other 6 physiology serum. At 3,7 and 30 days after, rabbits were sacrificed and ocular globes were enucleated inmediately.

RESULTS: On ocular globes control it was not observed significant histologic alterations both by optic and electron microscopy. In anterior segment of the ocular globes injected with ferrofluid it was observed important and generalizated inflamatory changes in the corneal epithelium , and ondothelial vacuolization; light endothelitis and focal endothelitis with presence of siderophages and, in some cases corneal ferrofluid extravasation Also inflamatory lesions were observed and presence of siderophages at esclerocorneal level. At posterior segment of silicone injected eyes appear abundant intra and extra macrophagic binded iron with an acute inflamatory response in ciliars process; escleral impregnation by ferrofluid; inflamatory and macrophagic cells with ferric pigment in vitreou. All the results were confirmed by the ultrastructural study. For all the cases, the inflamatory reaction is more important in enucleated eyes after 3 days decreasing at 7 and 30 days, however the presence of siderophages is higher at 30 days after enucleation and smaleer at 7 and 3 days.

CONCLUSION: The inflamatory changes found by using intraocular ferrofluids are associated with the ferric particles deposit and in this may they are observed initially in the wear point to tht are inyection hole and adjacent zones. The inflamatory response is higher when we used silicone like solvent medium.

## P-560

### DIAGNOSTIC ERRORS TIPE I AND TIPE II DISCOVERED AT AUTOPSY IN THE ICU

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**Aims**: To know and classify the diagnostic errors through an objective standard reference as it is the autopsy. **Design**: Prospective and observational study. **Patients**: 400 consecutive patients who died at the ICU in whom autopsy was performed.

**Method**: Clinical and post-mortem diagnoses were classified in accordance to the WHO definitions of fundamental diaseases and cause of death. Clnical diagnoses were made by the attending physicians previously to the performance of the autopsy and pathologists involved in the autopsy procedure were not aware of those clinical diagnoses. Physicians and pathologists confronted tehir diagnoses, established the diagnostic discrepancies and classified them into errors type I or type II according to the WHO definitions.

**Results**: The percentage of autopsies performed each year in patients who died at the ICU over the study period varied from 28% to 42%. The diseases that motivated type I errors varied among the four consecutive groups of 100 autopsies each one. The autopsy yielded a diagnosis of the fundamental disease in 100% of the patients, and a diagnosis of the cause of death in 96-98% of the patients.

| Autopsy nº | 1-100 | 101-200 | 201-300 | 301-400 |
|---|---|---|---|---|
| Error type I | 7 | 5 | 5 | 6 |
| Error type II | 15 | 10 | 5 | 10 |

**Conclusions:** Newer diagnostic techniques do not avoid the diagnostic errors. Autopsy is efficient in obtaining diagnosis and useful to determine the diagnostic errors..

## P-561

TELEPATHOLOGY-MODERN ADVANCE OR LUXUS?

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**Aims**: We analyze accuracy and viewing times using a dynamic-robotic telepathology system to render diagnoses on a test set of 47 consecutive routine surgical pathological cases and a preceeded lerning phase with 30 cases.

**Methods:** We used a so called „master" system with a robotic microscope of a telepathological system („HISTCOM") and a high speed communication link (ISDN) with dynamic images. Glass slides were placed on the stage of the robotic microscope in the operating room. Real time control of the motorized microscope was then transferred to a pathologist, who viewed images on a video monitor. Diagnostic concordance of telepathological frozen section diagnosis and diagnosis after paraffin embedding was analyzed.

**Results:** Accuracy of video diagnoses was categorized as I (correct), II (right dignity), III (deferred), IV (false negative) and V (false positive).In the lerning phase 18 cases were captured in set I, 5 in set II, 4 in set III, 3 in set IV and no case in set 5. In the test phase there were 34 cases in set I, 3 cases in set II, 9 cases in set III, 1 case in set IV and no case in set V. That means an elevated share of the deferral rate and an elevation of the accuracy in the test phase versus learning phase.

**Conclusions:** An accurate pathological diagnose was possible on the base of telepathological examination. *Advantages*: 1.Repeated frozen sections from one patient are possible. 2.Reduction of anesthesia interval for the patient and reduction of operation costs. *Disadvantages*: 1.High costs for the telepathological system. 2.Longer examination time for the receiving pathologist. 3.Lack of macroscopy and sampling of material by the pathologist. That's why a doctor who is thoroughly familiar with the technology has to be at the reffering end. This is necessary from the juridical point of view, too.

## P-562

USE OF ELECTRON MICROSCOPY IN DIAGNOSTIC PATHOLOGY RESEARCH REPORTS: A RETROSPECTIVE APPRAISAL.

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**Background**: Electron microscopy (EM) is a valuable tool in basic research and teaching. However, EM is not as popular as it used to be among general pathologists. This can be attributed to strategic and/or economic reasons. Decreased awareness of potential applications of EM can result in its being neglected as an ancillary, control or gold standard method to complement, support, or confirm research results in diagnostic pathology (RDP). The purpose of the present work is to evaluate the use of EM in RDP, as evidenced in reports from three top-ranking pathology journals mostly devoted to the communication of relevant diagnostic, prognostic, and therapeutic information.

**Material and methods**: Data were obtained by analyzing all articles (n=2531) in three top indexed journals (Am J Surg Pathol, Hum Pathol, Mod Pathol), from the last 60 months (July 1993- June 1998). They were classified by subject and divided into three categories: case reports, descriptive articles, and articles on new diagnostic strategies. Only articles in which use of EM would be pertinent (according to standard textbooks in surgical pathology) were selected. Both the actual and the potential EM content of each article were scored as follows: 0, none; 1, illustrative; 2, supportive; 3, confirmative (gold standard); 4, independent EM information; and 5, mostly EM content.

**Results:** A total of 448 articles (out of 2531) were selected. From these, 345 (13.6%) contained relevant ultrastructural information and the remaining 103 (4%) did not contain any in spite of being potentially indicated. The percentage of EM-containing articles in each of the three journals was 21%, 10.6%, and 9.6%. The percentage of articles not including potentially useful EM was respectively 8.7%, 2%, and 2%. Considering the total number of articles where EM was indicated, 77% of them made use of it (71%, 83.6%, and 85%). EM support was lacking most often for articles on serosal neoplasms and on new diagnostic strategies ($p<0.00005$). There was not a definite trend towards increase or decrease in the use of EM during the last 5 years.

**Conclusion**: Electron microscopy is generally included in most reports on diagnostic pathology, when it is indicated. However, a small but not negligible, percentage of articles could benefit from including EM as an ancillary, control, or gold standard method to complement, support , or confirm their research results.

## P-563

EPITHELIAL LESION OF THE BULBAR CONJUNCTIVA WITH IRRITATED SEBORRHEIC KERATOSIS PATTERN.

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**Aims:** The seborrheic keratosis is an benign epidermic lesion that can occur in any place of the skin, with the exception of palms and soles. In Oftalmology, it preferentially appears in eyelid, affecting mostly the palpebral edge. The revised literature did not reveal any case involving the conjunctiva.

**Methods:** A 71-year-old woman who, seven years ago, had undergone surgical removal of a pterigium in the conjuctiva of the right eye. Two years later, she presented an excrecent lesion in the right eye bulbar conjunctiva, close to the esclerocorneal limb of the temporal region. This lesion was also removed and sent to the Pathology laboratory. At this time, the patient has not relapsed.

**Results:** The microscopic study revealed conjunctival mucous covered with squamous epithelium showing superficial parakeratosis and prominent acanthosis due to the proliferation of basaloid cells, some of them with melanic pigment. There are numeous whorls composed of eosinophilic flattened squamous cells arranged in an onion-peel fashion ("squamous eddies").

**Conclusions:** In the revised literature, there are not previous references about a conjunctival lesion with irritated seborrheic keratosis pattern. Possibly, this lesion has its origen in a squamous metaplastic transformation, related with the previous surgical removal of the pterigium.

## P-564

PATHOLOGY IN THE NEW MEDICAL CURRICULUM.

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**Introduction:**In line with the requirements of the UK General Medical Council, the undergraduate curriculum has changed from a didactic, lecture-based course to one using problem-based learning, the new curriculum taking its first student intake in 1996. An important change has been the disappearance of separate subject teaching, and integration of clinical and preclinical studies, coupled with a reduction in the factual content of approximately 30 %.

**Aims:** To develop an appropriate method of providing the pathology knowledge required by the students.

**Methods:** We have adopted the following:

1. Ensuring pathology is included in the core of all the module case scenarios, which requires a pathologist member on each module planning committee, the content loosely based on the previous syllabus.
2. Providing special study modules per year, of one month duration or longer and part-time, attached to the Pathology Department, during which students observe surgical and autopsy pathology and write a dissertation.
3. Clinicopathological correlation teaching meetings with clinicians and pathologists discussing case examples, and extensive student involvement.
4. Ensuring pathology questions are provided and used in the student assessments. In keeping with the integrated curriculum, these questions are integrated with other subjects, as far as possible.
5. An extensive Pathology website for student use.

**Results:** The first cohort are scheduled to qualify in 2001. In-course assessments to date are encouraging, and student response to the pathology resources offered has been very positive.

**Conclusions:** Despite the disappearance of traditional didactic methods of teaching, pathology can maintain its vital place as a field of study in the undergraduate curriculum, thus ensuring that students will have the sound foundation which they will require.

## P-565

### The lost pathology specimen, a little-studied entity

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Aims: Millions of pathology specimens are successfully handled each year but small numbers get delayed, damaged or lost. Although loss of a specimen such as a suspicious breast lump or a pigmented skin lesion may have serious therapeutic, psychological and legal consequences there has been little analysis of the frequency and circumstances of specimen loss. Methods: An episode in which lost specimens were recovered is described. 30 further reports of specimen loss were collected from colleagues and from the literature and were categorised. Results: After two biopsy specimens had been lost, a subsequent lost specimen was traced to a specimen transport elevator and all three were found when the elevator shaft was searched. The types of specimen loss reported may be categorised as 1) observed loss due to mishaps in transport or processing, 2) apparent loss due to specimen delay or mix-up, 3) unexplained (mysterious) disappearance, 4) unrecognised loss (recovery of a specimen not reported lost). Conclusion: New technology for bar-code tracking and test-ordering by computer should reduce the incidence of delayed and lost specimens but for prevention it remains important that clinicians and pathologists be aware of the range of occurrences which may lead to specimen loss.

## P-566

### CORNEAL OPACITIES IN TWO SISTERS DUE THE PARTIAL DEFICIENCY OF REVERSE CHOLESTEROL TRANSPORT ENZYME

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**Aims:** LCAT ( Lecithin cholesterol acyltransferase) is a key enzyme in reverse cholesterol transport, the process by wich cholesterol is mobilized from peripheral tissue to the liver.

Mutations of LCAT are associated with certain abnormalities in plasma lipid profiles, such as hypoalphalipoproteinemia and an increased ratio of free to esterified cholesterol. There are two kinds of LCAT activity: alpha-activity , related to apoprotein A-I, wich promotes HDL esterification , and beta-activity ,related to apoprotein C – I , wich promotes VLDL and LDL esterification.Clinically, these mutations are expressed as FDL (familial LCAT deficiency ) and FED (fish eye disease).In FLD , there is a complete deficiency of both, alpha and beta activity,it is characterized by nephropaty, anemia, and corneal opacities.In FED there is absence of only alpha-LCAT activity, corneal opacities are the only clinical manifestation.Both are very rare disorders.

**Methods:** We present two histological studies of the queratectomy specimens of a 63-year-old woman and her 70-year-old sister with decreased vision due to corneal opacities.

**Results :**In corneal light microscopy the epitelium and Bowman's layer, endotelium and Descemet's membrane appear normal in the H&E sections.The whole stroma presents cytoplasmic micro-vacuoles, difficult to differentiate from artifacts,.negative for Alcian Blue, Congo Red and PAS.Electron microscopy shows spaces,some containing a lipidic membrane-like material, widespead throghout the entire stroma. They are extracellular.

**Conclusion:** Electron microscopy is a most useful tool for the diagnosis of those patients.LCAT can be underdiagnosed clinically because a partial deficiency of the LCAT enzyme related in our case , can not diminish the whole amount of alpha and beta cholesterol in the plasma.

## P-567

### HOBNAIL HEMANGIOMA: CLINICOPATHOLOGICAL REVIEW OF FIVE CASES.

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Hobnail hemangioma is an acquired vascular lesion with a distinct histopathological appearance. It may be confused with well-differentiated angiosarcoma and patch-stage Kaposi's sarcoma.

We report five cases of hobnail hemangioma presenting in three males and two females whose ages ranged from 13 to 56 years. In four cases there was a previous history of inflammation and relapses. The most constant microscopic feature was the presence of capillary vessels lined by prominent hobnail endothelial cells. Another steady finding was dissection of dermal collagen with frequent appearance of the promontory sign, considered to be characteristic of Kaposi's sarcoma. Endothelial cells were epithelioid in two lesions and very vacuolated in another two. Red blood cell extravasation and/or hemosiderin deposition was found in four cases, in two of which the presence of a distinct ecchymotic ring justified the descriptive term targetoid hemosiderotic hemangioma. Papillary projections of endothelial cells (Dabska's tumor-like) were observed only in one case. A scanty lymphoid inflammatory infiltrate was present in all instances. Additionally, a granulation tissue nodule overlied the 13-year-old boy hemangioma and one case contained a few epithelioid granulomas. The proliferation index of intralesional endothelial cells was evaluated by Ki67 immunohistochemistry and was found to range from 1.0 to 16.5%, probably reflecting fluctuations in activity.

Trauma has been proposed as the cause of capillary hemangiomas. In favor of this hypothesis are the chronic inflammation, granulomas, and increased Ki67 index found in our series. Also supportive of this connection is the fact that angiolymphoid hyperplasia with eosinophilia, considered to be another reactive vascular process, shares some microscopic features with hobnail hemangioma.

## P-568

### NUCLEOLAR ORGANIZER REGIONS PROGNOSTIC VALUE IN THE MALIGNANT MELANOMA OF THE UVEAL TRACT

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The aim of this study was to establish the contribution of the Nuclear Organizer Regions (Ag NORs) to modified Callender histopathological types for the prognosis of the malignant melanoma of the uveal tract (MMUT).

Materials and Methods: We investigated 36 patients diagnosed with uveal malignant melanomas (19 males, 17 females). 29 tumors were located in the posterior pole and 7 were located at the equator. Fragments obtained after enucleation were prelucrated by paraffin-embedding, followed by usual stainings and by argyrophilic method for Ag $NOR_s$.

Results: In order to obtain more informations concearning the prognosis of MMUT, we added the aspects provided by Ploton technique to dates offered by the modified Callender classification (cellular types, mitoses, vascular pattern, invasion into the sclera). Ag $NOR_s$ counting revealed an increased mean number beginning with the spindle shaped cellular type A and B to mixed cellular type, with the maximum value in the epithelioid cellular type.

Conclusions: The Ag NORs investigation represents an useful tool for assesing the prognosis in MMUT. The lower values found in A and B spindle shaped forms correspond to the favorable prognosis in comparison with the epithelioid and mixed forms with higher Ag NORs values and poor prognosis.

## P-569

**POST-MORTEM DIAGNOSIS OF DAD AND ITS CORRELATION WITH CLINICAL DIAGNOSIS OF ARDS.**

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**Aims**: a) To determine the correlation among histologic criteria of diffuse alveolar damage (DAD) and clinical criteria of ARDS, b) To assess the cause of death in ARDS. **Design**: Prospective study. **Patients**: a) Consecutive patients meeting criteria of ARDS in whom autopsy was performed (n=54); c) Patients with post-mortem diagnosis of DAD and without clinical diagnosis of ARDS

**Method**: Diagnosis of DAD (hyaline membranes, interstitial and alveolar edema, alveolar lining cell hyperplasia, fibrine trombus) was made by pathologists who were not aware of the clinical diagnosis previously established by the pshycians according to the consensus conference criteria. The cause of death was defined as follows: a) Refractary hipoxemia ($SaO_2$ <85% and systolic arterial pressure >100 mmHg for at least 6 hours before death); b) Hypotension ($SaO_2$ >85% and SAP <90 mmHg) and c) Myxed (a+b)

**Results**: Autopsy was performed in 38% of the patients admitted to the ICU over the period of the study. Clinical-histologic concordance was observed in 44 (71%) patients of the 54 patients with clinical diagnosis of ARDS. Forty-four had DAD, 10 showed pneumonia, and 1 presented alveolar hemorrhage. Eight patients with DAD had clinical diagnosis of penumonia (4) and pulmonary edema (4). Post-mortem study showed areas of pneumonia in 62% of patients with DAD. Ten percent of the 56 patients with DAD died because of refractary hipoxemia, 80% because hypotension and 10% because hipoxemia and hypotension.

**Conclusions:** In two thirds of patients meeting criteria of ARDS, post-mortem study shows DAD. Refractary hipoxemia is not a frequent cause of death in ARDS patients. A high percentage of patients with DAD also have pneumonia.

## P-570

**DEVELOPMENT OF AN IMAGE BASED DOCUMENTATION PROGRAM TO ASSIST QUALITY CONTROL IN SURGICAL PATHOLOGY LABORATORY**

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3. Higher Education Institute of Maia, Portugal

**Aims:** To evaluate the possibility of perform quality control by reviewing digital images from surgical pathology randomly selected cases acquired during the microscopic analysis. Problems such as the amount of space needed (file size, number of images, resolution and compression) and the chromatic quality of the images to properly document a case are also analyzed.

**Methods:** An acquisition program was developed to acquire images directly from a digital microscope camera with a TWAIN driver. The program allows acquiring up to 12 images by case. All the images of a case were stored in a multipage standard TIFF file and automatically tagged with the identification. JPEG compression functionality was implemented to allow the users to optionally compress the images during the acquisition with several degrees of compression (quality vs. compression). The program allows storing the opinion of different specialists about the diagnosis and about the quality and interest of each image for the documentation of the case.

**Results:** A total of 315 cases were documented with 748 images. The images of all routine cases of our laboratory were acquired. We selected to review the cases monthly chosen for quality control. The redundancy of information was evaluated in all cases. The amount of information (images) to proper document a case was also evaluated.

**Conclusions:** The diagnosis based on images was possible in all reviewed cases. Information redundancy was detected in 13% of the cases. In 10% of the cases the diagnosis was not possible due the lack of essential information and in 6% due the use of inappropriate magnification.

## P-571

SINGLE CELLS SEPARATED BY THE LASER MICROBEAM TECHNIQUE

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**Aims:** Molecular biological methods have been introduced into the area of medicine to analyze genetic defects. Due to the extremely high sensitivity of molecular techniques sample separation without contamination is extremely important. We combined Laser MicroBeam microdissection (LMM) with Laser pressure catapulting (LPC) to separate single cells from various sources. PCR-based DNA amplification or RT-PCR was used to examine single or a few separated cells for genetic analyses.

**Methods:** A pulsed 337nm laser is interfaced with a research microscope and focused to yield a laser spot size of less than 1 µm in diameter. The cells of interest are isolated from their surroundings by circumscission with the focused laser beam (LMM). Cells were isolated from tumour preparations, peripheral blood and female transcervix samples. With single laser shots the isolated cells are ejected from substrate and catapulted directly into the cap of a PCR tube.

**Results:** PCR and RT-PCR methods were used on single or few cells for tumour cell determination, fetal sexing, RhD genotyping and individual genotyping by small tandem repeats (STRs).

**Results:** Most of the Laser separated cells show a specific PCR signal. DNA and mRNA information of the selected specimen as well as of the remaining sample are well preserved.

**Conclusions:** The Laser MicroBeam microdissection (LMM) and Laser pressure catapulting (LPC) technique allows is the state of the art technology to isolate single cells free of contamination. The procurement of single cells from different sources for subsequent genetic analyses increasingly gains importance in a wide field of cell biology and molecular medicine.

## P-572

TUBERCULOSIS AT AUTOPSY: THE HIDDEN PART OF AN ICEBERG?

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**AIM and METHODS**: In many western European countries and in USA an increasing proportion of tuberculosis [TB] notifications is reported, but very few studies are based on autopsy material. In Trieste, where the autopsy rate is presently almost 80% of all hospital deaths, we have a very large volume of reports of autopsies. We are therefore able to study retrospectively changes in some of the main human pathological conditions between 1901 till today.

**RESULTS:** In 1901, tuberculosis was the cause of death of 22.4% of subjects and 41.6% of people who died before the age of 40. The introduction of chest X-ray examinations, the improved living conditions and the introduction of streptomycin and isoniazid resulted in a dramatic reduction of death rates from TB: in 1993 the autopsy rate of TB was 0.66%. In the last five years a significant, progressive increase in autopsy frequency of active TB has been evidenced. In 1998, 22 out of 1740 autopsies of adult patients (1.26%) were found to have a pulmonary or a systemic TB (mostly miliaries or tubercular bronchopneumonia) and in almost 80% of these cases the lesion was the underlying cause of death. In these patients clinical signs and symptoms often weren't very clear and didn't reach the "minimum standard" necessary to request the microbiologic isolation of the *Micobacterium Tuberculosis* or the tuberculin skin test. In no one of these subjects there was a clinical diagnosis or a clinical suspect. The mean age of autopsied subjects with TB at present is 78 years, but the autopsy frequency of the lesion increased for all the age groups and particularly in females (the autopsy rate in the two sexes is presently 0.73% for males and 1.66% for females).

**CONCLUSIONS:** Of the all patients found to have TB in Trieste, over 60% were diagnosed by autopsy. These data emphasise the need for improved surveillance of TB in Trieste as in other parts of the developed world.

## P-573

**MICROSCOPIC FOREIGN MATERIAL IN SURGICAL AND FORENSIC PATHOLOGY.**

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**Aims**: Foreign or exogenous materials are a common finding in the histopathological study of biopsy or necropsy tissues. Usually, diagnosis is limited to stablish its general nature (inorganic, vegetal, iatrogenetic or alimentary). However, a more accurate identification allows to assess the source of this kind of material, as well as its possible involvement in the pathogenetic process of the patient.

**Methods:** Two series of 176 hospitalary autopsies and 48 forensic autopsies performed over 3 years, were analyzed microscopically, using routinary stains and birefringency.

**Results:** Overall findings of foreign material are comparable to previous data (21%). Vegetal materials were most often demostrated (72%), followed by inorganic materials (16%), commonly in the lung tissues and upper respiratory tract. The localization, amount, and morphological characteristics of the different materials were evaluated in forensic and surgical tissues, showing a group of 28 foreign materials (seeds, algae, peels, medicaments, etc.), some of which have not been described to date.

**Conclusions:** The routinary identification of foreign material by histological study is often incomplete or obscure. An accurate diagnosis of the specific type of material may provide important clinic and forensic clues.

## P-574

EXPERIMENTAL CRONIC RENAL DISEASE SLOWED BY CARVEDILOL TREATMENT IN SPRAGUE-D RATS.

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Hypertensive mechanisms are postulated to play a role in the progressive glomerulosclerosis after renal mass reduction. Previous studies have demonstrated differences in the progression to glomerulosclerosis with the use of anthypertensive drugs. We analyzed wether the use of Carvedilol (CVD), a new beta adrenoceptor antagonist and vasodilator slows de evolution of experimental glomerulosclerosis.

Fifty-four adult Sprague-Dawley rats were distributed among five groups: Sham, 5/6 Nephrec, 5/6 Nx 5mg CVD, 5/6 Nx10mg CVD, 5/6 Nx 20 mg CVD/day. Tail-cuff blood pressure, serum cratinine and urine protein were measured with an optical and morphometric study of glomerulosclerosis evolution.

Rats treated with 10 ant 20 mg/day/CVD showed controlled systemic blood pressure. The prevalence of glomerular lesions was closely associated with the degree of proteinuria. Vehicle-treated rats presented more than 75% of glomerular injury in 30% of glomeruli by contrast those treated with 20 mg/day/CVD showed it in the 10% of glomeruli. Renal injury was better prevented in those groups treated with 10 and 20 mg/day/CVD. Tuft enlargement was more prominent in vehicle-treated group, 1.5 times higher than group treated with 20mg/day/CVD.

Although, these data demonstrated the importance of systemic blood pressure control in the renal protective efficacy of Carvedilol, other less known mechanisms participate in the clinical and morphological evolution of this process and must be investigated.

## P-575

**SCLEROTIC FIBROMA. CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF 4 CASES.**

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**Aims.-** The Sclerotic Fibroma {SF}(also called Storiform Collagenoma) clinically appears in adult as a small pigmented nodule, distributed in a wide range of anatomic sites, with an invariably benign biological behaviour. Their histopathological findings are very distinctive. The SF can occurre as a solitary lesion or as multiple papules or nodules in patients with Cowden disease.

**Methods.-**Four patients presented with a solitary pigmented nodule, in varying locations, less than 1 cm. in diameter. In any case a clinical dignosis of SF was made. A conventional pathological study was performed. Immunohistochemical stains were carried out, using the ABC method, including the following monoclonal antibodies: VIM, DES, Collagen IV, A1-AT, A1-AQT, F-XIIIa, CD 34, NSE, and S-100.

**Results.-** All cases schowed similar histopathological findings.consisting in a dermal nodule, compose of interwoven fascicles of collagen bundles, arranged in a storiform pattern, with many clefts due to an artefact retraction between them The proliferating cells were positive for VIM, Collagen IV and F-XIIIa.

**Conclusions.-** The SF is a peculiar lesion that can be easily identified by their characteristic microscopical pictures, although, occasionally, a differential diagnosis must be made with others bening tumors, such as regressing dermatofibroma, tendon sheath fibroma, fibrolamellar nerve sheath tumor, and solitary myofibroblastoma. It has been pointed out that multiple SF is a cutaneous marker of Cowden disease The four cases of this report appeared as a solitary lesion.

## P-576

P53 GENE MUTATIONS AND P53 PROTEIN OVEREXPRESSION. THE MARKERS OF POOR PROGNOSIS IN BREAST CARCINOMAS

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**Aims**: Alteration in the *P53* tumor suppressor gene are the most frequent genetic changes found in breast cancer with an incidence in a range of 15% - 52% .The huge variations concerning the percentage of breast cancer cases with P53 changes are due to clinical staging of disease, histological [illegible] carcinomas, and different techniques used for detection. Till now, there has been no satisfactory explanation of discrepancy between the accumulation of P53 protein and *P53* gene changes. Additionally, there is a disagreement concerning the prognostic significance of *P53* gene mutations as well as P53 protein expression.

**Materials and methods:** The analysis is based on 75 unselected breast cancer patients. The immuno-histochemical reactions were performed on formalin-fixed, paraffin-embedded material using two different monoclonal antibodies: Pab1801 and BP53-12. Simultaneously, fresh material from the same tumours was investigated for the presence of *P53* mutations basing on analysis of exons 4-8 by PCR-SSCP technique. The results of these studies were correlated to other clinico-morphological parameters like histological grading, MiB-1 index, ploidy, ER and PgR status, expression of c-erbB-2 protein and survival of patients.

**Results:**

1. A significant proportion (app. 50%) of human breast carcinomas shows overexpression of p53 protein. The *P53* gene mutations were observed in 15% of cases studied.
2. The deletion within the splice acceptor site or the mutation in the immediate vicinity of the splice acceptor site leads to break in the protein production.
3. The abberant accumulation of p53 protein cannot be exclusively explained by p53 gene mutation or by overexpression of mdm2. Some additional factors must play a role in the stabilization of p53 protein during mammary carcinogenesis.
4. Patients with p53 overexpression have a significantly poorer disease-free- and overall survivals. The analysis of p53 protein expression together with the estimation of ploidy and c-erbB-2 overexpression allows to discriminate the group of patients in stage I and II with a higher risk of recurrence after surgical treatment.

## P-577

### EXPRESSION AND LOCALIZATION OF METALLOPROTEINASES IN COLORECTAL CANCER PROGRESSION

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Degradation of the extracellular matrix is one of the initial steps in invasion of malignant tumor cells. One group of proteases that have been particularly implicated in this process are the matrix metalloproteinases (MMPs).
The aim of this study was to investigate whether the expression of MMPs can be predictive of tumor invasion and metastasis (tumor stage).
We have studied the immunohistochemical localization of MMP-1, MMP-2, MMP-3 and MMP-9 in paraffin embedded sections from 70 colonic adenocarcinomas (Duke's stage A: n=10; stage B: n=29; stage C: n=27 and stage D: n=4). Immunohistochemical stains were performed with the ABC method.
We found that the presence of MMP-1 and MMP-2 in cancer cells was associated with grade of differentiation, and not with Duke's stage. The intensity and distribution of MMP-3 in carcinoma cells and MMP-9 in inflammatory cells, surrounding carcinoma correlated with cell differentiation, irrespective of tumor stage. The immunoreactivity in macrophages was weak or negative, and independent of tumor differentiation and stage.
These findings indicate that MMPs may play multiple roles in tumor progression.

## P-578

### THYMIC BASALOID CARCINOMA ARISEN FROM LATEROCERVICAL THYMIC CYST. DOES IT REALLY EXIST?

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We report a case concerning a 35 year old female patient who showed a right laterocervical mass. Clinically, this lesion was not painful and it rapidly had been going on rising a few months. Macroscopically it was mainly cystic, containing a milky fluid and it measured 3 cm wide as a maximum. Microscopically, the lesion mainly presented two types of cellular population: the first was composed by lymphocytes which resulted positive from immunostaining with CD-1a monoclonal antibody, and the latter by neoplastic, basaloid epithelial cells, resulted positive from immunostaining with PanCK and EMA monoclonal antibodies. Moreover, the epithelial cells were morphologically and histologically structured to form a manifest carcinoma, surrounding a probable pre-existent thymic cyst. Our diagnosis arises from an ENT examination, many "blind" biopsies (in absence of any suspicious areas) and a follow-up throughout 1 year, which all have presented results negative of occulted carcinomas. We know that some cases of laterocervical cystic metastasis of carcinomas exist, mainly arisen from upper aero-digestive tract (nasopharynx, retromolar trigone and posterior tonsillar pillar), but nowadays, we don't have any tangible evidence of our excluding the possibility that our diagnosis might be exact, except for just statistical information, even if, in literature, some cases had been described some cases concerning laterocervical cystic metastasis of carcinomas arisen from upper aero-digestive tract, with a follow-up throughout 11 years.

## P-579

### ANALYSIS OF THE EXPRESSION AND THE STRUCTURAL ALTERATIONS IN THE 5'NON- CODING REGION OF THE BCL-6 PROTO-ONCOGENE IN T CELL NON-HODGKIN'S LYMPHOMAS

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**Introduction:** BCL-6 proto-oncogene is a transcription factor with repressor function regulating B-cell development and differentiation as well as T cell dependent antibody response and the Th2 inflammatory response. The protein is exclusivly expressed in germinal center B lymphocytes and in a subpopulation of germinal center and perifollicular T lymphocytes. Rearrangements and/or mutations of the 5'noncoding region of the BCL-6 gene have been demonstrated in virtually all diffuse large B cell lymphomas (DLCL). Some but not all of these genetic alterations lead to overexpression of the protein, however it can also occur independently with the regard to the presence of a structural alteration. Lymphomas other than DLCL with germinal center cell origin and nodular, lymphocyte predominant Hodkin's disease also exhibit BCL-6 overexpression. Recently, anaplastic large cell lymphomas (ALCL) with T and null phenotype have been reported to show immunoreactivity with the BCL-6 antibody.
**Methods:** We collected a total of 35 T-NHL and subjected them to SSCP and Southern-Blot analyses to investigate the organization of the BCL-gene. Furthermore, immunohistochemistry was performed to detect the expression of the protein. The moleculargenetic and immunohistochemical analyses were then correlated.
**Results:** Wild type BCL-6 gene first exon-intron boundary region structure was detected in all but 3/13 (23%) peripheral T cell lymphomas (PTCL) by SSCP. Immunohistochemistry demonstrated the expression of the BCL-6 protein in 3/8 (37.5%) of T-lymphoblastic, 4/11 (36%) anaplastic large cell, 4/13 (31%) peripheral T cell and 1/2 (50%) angioimmunoblastic lymphadenopathy with dysproteinemia-like T cell lymphomas. The BCL-6 positive malignant cells exhibited various CD4+, CD8+ or CD4+/CD8+ phenotype. One case of PTCL harbouring mutations exhibited BCL-6 protein overexpression
**Conclusion:** BCL-6 protein expression can be detected in T-NHL's other than ALCLs. BCL-6 positivity is not restricted to the CD4+/ CD30+ cell population in all of the cases. Similarly to B-NHLs, the BCL-6 overexpression can be demonstrated independently of genetic alterations of the coding gene.This further suggests that molecular mechanisms other than rearrangements and/or mutations of the gene can result in the overexpression of the protein. Finally, structural alterations of the BCL-6 gene are rare in T-NHLs but mutations do occur in the 5'non coding region and seem to cluster in the PTCL category.

## P-580

### PALISADED MYOFIBROBLASTOMA OF THE LYMPH NODE – REPORT OF TWO CASES

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Palisaded myofibroblastoma (intranodal hemorrhagic spindle cell tumor with amianthoid fibres) is a very rare benign tumor of the lymph node.
We report two cases occurring in two adult women which were biopsed for lymph nodes enlargements in their right inguinal region. The tumoral masses were 3/2/2 cm, respectively 4/3.5/3 cm, well circumscribed, gray-whitish with hemorrhagic areas. The microscopic appearance was that of a tumor with blind-looking spindle cells arranged in intersecting fascicles with scattered areas of rosette-like collections of modified collagen fibres (amianthoid fibres). The spindle cells were positive for actin and vimentin and negative for desmin, S-100 protein and factor VIII-related antigen, features suggestive for a myofibroblastic origin of this tumor.
Due to the rarity of spindle-cell tumors in the lymph nodes (most of them are malignant, especially metastases from malignant melanomas, sarcomas or carcinosarcomas or, in HIV patients, primary Kaposi's sarcoma) the positive diagnosis of this entity is very important in order to avoid a useless and aggressive treatment.

## P-581

PATHOLOGY OF POST-CHERNOBYL THYROID CARCINOMA IN CHILDREN FROM THE BRYANSK OBLAST OF RUSSIA

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**Aims:** A considerable increase in childhood thyroid carcinoma (TC) has been observed after the Chernobyl nuclear accident (CNA). A study of the post-Chernobyl TC requires careful verification of morphological diagnosis. We carried out histological diagnosis verification and morphological study of TC in children from Bryansk Oblast (one of the areas of Russia contaminated with radionuclides after the CNA).

**Methods:** Paraffin blocks and histological sections from patients aged 0-19 at the time of the CNA, living in the Bryansk Oblast and surgically treated for thyroid carcinoma were obtained from different hospitals. At least three independent pathologists from Russia and the UK confirmed a diagnosis of the thyroid malignancy.

**Results:** One hundred thirty-seven cases of childhood thyroid carcinoma have been officially registered in the Bryansk Oblast from 1990 to 1998. Material from 105 cases was obtained for diagnostic review. A diagnosis of thyroid carcinoma was confirmed in 76 cases. Material for the remaining 29 cases was insufficient to confirm a diagnosis. Of the 76 confirmed cases, 5 were follicular thyroid carcinoma (FTC), 1 was medullary thyroid carcinoma (MTC), 66 were papillary thyroid carcinoma (PTC), 3 were classified as TC, not otherwise specified (NOS), and 1 was oxyphilic cell type of TC. Primary PTC was confirmed in 60 of the 66 PTC cases, and metastatic PTC - in the other 6 cases. The architecture of the primary PTC cases was as follows: 27 with classic papillary variant (CPV); 31 with solid-follicular variant (SFV); and two classified as papillary microcarcinoma. Primary PTC in younger patients at the time of surgery (8-14) was diagnosed as SFV more often (40%) than CPV (12%). PTC in patients over age 15 at surgery was diagnosed as CPV more often (33%) than SFV (12%).

**Conclusions:** PTC have been diagnosed in 86,9%; FTC - in 6,6%; TC NOS in 3,9%; oxyphilic cell type of TC - in 1,3%; MTC - in 1,3%. SFV of PTC is more frequent in patients under age 15; CPV of PTC is more frequent in patients over age 15 at surgery.

The study is supported by the International Consortium for Research on the Health Effects of Radiation, USA

## P-582

ADAPTATIVE INTIMA FIBROSIS OF TUMOR VESSELS AFTER PREOPERATIVE CHEMOTHERAPY OF CERVICAL CARCINOMA

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**Introduction:** After effective chemotherapy tumor parenchyma of cervical carcinoma is replaced by a fibrous connective tissue. Chemotherapy first eliminates fast proliferating tumor cells. Later, the small blood vessels regress. Larger arterioles and arteries are not easily destroyed. Due to the tumor regression and reduced need for blood, this blood vessels of a larger caliber would lead to increased tortuousness and narrowing of the lumens. The narrowing of the vessel lumens results from fibrosis of the intimal layer, which is called adaptative intima fibrosis (AIF). We describe AIF in three patients with cervical cancer after a complete or partial response to preoperative cytotoxic chemotherapy.

**Case reports:** Pt.1: A 44-year-old woman had FIGO stage IIIB squamous cell carcinoma of the cervix. The tumor volume was 100 ccm. After 6 courses of cytotoxic chemotherapy the tumor had regressed clinically and radical hysterectomy was performed. Histology of the cervix showed severe AIF and no residual tumor. Pt.2: A 37-year-old woman presented with FIGO stage IIIB adenosquamous carcinoma of the cervix. The tumor volume was 720 ccm. After 6 courses of cytotoxic chemotherapy radical hysterectomy was performed. Histology of the cervix showed moderate AIF and a residual tumor of 2x 4x3 cm. Pt.3: A 47-year-old woman had FIGO stage IVB squamous cell carcinoma of the cervix. The tumor volume was 250 ccm. After 4 courses of cytotoxic chemotherapy radical hysterectomy was performed. Histology of the cervix showed severe AIF and no residual tumor.

**Conclusions:** Our findings suggest that AIF, which has not been described in cervical carcinoma, is a morphologic correlate of tumor regression after chemotherapy. It may also be a prognostic factor. In cases with clinical and histologic complete response to preoperative cytotoxic chemotherapy (patients 1 and 3), the AIF constitutes a simple recogniable and predictable histological finding, referring to the therapy success. If residual disease will be found (patient 2), it is possible to define the reduction grade of tumor parenchyma according to the AIF.

## P-583

**QUANTITATIVE ANALYSIS OF NUCLEOLAR ORGANIZER REGIONS IN BREAST TUMORS**

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The aim of this study was the evaluation of morphometric measurements of the proliferation index - Ag $NOR_s$ in breast tumors.
Materials and Methods: 69 surgically removed breast tumors were studied. Paraffin-embedded specimens were stained with usual stainings and with argyrophilic Ploton method for Ag $NOR_s$. Morphometrical analysis was performed with a computer assisted image analyser- KS 400 program.
Results: The investigated specimens were classified as following: 9 cases of atypical ductal hyperplasias (ADH), 6 cases of ductal carcinomas *in situ* (DCIS), 27 cases of invasive ductal carcinomas, 5 cases of medullary carcinomas, 8 cases of lobular carcinomas *in situ* (LCIS), and 14 cases of invasive lobular carcinomas. Ag $NOR_s$ variables: number per nucleus, mean Ag $NOR_s$ area, coefficient of variation of Ag $NOR_s$ area and number, presented increasing values according to the increased degree of dysplasia and of invasion. Differences between histologic types, grades, and lymph node metastases ( $N_0$ / $N_{1-3}$ ) were recorded.
Conclusion: Ag $NOR_s$ morphometric variables represent significant valuable factors in differentiation dysplasias from carcinomas and in evaluating the degree of invasion of carcinomas, creating the premises of their application on materials obtained by fine needle aspiration cytology (FNAC), as a screening examination or in order to guide the surgical treatment.

## P-584

**DEMONSTRATION OF LAMININ, COLLAGEN IV AND α-SMA IN INTRADUCTAL SPREAD OF BREAST CARCINOMA**

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**Aims:** Studies on the histological characteristic and biological behavior of intraductal spread are critically important in that they may lead to the identification of a unique spread pattern rather than a noninvasive lesion in breast carcinoma.

**Methods:** Three groups of primary breast carcinoma, that are noninvasive ductal carcinoma, invasive ductal carcinoma with a predominant intraductal component (PIC) and invasive ductal carcinoma with intraductal spread (IS) were demonstrated. Laminin, collagen IV, α-SMA, MIB-1 and c-erbB-2 were assessed in 51 paraffin embedded specimens by using immunohistochemistry. Chi-square with Fisher exact tests was applied to evaluate significant differences by the Macintosh for Expert StatView 4.0 system.

**Results:** The basement membrane (BM) around intraductal lesion showed two patterns. One appeared as a thick membrane (lace pattern) composed of periductal angiogenesis. Another one appeared as a thin smooth membrane (linear pattern). Noninvasive ductal carcinoma as an early cancer, was well surrounded by BM, predominantly linear pattern. But PIC or IS showed fragmented or absent BM (both lace and linear) in some area or in most area of intraductal component in some cases, which indicates that some IS may in reality be invasive but mimic intraductal growth. A higher MIB-1 index and c-erbB-2 overexpression were also found in PIC or IS of invasive ductal carcinoma than in noninvasive carcinoma ($p<0.05$). Therefore, IS may be not only extended from noninvasive ductal carcinoma but also from invasive carcinoma.

**Conclusions:** IS may progress with higher potential of invasion and metastasis than noninvasive ductal carcinoma, because lack of BM or α-SMA in part of IS as well as higher c-erbB-2 expression in IS was recognized. Further investigations are needed to explain the different types (lace and linear patterns) of basement membrane around the carcinoma cells.

# P-585

## THE ESTIMATION OF RELAPSE- AND DEATH- RISKS AMONG PATIENTS WITH BREAST CARCINOMAS BASING ON HISTOLOGICAL AND IMMUNOHISTOCHEMICAL PARAMETERS

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**Aims:**It is now widely accepted that traditional factors of tumour size and lymph node stage can be used to predict biological behaviour in the form of overall survival and local recurrence in women with primary breast carcinoma The value of the other factors is still controversial.

**Materials and methods:** A total of 178 unselected consecutive breast cancer patients treated surgically at the Center of Oncology in Cracow, were evaluated. The paraffin slides from primary tumours were stained immunohistochemically and assessed for the presence of ER and PgR receptors, p53 protein, c-erbB-2, vimentin, as well as the expression of Ki67 (MiB-1) antigen.

**Results:**

1. Independent prognostic factors for relapse-free survival

| independent factors | relative risk | p-value |
|---|---|---|
| Number of involved lymph nodes | 35,12 | 0,00000 |
| Tumour growth pattern | 14,80 | 0,00012 |
| Ki67 (MiB-1) index (cut-off point 15%) | 8,18 | 0,0042 |
| Oestrogen receptor | 9,79 | 0,0018 |
| P53 protein score | 5,46 | 0,019 |
| DNA index | 4,57 | 0,033 |

2. Independent prognostic factors for overall survival

| independent factor | relative risk | p-value |
|---|---|---|
| Number of involved lymph nodes | 27,63 | 0,00000 |
| Tumour diameter | 8,54 | 0,0035 |
| Oestrogen Receptor | 5,92 | 0,015 |
| P53 protein score | 5,27 | 0,022 |

3. Basing on the above-mentioned parameters it is possible to plot the curves presenting the risk of relapse and death for an individual patient who underwent surgery for primary breast carcinoma.

# P-586

## THE EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 IN BREAST CARCINOMA

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**Aims:** The aim was to analyse the expression of intercellular adhesion molecule-1 (ICAM-1, CD54) in normal breast tissue and breast carcinoma cells, and to investigate the prognostic significance of this molecule.

**Methods:** The expression of ICAM-1 was analysed by immunohistochemistry and flow cytometry. Immunohistological analysis was performed on frozen sections of 81 breast carcinomas, among which 67 were of ductal NOS type and 14 were of special types (5 medullar, 5 lobular and 4 mucinous carcinomas). Flow cytometry was performed on 45 primary carcinoma samples and 20 carcinomas from lymph node metastasis. The results were compared to well-known prognostic parameters such are tumor size, lymph node status, mitotic index and tumor gradus as well as to the extent of lymphocyte infiltration.

**Results:** In normal breast tissue, endothelial cells, stromal fibroblasts and lymphocytes expressed ICAM-1, while ductal and acinar epithelial cells were negative. The breast carcinomas were heterogenous regarding ICAM-1 expression. The results of ICAM-1 expression, obtained by immunohistochemistry on the 67 ductal NOS carcinomas, indicate the correlation between the level of ICAM-1 expression and the extent of lymphocyte infiltration. By flow cytometry we found higher ICAM-1 expression in tumors with high mitotic activity, tumors with diameter over 2 cm and less differentiated tumors. In addition, medullary carcinoma cells expressed a higher level of ICAM-1 compared to the ductal NOS type. No difference in ICAM-1 expression between the primary carcinoma cells and cells from lymph node metastasis was observed.

**Conclusions:** The results suggest that ICAM-1 is upregulated in breast carcinoma and may influence the tumor progression.

# P-587

## CARCINOMA ARISING IN FIBROADENOMA

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**Aims:** The aim was to present five cases of carcinoma arising in fibroadenoma.

**Methods:** In all cases the clinical records, the pathologic protocols, the original slides and the paraffin blocks were available. In addition to examination of the original slides, selected blocks were recut and stained with hematoxylin and eosin. Every slide from each was screened by one of the thee pathologist for any evidence for ductal or lobular hyperplasia, atypia, carcinoma in situ or infiltrating. Infiltrating ductal carcinoma were typed according to Elston and Ellis in primary tumor site.

**Results:** The five patients were women whose ages ranged from 37 to 70 years (average 53,5 years). The size of the fibroadenomas varied (greatest dimension 1,1 - 5,8 cm, average 3,45 cm). All of them displayed the usual gross features of ordinary fibroadenomas, cut surface being gray-white, slightly firm, and fibrous. The histological appearance: lobular carcinoma in situ with infiltrating lobular carcinoma (four cases), infiltrating ductal carcinoma (one case), and two cases with contralateral infiltrating ductal carcinoma.

**Conclusions:** The prevalence of carcinoma within fibroadenomas in a screened population was reported as 0,02% by Deschenes and associates. Buzanowski-Konakry and associates identified five cases in review of 4,000 fibroadenomas examined over a 43-year period. The behaviour of these tumours is not well established, given that only a small number of cases with meaningful follow-up data have been reported.

# P-588

## QUANTIFICATION AND PROGNOSTIC VALUE OF BREAST TUMOR ANGIOGENESIS ASSESSED USING THE PHOTOSHOP-BASED IMAGE ANALYSIS.

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**Aims:** We performed a retrospective immunohistochemical evaluation of the tumor angiogenesis in 60 patients with breast carcinomas (40 with progression and 20 patients with stabilization of the disease).

**Methods:** Paraffin sections were immunostained using antibodies to FVIII-RA and the standard ABC/hpr method with DAB/$NiCl_2$ chromogen, without nuclear counterstaining. Using a PC-based image analysis system equipped with Adobe Photoshop 5.0 graphics software, microvessel density (MVD = no.vessels/$mm_2$) and the endothelial area (EA = no.pixels/$mm_2$) were evaluated in 20x adjacent fields (equivalent to 0,86 $mm_2$) in the tumor area of great vessel density. Results were analyzed using statistical method (SPSS program).

**Results:** Using the statistical method, the best cut off value which separated patients with progression of the disease from those with stabilization was 78,84 vessels/$mm_2$ for MVD and 19759 pixels/$mm_2$ for EA. A statistical significant correlation was observed between values of MVD and EA under the cut off and histological grading (G1), tumor size (pT1), lymph node status (pN0), stage of disease (I and IIa) and disease free survival (DFS). In particular: (1) among the pN0 breast tumors, all cases with MVD and EA values lower than the cut off had stabilization of the disease (no local or distant recurrence, and alive), while all cases with MVD and EA values greater than the cut off had progression of the disease (recurrence or death for disease); (2) all tumors with MVD and EA values under the cut off had a longer DFS.

**Conclusions:** Both MVD and EA at the cut of values of 78,84 vessels/$mm_2$ and 19759 pixels/$mm_2$ are prognostic indicators for disease progression in lymph node negative breast carcinomas and for DFS.

## P-589

**ANGIOSARCOMA OF THE BREAST ASSOCIATED WITH PREGNANCY**

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**Aims:** to present the case of a 23 years old woman with a breast tumor dignosed post-partum.

**Materials and methods**: After mastectomy and axillary lymph nodes exeresis, the tissue was fixed in formalin and embedded in paraffin. We performed HE staining for the breast tissue and for the lymph nodes and immunohistochemical method for the endothelial cells ( CD 34, vimentin, cytokeratin ), tumor hormonal receptors, and tumor proliferating factors.

**Results**: The tumor mass was multicentric, friable, hemorrhagic with necrosis and occupied the entire breast tissue. We found 6 axillary lymph nodes. Microscopically the tumor exhibits irregular vascular spaces with prominent endothelial tufting and papillary formations that contain cytologically atypical endothelial cells, solid atypical endothelial cell areas, areas of hemorrhage and necrosis. The tumor cells exhibit a high number of mitosis. The peripheral vascular component has neoplastic vascular channels that are structurally indistinguishable from the normal capillaries. Immunohistochemically, the tumor cells exhibit CD 34 and vimentin positivity, cytokeratin negativity. The lymph nodes were negative for metastasis.

**Conclusions**: The pattern of this tumor associated with pregnancy, the hormonal profile and tumor proliferating factors, strongly suggested a high grade angiosarcoma of the breast.

## P-590

**Medullary Carcinomas of the Breast Display Unique Characteristics with Respect to Proliferation and the Frequency of Apoptosis**

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**Aims**: Medullary carcinomas of the breast are encountered in less than 5% of mammary tumors in most series. Despite their poorly differentiation, these tumors exhibit a favorable clinical outcome and tend to have a lower overall frequency of axillary lymph node metastasis.

**Methods**: We determined the proliferation rate and apoptotic activity of medullary carcinomas of the breast as opposed to non-medullary tumors by means of MIB-1 immunohistochemistry and in situ detection of oligonucleosomal fragments (TUNEL reaction). The retrospective biopsy series included 11 medullary carcinomas as well as 15 randomly selected cases of invasive ductal carcinoma. Two patients in the medullary group and seven patients in the control cohort developed lymph node metastasis.

**Results**: The MIB-1 labeling index of medullary carcinomas averaged 83%, while that of the controls was of 30.03%. Apoptotic nuclei were present in a mean of 7.05% of medullary carcinoma cells. The control tumors exhibited an average apoptotic frequency of 5.85%. Tumor size, hormone receptor status and presence or absence of lymph node involvement were found not to correlate with either proliferation or apoptosis.

**Conclusion**: We conclude that medullary breast carcinomas are characterized by a peculiar "high proliferation-high apoptosis" situation. The more indolent clinical behavior of these neoplasms may possibly be accounted for by an effective apoptotic elimination of otherwise highly proliferating tumor cells.

## P-591

THE EXTENT OF APOPTOSIS AND PROLIFERATION IN BENIGN, PREMALIGNANT AND MALIGNANT EPITHELIAL BREAST LESIONS DETECTED BY TdT MEDIATED dUTP-DIGOXIGENIN NICK END LABELLING (TUNEL) AND KI-67 ANTIBODY.

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**Aims:** Disruption of tissue homeostasis may be a major factor in the multistep process of tumorigenesis. In this study we examined the extent of apoptosis and cell proliferation in benign, premalignant and malignant epithelial breast lesions in order to investigate the in vivo role of cellular turnover in the pathogenesis of breast cancer.

**Methods:** A total of 106 areas of breast lesions were studied from 40 cases. Apoptosis was quantified in tissue sections using the TUNEL assay, and cell proliferation using immunostaining with the Ki-67 antibody. Apoptotic index (AI) and proliferative index (PI) were expressed as the percentage of TUNEL and Ki-67 positive/100 cells.

**Results:** The AI range from 0.00 to 3.69.The extent of apoptosis was lower in apparently normal epithelium and low hyperplasia than in severe and/or atypical hyperplasia (p=0.000). The AI was higher in-situ carcinomas than in severe and/or atypical hyperplasia and in invasive than in in-situ. carcinomas (p=0.001 and p=0.002 respectively).The extent of proliferation was higher than apoptosis and the PI ranged from 0.00 to 33.2. The PI presented correlations similar to AI . A strong positive correlation between AI and PI was found (p=0.000). It was obvious from the mean values that PI increases much more than AI with the progression of epithelial lesions.

**Conclusions:** Our results showed that there is a gradual increase in the extent of apoptosis and cell proliferation from benign to premalignant and malignant lesions and a positive correlation between apoptosis and cell proliferation. These findings support the view that in the multistep process of breast carcinogenesis there is a deregulation of cell proliferation and induction of apoptosis.

## P-592

**HISTOLOGIC CHARACTERISTIC OF A TELANGIECTATIC OSTEOGENIC SARCOMA**

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**Aims:** to study histologic particulars of the telangiectatic osteogenic sarcoma in different age groups patients.

**Methods:** histologic sections of the bioptic and surgical materials of 20 patients (in ages ranged from 3 to 46 years) with diagnosis of the telangiectatic osteogenic sarcoma have been studied.

**Results:** histologic analysis reveals one or numerous cysts containing blood and (in thin septum separating cysts or in solid tissue areas) anaplastic mono- and multinuclear cellular elements intermittent with multinucleated giant cells of osteoclast-like type. In tumour cells with signs of anisocytosis and anisokaryosis there are noted the hyperchromatic nuclei with large single or numerous nucleoli, as well as significant increase of nucleo-cytoplasmic ratio and relatively numerous mitoses, including the abnormal ones. The numerous in some cases osteoclast-like cells sometimes have abnormal form. It is typical for them to be located at cysts edges and tumors cavities. In childhood age the telangiectatic osteogenic sarcoma are often characterised by: 1) not numerous anaplastic cells lining mainly the lumens of cysts and tumors cavities; 2) high degree of malignant cells differentiation; 3) osteoid unconsiderable formation; 4) presence of typical fields of aneurysmal cyst of bone.

**Conclusions:** histologic diagnosis of telangiectatic osteogenic sarcoma may be relatively uncomplicated (in presence of high degree of mononuclear cellular elements anaplasia). However in some cases, especially in children and adolescents (due to insignificant content of malignant cells and tumour osteoid and presence of tissue locations having structure of aneurysmal cyst of bone) diagnosis of telangiectatic osteogenic sarcoma is in great measure problematic and fraught with difficulties.

## P-593

**Alveolar Soft Part Sarcoma (ASPS) : an immunohistochemical study**

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**Aims**: ASPS have been considered of skeletal muscle origin because of the reported expression of desmin and myoD1 protein (Tallini *et al.* Am J Pathol 1994, 144: 693-701). To verify this histogenetic hypothesis we have performed an extensive immunohistochemical study with a panel including 3 recently produced anti myogenic regulatory proteins antibodies on 6 typical cases of ASPS.

**Material**: Representative paraffin-embedded neoplastic sections were stained with the anti MyoD1/myf3 antibodies 5.8A and 3A11 and with the anti-myogenin /Myf 4 antibody L026 using an unmasking method previously described (d'Amore *et al.* Pathologica 90, 700-701, 1998.). The expression of sarcomeric actin and desmin was also studied in all the tumors, whereas muscle specific actin (HHF35) and smooth muscle actin in selected cases. Finally the nuclear staining with the Mib1 antibody was measured with a Zeiss-Contron cytometer and the software "histology".

**Results**: The anti-MyoD1 antibody 5.8A resulted in a granular cytoplasmic stain of 5/6 ASPS; however no nuclear stain, typical of skeletal muscle differentiation, was observed. Completely negative results were seen with the antibody 3A11 and L026. Desmin and sarcomeric actin were focally detected in 1/6 ASPS (same tumor). Focal positivity was detected for smooth muscle actin (2/3) and HHF35 (1/2). Nuclear Mib1 staining was always low (range: 1-20% of the cells).

**Conclusions**: ASPS may express muscular antigens such as desmin and various actins, although in a minority of the cases; however in our series none of the myogenic regulatory proteins of the myoD family can be detected with a sensitive immunohistochemical method and these results casts doubts on the presumed skeletal muscle line of differentiation of ASPS. In addition their proliferative activity is always low despite the fact that ASPS behave usually as high grade tumors.

## P-594

PAPILLARY FIBROELASTOMA: CLINICOPATHOLOGIC ANALYSIS IN FIVE CASES.

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**Aims:** Cardiac papillary fibroelastoma ranks third among the primary benign heart tumors. It has long been considered an incidental autopsy finding. Recently these tumors can be detected in vivo by echocardiography thus enabling surgical removal.

**Methods:** Between 1992 and 1998 five cases of papillary fibroelastoma were observed at our Institute. Clinical diagnosis was achieved in four of the tumors by 2D-transthoracic and transesophageal echocardiography and were removed by surgery, one tumor was incidentally found during autopsy. Tumor-related symptomatology, echocardiographic features as well as histopathology of these tumors were reviewed. Immunohistochemical investigation included factor VIII-related antigen, CD-34, CD-68, S-100 protein, α-smooth-muscle actin, collagen IV and HLA-Dr antibody. Scanning and transmission electron microscopy (EM) were performed.

**Results:** There were no symptoms associated to the tumors in 3 cases, 1 had a transient ischemic attack and 1 had pulmonary embolism. Echocardiography revealed 11-19 mm sessile masses attached to the tricuspid leaflet, papillary muscle, mitral chordae and left ventricular outflow endocardium. Surgical specimens had similar appearance. In one case an 8 mm mass was found at autopsy on the tricuspid valve. Scanning EM revealed microvilli covered by plump endothelial cells. Acellular collagen accumulations and fragmented elastin fibrills, macrophages and fibroblasts were seen on transmission EM. Immunohistochemistry evidentiated vascular intimal markers.

**Conclusion:** The in vivo diagnosis of papillary fibroelastoma can be obtained by echocardiography. Pathologically these tumors were defined as endocardial hamartomas. Definitive recovery was achieved with surgical removal.

## P-595

SUCCESSFULL ORTHOTOPIC HEART TRANSPLANTATION FOR ACUTE MYOCARDIAL INFARCTION COMPLICATING CORONARY ARTERY ANEURYSM IN STAGE IV KAWASAKI DISEASE.

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Aims: We describe the pathologic features of multiple coronary artery aneurysms (CAA) in a case of orthotopic heart transplantation (HT) performed in a young female for acute myocardial infarction (AMI), complicating undiagnosed stage IV Kawasaki disease (KD).

Methods: A 30 year old female was admitted to our Hospital for AMI. Emergency coronary angiograms showed occlusion of the.major subepicardial coronary arteries .After PTCA and saphenous vein graft coronary artery by-pass critical haemodinamic parameters persisted. Emergency HT was successfully performed because of a compatible donor graft could be obtained .

Results: The heart was dissected and multiple CAAs were demonstrated , affecting the proximal branches of major subepicardial coronary arteries . CAAs were occluded by stratified thrombi .No lesion could be identified in the remaining coronary artery branches. The left ventricle was affected by transmural AMI involving 80% of the ventricular mass.Histological examination of the CAAs wall showed evidence of coronary artery vasculitis in the healing phase,consistent with stage IV KD. Pathologic diagnosis was eventually confirmed by accurate rewiew of the clinical history that showed 2 previous admittance to our institution 25 and 19 years respectively for fever,mucocutaneous rush , and lymphoadenopathy.

Conclusions:KD is a rare cause of ischemic heart disease and sudden death.We describe the pathologic findings in a case of previously undiagnosed stage IV KD successfully transplanted for AMI

## P-596

**HISTOLOGICAL CHANGES IN THE HEART WITH IMPLANTED ARTIFICIAL HEART VALVES (AHV)**

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**Aim:** Fifteen cases with implanted AHV were analysed from autopsies performed in the Pathological Institute at the Faculty of Medicine in Skopje.

**Methods:** In this analysis the standard autopsy tehnique, autopsy protocols, gross heart specimens, histological sections as well as clinical data of each case, were used. Seven cases had AHV of Bjork-Shilley type, 6 Starr -Edwards, 2 Medronic, and in one case a bioprothesis was used. Isolated mitral AHV was seen most frequently (9), double aortal and mitral valve was found in three cases, and the mitral AHV with tricuspid correction by annuloplastc or commissurotomy were found in 4 cases.

According to the clinical data, the most frequent disease was mitral tenosis caused by rheumatic endocarditis.

**Results:** The group represented patients of the age from 22 to 58 years. The postsurgical period was up to 30 days in 3 cases and up to 36 months in 12 cases. Thrombosis of the valves in 8 cases, malfunction in 2 cases and haemorrhage in 1 case were the complications associated with the artificial heart valves.

Histological analysis on the junctional area of the prothesis and the ring presented granulomas in 3 cases, degenerative calcifications in 4 cases and endocarditis in 2 cases. Hypertrophy on the myocytes in 9 cases and fibrosis of interstitial, perivascular and subendocardial type in 9 cases were also found. In some areas myocytes were damaged: band necrosis and myocytolysis were seen, as well as changes suggesting acute myocarditis 2 cases.

**Conclusion:** Our analysis leads us to the conclusion that the previous condition in the heart, valvular disease and the congestive heart failure are influence on the effect of the reconstructive valvular surgery.

## P-597

**The Pathogenesis of Graft Vessel Disease after Heart Transplantation: Immunohistochemical Evidence of Early Endothelial Injury**

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*Aims*: Graft Vessel Disease (GVD) after heart transplantation (HTx) is characterized by diffuse proliferation of vascular smooth muscle cells (SMCs) in the large and small vessels, but the mechanisms of this phenomenon still remain unknown. The aim of this study was to ascertain whether investigations of right ventricular endomyocardial biopsies (EMBs) sustain the hypothesis that endothelial injury is associated with a proliferation of SMCs within the terminal vascular system, and whether there is the possibility of quantifying the differences in the number of affected blood vessels in the studied population.

*Methods*: The population consisted of 41 patients who underwent HTx due to terminal heart failure: 15 patients (four women, 11 men; mean survival time 57 months) with angiographic evidence of GVD and 26 patients (five women, 21 men; mean survival time 59 months) with no angiographic signs of GVD. The paraffin-embedded right ventricular EMBs (n=272) were immunohistologically stained with α-actin (SMCs), factor (F) VIII (250 000 kDa, von Willebrand factor in endothelial cells (EC), indicator for membrane injury) and H & E. The observation time included the first 14 months after HTx. All results were analyzed using $\chi^2$- and Mann-Whitney tests.

*Results*: 1. There were no significant differences between the two groups with regard to their demographic data. 2. The angiographic evidence of GVD was associated significantly with an increased number of endomyocardial α-actin- and F VIII-positive blood vessels ($p<0.01$). 3. The proliferation of vascular SMCs showed a time dependent increase while the number of F VIII-positive blood vessels did not change. 4. During the observation time there was a significant positive correlation ($p<0.05$) between α-actin- and F VIII-positive vessels.

*Conclusions*: 1. These results permit the hypothesis that the interaction of EC and SMCs play a crucial role in the development of GVD after HTx. 2. In this context, F VIII-positive membrane injury seems to initiate/promote the proliferation of vascular SMCs. 3. An increased number of α-actin- and F VIII-positive blood vessels seem to have a prognostic value for the development of GVD after HTx and completes the present diagnostic tools.

## P-598

ORGINISATION OF POLYCOMPONENT VASCULAR PROTHESIS IN EXPERIMENT

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**The aim** of the present investigation was to study the organization dynamic of polytetrafluorine-ethylene (PTFE) porous vascular prosthesis.

**Material and Methods:** In 56 dogs (15-20 kg) the implantation into the abdominal aorta, femoral arteria of vascular prosthesis 5-9sm long and 6-10mm in diameter was performed. The prosthesis consist of two membranes - internal fenestrated PTFE and external PTFE without pores. The duration of the prosthesis functioning was 1-300 days. The specimens of vascular-prosthesis anastomosis were obtained in the different time of experiment. The paraffin-embedded sections were stained by haemotoxylin -eosin and by picrofuxin.

**Results:**The The formation of connective tissue adventicia between the layers could be seen in 3-4 weeks without connecting the prosthesis to surrounding tissues and inflammation. It can improve the biological inertness of the material. At the same time the internal membrane was covered with hin fibrin layer. After 8 weeks the internal layer was covered with young connective tissue containing fibroblasts and collagen fibers, growing through the pores. After 8 month period the of connective tissue was firmly connected with the internal layer of the prosthesis and covered with the endothelium from the inner part. The external layer stayed not connected to the formed inner part. Inflammation was not detected in all periods.

**Conclusion** The present results can proof the organization of this type prosthesis by mean of connective tissue growth through the pores of the inner membrane. Also the PTFE prosthesis seems to be biologically inert.

## P-599

**Computer-based Analysis of the Terminal Vascular System – Novel Approach in Diagnosis of Graft Vessel Disease after Heart Transplantation ?**

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*Aims*: The prevention of disease depends on its diagnosis, which should be early, easy to make and feasible. Diagnosis of Graft Vessel Disease (GVD), the main limiting factor to the long-term survival after heart transplantation (HTx), lacks of all these. The aim of our study was to investigate the morphological changes of the terminal vascular system in order to create a new method of diagnosis for small vessel disease in endomyocardial biopsies of right ventricles.

*Methods*: We studied 28 patients (six women, 22 men; mean age at HTx 47 years) who underwent HTx due to terminal heart failure. They underwent right heart catheterization in post-transplant management. The paraffin-embedded rejection control samples (n=162) were immunohistologically investigated with α-actin (smooth muscle cells, SMCs), CD 105 (transforming growth factor-β receptor), CD 31 (glycoproteine in endothelial cells (EC)) and factor (F) VIII (indicator for membrane injury of EC). The observation time was 14 months after HTx. Computer-based quantitative morphometry was done at x200. All data were analyzed using $\chi^2$ - and Mann-Whitney tests.

*Results*: 1. There was an increase in α-actin, CD 31 and CD 105-positive blood vessels in all patients, whereas the number of the F VIII-positive vessels did not change. 2. In all seven patients with angiographic evidence of GVD there was a higher amount of α-actin-, CD 31-, CD 105- and F VIII-positive cells than in patients without large vessel disease. 3. After laying down a fixed limit of positive vessels in all four stainings in the first month after HTx, patients were rearranged in two new groups: one with and one without evidence of small vessel disease. 4. The group with evidence of small vessel disease showed significantly more α-actin-, CD 31-, CD 105- and F VIII-positive vessels within the first three months after HTx ($p<0.05$). 5. All patients with GVD on coronary angiography were also present in this morphometrically scored GVD group.

*Conclusions:* 1. Computer-based morphometry could be performed in all right ventricular rejection control samples after HTx. 2. Combined with immunohistochemistry it is an easy, fast and feasible method for quantifying changes within the terminal vascular system. 3. Computer-based morphometry completes the present tools used in diagnosis of GVD after HTx. 4. It may offer the possibility of prediction of GVD independantly from coronary angiography.

## P-600

IMMUNE INFLAMMATION OF ARTERY WALL DURING ATHEROGENESIS.

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**Aims:**Investigation of cellular and molecular aspects of atherogenesis

**Methods:** The material of 26 urgent autopsies (within 1.5 - 3 h) after death from acute cardiovascular insufficiency, together with IHD of patients aged 45-65, was mainly used in the study. Scanning and transmission electron microscopy was applied. We used mono- and polyclonal antibodies against CD4, CD8, CD68, CD40, CD40L, IL-1β, TNF-α.

**Results:** Immunoregulatory signaling molecules (CD40-CD40L) are shown to play important, and probably key role in the initiation of atherosclerotic lesions of arteries. Adhesion and migration of monocytes and T-lymphocytes (Th1) occur on endothelium, producing CD40L and IL-1β. Monocytes, not transformed into foam cells, T-lymphocytes ($CD4^+$) and smooth muscle cells express CD40 CD40L and produc TNF-α. It is likely that a pathological response similar to the delayed-type hypersensitivity reactions is brought forth via self-regulation mechanisms. Macrophages and T-lymphocytes producing pro-inflammatory cytokines and free radicals provoke a peroxide modification of apo B-containing lipoproteins.

**Conclusions:** Focal development of immune inflammation is considered as the important condition in initiation and progress of atherosclerotic damage of arteries.

# P-601

**ISCHEMIC AND REPERFUSIONAL ULTRASTRUCTURAL CHANGES OF MYOCARDIAL CAPILLARIES AND CARDIOMYOCYTES DURING OPEN HEART SURGERY**

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**Aim:** To assess ultrastructural changes of myocardial capillaries and cardiomyocytes due to ischemia and reperfusion during open heart surgery.
**Methods:** We evaluated 61 consecutive patients (pts) who underwent open heart surgery. 215 byoptical samples were taken from right ventricle anterior wall, and from the apical region. They were taken during ischemic period (5, 10, 20, 30 min starting cardyoplegia (CPL), and at the end of CPL) and during reperfusion period ( 5-30 min after beginning of this period). All samples were analyzed by electron microscope using quantitative stereological analysis. Volume fraction of the pinocite vesicles (PV), mitochondria (M), and endoplasmatic reticulum (EPR) were calculated. All pts were devided into 2 groups based on duration of CPL - ischemia; group 1( CPL < 65 min, 19 pts), and group 2 ( CPL>65 min, 42 pts).
**Results**: Damages of the endothelial cells in all samples occurred 5 minutes after introduction of CPL and progressively increased during ischemic phase. The worse damage was found after 65 min of CPL usage: capillaries obstruction due to microclasmatosis, edema of the endothelial cells, widening of the cell junctions, degenerative changes of the cardiomyocytes. Stereological analysis confirmed that the larger damages were found during reperfusion in group 2 (Table).

| Variable | Group 1 | | | Group2 | | |
|---|---|---|---|---|---|---|
| | ischemia | reperfusion | Pvalue | ischemia | reperfusion | Pvalue |
| PV(Vv) | 0.052±0.002 | 0.043±0.001 | <0.05 | 0.033±0.001 | 0.028±0.001 | <0.01 |
| MV(Vm) | 0.024±0.002 | 0.025±0.001 | NS | 0.022±0.001 | 0.017±0.001 | <0.01 |
| EPR(Vr) | 0.026±0.001 | 0.026±0.001 | NS | 0.025±0.001 | 0.023±0.001 | <0.01 |

**Conclusion**: Our study has shown that endothelial cells and cardiomyocytes are very sensitive to hypothermia, hypoxia and reperfusion during open heart surgery. Ultrastructural changes depend on the length of CPL, and are particularly induced by reperfusion.

# P-602

**THE ROLE OF ENDOMYOCARDIAL BIOPSY IN PATIENTS WITH HYPERTROPHIC CARDIOMYOPATHY**

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The aim of this study was to determine the role of pathohistological (PH) findings in endomyocardial biopsy (EMB) samples obtained from pts with clinicaly suspected hypertrophic cardiomyopathy (HCM).
**Methods:** During a 15 year period (1984–1998) we have analysed over 1050 cases of EMB. In 42 pts. (28 males and 14 females, aged from 16 to 66 years, mean 55.3) clinicaly suspected diagnosis of HCM was established. The samples were taken from the left ventricle, with an average of 3.1 sample per pts., routinelly processed and stained with H&E and ElvG, Masson, PAS. In 10 cases electron-microspcopy analysis was performed. HCM was diagnosed by using the semiquantitative index. Points from 0 to 3, for each of the 5 following changes were evaluated: disarray, "short runs" of myocardial fibers, degree of hypertrophy, perinuclear haloes, and amount of fibrosis. The maximum that could be obtained was 15, and results over 50% of the score, confirm a diagnosis of HCM. Results from 30% to 50% of the score were suggestive of HCM, and findings below 30% means no PH confirmation of HCM.
**Results:** EMB of 24 pts showed histological findings characteristic for HCM (57.1%); in additional 12 pts (28.6%) clinical diagnosis was changed, and nonspecific findings were present in 6 pts (14.3%). In 5 pts findings of early stage of dilated cardiomyopathy was found (transition process in advanced cases); in 3 pts diagnosis was changed to myocarditis (2 focal and 1 border-line), and in remaining 4 pts, amyloid heart disease (2 pts), glycogenosis and small vessel disease was diagnosed.
**Conclusion:** Our results show that EMB is usefull in determining diagnosis of HCM, especially when changing suspected clinical diagnosis. No PH feature alone is pathognomonic, and only combination of findings allow confident assesment of HCM.

# P-603

**LESIONS OF SINOATRIAL NODE IN CORONARY HEART DISEASE**

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**Aim:** To study the morphological lesions of sinoatrial node (SAN) in coronary heart disease (CHD).
**Methods:** The SAN and adjacent myocardium in 36 necropsy cases of CHD were examined by complex of histological, histochemical and fluorescent microscopic methods.
**Results:** In chronic myocardial ischemia the volumetric correlation "muscular parenchyma/stroma" in nodal tissue is modified, prevailing the fibroconnective component. A similar tendency was observed in microcirculatory system, the density of capillaries being 3-4 times lower than in the control group of the same age. Significant distrophic lesions have been revealed in the central artery of SAN, particularly in its elastic membrane. These lesions cause the thickening of arterial wall, the reduction of its elasticity and the increase of rigidity, that impairs the pulsation of sinusal artery. Considering that the structural elements of SAN tightly interwoven with the external membrane of the sinusal artery, are really "an enormous adventitia" of this vessel, the mentioned lesions may perturb the function of SAN. In cases of myocardial infarction acute heterogenous distrophic and haemodynamic disturbances manifested by inomogenity of enzymatic activity and intensity of specific cardiomyocites fluorescence, focal destruction of endothelium, the dissociation and plasmatic infiltration of vascular wall, the punctiform haemorrhagies have been observed in SAN.
**Conclusions:** The morphological lesions of SAN in CHD may cause acute disturbances in the process of cardiac rhythm generation.

# P-604

**SUDDEN DEATH IN CASES OF HYPOPLASIA OF THE LEFT CORONARY ARTERY IN YOUNG PEOPLE DURING PHYSICAL ACTIVITY**

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**Aims:** Detection of the implications of hypoplasia of the left coronary artery on the myocardium function.
**Methods:**Anatomical dissection of coronary arteries, seriatim, in order to detect their distribution.
**Results:** During 1966-1998 period, extreme hypoplasia of the whole left coronary artery (LSD) was diagnosed in 25 cases.
In 12 cases (10 men and 2 women; between 16-28 years old, at the moment of death), extreme hypoplasia of LCA was isolated as anomaly.Its origin was in the stenotic and thick-walled main trunk, with stenotic lumen, 3.5 mm long. Both main branches were also very thick-walled and also had stenotic lumen. Left circumflex coronary artery (LCxA) ended in the medial half of the left anterior atrio-ventricular sulcus, and anterior descending coronary artery-LAD dominated and ended as posterior descending coronary artery.
In 7 cases , the cause of death was masive transmural infarction of myocardium of left ventricle which occurred suddenly without previous clinical symptoms.
In 13 cases ( 9 male and 4 female, aged between 45 minutes and 4 years, average age at the time of death 6.5 momths), extreme hypoplasia of SCA was accompanied with other congenital heart diseases (CHD): complete transposition of great arteries-4, atresia of aorta-3, hypoplasia of the left heart-2, aortal and mitral atresia-1,double outlet of right ventricle-1, mitral atresia-1, and anomalous connection of all pulmonary vein in left vein antonym-1. In 1 case, the cause of death was ischemia of myocardium of the left ventricle.
**Conclusions:** Sudden death in case of hypoplasia of LCA has specific significance in young , healthy people, where it was not previously diagnosed, in case of higher physical strain.

## P-605

CYTOLOGY OF CLEAR-CELL CARCINOMA OF THE GENITAL TRACT

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**Aims:** To define the cytomorphologic features of clear-cell carcinoma of the genital tract.

**Methods:** The study consisted of 4 clear-cell carcinoma of the genital tract with clinical and histological confirmation performed. Cytologic findings were obtained from May-Grünwald-Giemsa (MGG) stain of 3 fine needle aspiration materials and a peritoneal fluid cytology. All of the fine needle aspiration materials were obtained from supraclavicular lymph nodes with metastasis.

**Results:** Primary tumors of the patients in which the supraclavicular lymph node aspiration material had been examined were in ovary in two patients and one in vagina. The peritoneal fluid cytology was obtained from the patient with uterine cervical tumor. Cytologic findings were similar in all preparations. The cells had abundant, pale, finely vacuolated cytoplasm with indistinct cytoplasmic membrane. the nuclei were round to oval, with fine chromatin and small, round nucleoli. The characteristic feature of clear-cell carcinoma of the genital tract was basement membrane-like substance. This hyaline extracellular material was stained pinkish to purple red in MGG preparations, and frequently observed within the cancer cell clusters. In ascites fluid psammoma bodies were also observed.

**Conclusions:** The cytomorphologic characteristics of clear-cell carcinomas of the genital tract are distinctive, and the entity may easily be diagnosed even on the metastatic locations.

## P-606

**THE ACTUAL ACTIN POLYMERIZATION DEGREE AND REACTIVITY IN CLINICAL DIAGNOSTIC, DEMONSTRATED IN WHOLE BLOOD WITH A NEW METHOD**

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**Aims:** Reorganization of polymerized filamentous (F)-actin in polymorphonuclear leukocytes (PMN) occurs within seconds. For diagnostic purposes it is necessary to conserve the actual, native polymerization state. We have developed a simple method which makes it possible to fix whole-blood leukocytes within fractions of seconds and preserves F-actin in it's native state. The basal and chemoattractant-activated PMN F-actin content in healthy test persons and patients with severe trauma and sepsis was investigated.

**Methods:** F-actin polymerization was stimulated by adding FMLP to fresh whole blood before fixation; controls remained unstimulated. 100µl whole blood were shock-cooled to the freezing point by mixing it with 1ml of a formaldehyde-glycerol-mixture precooled to -8°C. After fixation the red blood cells were osmotically lysed. F-actin was stainded with FITC-phalloidin and quantified by flow cytometry.

**Results:** PMNs of control persons showed a uniform F-actin content while stimulated cells showed less uniformity and increased F-actin content. Unstimulated PMNs of patients with severe trauma showed marked interindividual variabilities in F-actin polymerization. FMLP stimulation resulted in a heterogenous F-actin pattern, characterized by the formation of two PMN populations with different F-actin content. During recovery the F-actin conformation normalized.

**Conclusions:** Shock-cooling and low temperature fixation conserves F-actin in or near the native state and can be used to define an organism's inflammatory state. The simplicity of the method enables a broad applicability for the clinical routine. Measurement of F-actin contents in PMNs may be a useful tool to for diagnosis and therapy monitoring of inflammatory deseases.

## P-607

CAVITY LYMPHOMA AND KAPOSI'S SARCOMA IN A VIH SEROPOSITIVE PATIENT

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**Aims:** this type of lymphoma involves pleural, pericardial and peritoneal cavities without mass lesion; usually the diagnosis is cytological and biopsies are negative.

**Methods**: a 31 year's old male, homosexual, VIH seropositive and with a diagnosis of Kaposi's sarcoma with nodal involvement. Two months later, the patient developed a pleural effusion. A biopsy and cytology were performed; apart from routine diagnostic methods, immunocytochemical (CD20, CD79a, CD3, UCHL1, CD45, CD30, ALK and EMA), heavy-chain rearrangement, Epstein-Barr virus (EBV) and herpesvirus 8 (HHV8) by PCR were done.

**Results**: pleural biopsies were negative; the cytology showed lymphoid cells with irregular and lobulated nuclei with conspicuous nucleoli and often with two nucleoli; cytoplasm was *amphofilus* and abundant with occasional vacuoles and *perinuclear halo*. Immunocytochemical profile of these cells was: CD45, CD30 and EMA positive. Heavy-chain rearrangement, EBV and HHV8 virus were demonstrated by PCR. CHOP chemotherapy produced a partial remission, but nowadays (4 months after diagnosis) the pleural effusion still persists.

**Conclusions:** clinical, morphological, immunological and molecular findings are unique in this entity. As in our case, the diagnosis is made cytologically with persistent negative biopsies of pleura, pericardium or peritoneum. This type of lymphoma is always associated with HHV8, the same virus related to Kaposi's sarcoma. The most part, are diagnosed in VIH patients. EBV association is only present in VIH patients, so pathogenic implication of this virus is not probably. Prognosis is poor with median survival of 2-4 months; local chemotherapy is discussed as an alternative to classical therapy because this is an entity without dissemination.

## P-608

**CORRELATION BETWEEN CYTOLOGICAL AND HISTOLOGICAL DIAGNOSES OF INTRAOCULAR TUMOURS**

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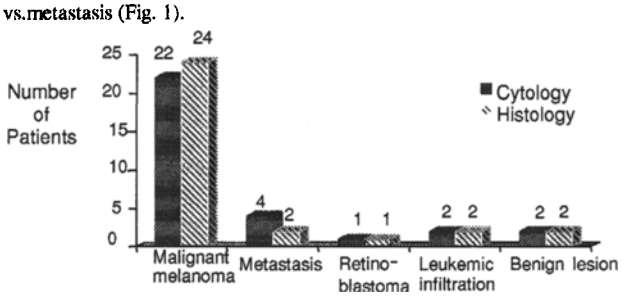
A correct diagnosis is essential when selecting therapy for intraocular tumours. In the period 1991-1998, we performed 95 Fine Needle Aspiration Biopsies (FNAB) together with the Eye Dept., The National Hospital, Norway. Of these, 31 also had a histological diagnosis.

**Material and methods:** All 31 patients were examined with FNAB which was performed through transscleral,translimbal or transvitral route by cooperation between ophthalmologist and cytologist. These eyes were later also examined histologically based on biopi/excision/enucleation. The material was prepared for standard staining and for immunohisto-, cyto-chemistry staining.

**Results:** There were only 2 cases with discrepant results melanoma vs.metastasis (Fig. 1).




**Conclusions:** There is good correlation between the cytological and the histological diagnoses. FNAB is simple, gives quick preoperative information which is of great importance for the determination of future treatment. Cooperation between experienced ophthalmologists, cytologists and pathologists is important.

## P-609

ANALYSIS OF CD43 POSITIVE CELLS INFILTRATIONS IN THYROID ASSOCIATED WITH FOCAL AND MIXED HASHIMOTO'S THYROIDITIS

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Hashimoto's thyroiditis is by far the most common of thyroid disorder, usually with no clinical manifestations. CD43 molecule is sialoglycoprotein which provides anti-adhesive barrier on cell surfaces, impending the function of several types of receptors.
The aim of the study was to analyse quantitatively of CD 43 positive cells in 40 cases (20 mixed and 20 focal thyroiditis). Measurements were performed in fixed paraffin-embedded and immunostained representative sections of thyroid tissue.
The CD43 values for mixed [MT] and focal thyroiditis [FT] were, respectively: 338+/- 109 cells/cm2 and 102+/-39cells/cm2 in intraepithelial compartment, 7670+/-1810cells/cm2 and 1074+/-249cells/cm2 in stroma compartment, 9689+/- 2732cells/cm2 and 459+/-114cells/cm2 in fibrosing stroma ( scattered cells). In stromal infiltrates CD43 fomed 20% of all cells in MT and FT. Characteristic CD43 cords were observed around atrophic thyroid follicles.
It is concluded that, owing to the abundance of CD43 positive cells only in mixed form of Hashimoto's thyroiditis, measurement of the cells has a diagnostic value, and CD43 should therefore be included in the cytological antibody panel.

## P-610

THE PECULIARITIES OF METABOLISM IN ADENOHYPOPHISIS AND ADRENAL CORTEX CELLS IN ACUTE PERITONITIS

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The aim of the investigation: the study of enzyme activity in the cells of adenohypophisis (AH) and cortical adrenal zone (CAZ) taking part in hormone synthesis and secretion in acute experimental peritonitis.
Methods: fecal peritonitis (FP) was simulated on white rats acid phesphatase (acid p'tase), alkaline phosphatase (alk p'tase), NAD, NADP, succinate – denydrogenase (SDH) and key enzyme of steroidogenesis – 3-b-ol steroiddehydrogenase (3-b-ol-SDH) were revealed on cryostat sections, in AH and CAZ cells of intact and experimental animals. A quantitative evaluation of histochemical reactions was done by scanning method using radiation microspectrophotometer apparatus. ACTH and corticosterone were determined by radioimmunologic method and molecules of mean mass – by spectrophotometer. The information authenticity was evaluated by student.
Results: endotoxicosis results in increasing progressive dyscirculatory impartments in microvessels and destructive changes in the cells of endocrine glands.In addition to it autherntic decrease of SHD is noted there, in adrenal glands NAD and NADP levels decrease, but 3-b-ol-SHD level doesn't change. On the contrary acid p'tase and alk p'tase activity increases. Fluctuations of enzyme activity do not prevent from synthesis and secretion of ACTH and corticosterone. Their concentration in blood increases for by certain 24 hours. By the third day of the experiment enzyme activity in AH and CAZ cells approaches to the initial level. This is followed by concentration decrease of adaptive hormones.
Conclusions: in spits of the decrease of oxidative phosphorylation in adenocytes and corticocytes in toxemia AH and CAZ cells can participate in the reactions of organism non – specific resistance when abdominal cavity is infected.

## P-611

MORPHOLOGICAL CHARACTERISTICS OF SKIN DIABETIC MICROANGIOPATHY IN CORRELATION WITH RETINOPATHY

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**Aims:** to find correlation between skin biopsies microangiopathy and retinopathy in diabetes.
**Methods:** Skin biopsies from 120 diabetic patients (33 with NIDDM treated with diet only or with oral hypoglycemic; 38 NIDDM treated with insulin and 49 with IDDM) were analyzed hystologicaly and hystochemically in an attempt to determine diabetic microangiopathy. All biopsies were stained on HeEo, PAS and elastic tissue (Weigert resorcin fuchisin)
All patients were exanimate on presence of diabetic retinopathy by ophtalmoscopy. We analized also the age, the duration of the disease.
**Results:** Microangiopathic changes were presented in all skin biopsies. They were characterized by a constant homogeneous or fibrillary thickening of the basement membrane of the vessels and arteriole hyalinosis, activation of endothelial cells and vascular permeability disturbance. The percentage of PAS positive capillaries was in correlation with the degree of diabetic microangiopathy. Biopsies were positives on elastic tissue staining. The degree of microangiopathy was in correlation with the dura tion of the diabetes. Retinopathy was present in: NIDDM on diet or oral hypoglycaemics in 22 ; in the group of NIDDM treated with insulin 27, and in 26 with IDDM.
**Conclusions:** We found strong correlation ($p<0,05$) between the presence of the diabetic retinopathy, the degree of the skin diabetic microangiopathy and duration of Diabetes. There was no correlation found with the type of Diabetes.

## P-612

A RETROSPECTIVE STUDY OF THYROID GLAND PATHOLOGY AND THE ENVIRONMENT OF THE INDUSTRIAL REGION.

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**The aim** of the present study was to examine the dynamics of incidences of thyroid gland pathology over the period of the last 15 years in industrial region of Ukraine.
**Methods:** 720 cases of thyroid gland disease during the period from 1983 to 1997 have been studied in the industrial region of Donbass (Lugansk, Ukraine). The dynamics of specific weight of thyroid gland malignant tumors among the other pathology is given in the table.

| Type of pathology | 1983-1985 | 1986-1988 | 1989-1991 | 1992-1994 | 1995-1997 |
|---|---|---|---|---|---|
| Benign tumours | 21 | 30 | 14 | 12 | 12 |
| Malignant tumours | 15 | 44 | 36 | 46 | 30 |
| Goitre | 77 | 51 | 95 | 82 | 60 |
| Thyroiditis | 13 | 9 | 14 | 25 | 34 |
| Total number | 126 | 134 | 159 | 165 | 136 |

**Conclusions:** The data presented here show an increased incidence of thyroid gland pathology including malignant tumours as a result of an accident at Chernobyl Atomic Station.

## P-613

**FINE NEEDLE ASPIRATION IN THE DIAGNOSIS AND THERAPY OF THE THYROID NODULE**

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**Aims:** The role of fine needle aspiration (FNA) is commented, as representing one of the most important methods for thyroid nodule diagnosis, which is able to provide valuable information about the benign or malignant nature. The usefulness of FNA, as a therapy procedure for some types of nodules (e.g. kystiques, haemoragic) is also discussed.
**Methods:** We performed FNA under echographic or scyntigraphic control in 336 cases with unique or multiple thyroid nodules. The smears obtained by aspiration were stained by Giemsa method. The number, type and origin of the cells, as well as the normal or pathological aspects were studied by light microscopy.
**Results:** A number of 141 cases presented uninodular goitre and 104 cases, multinodular goitre; in 75 cases we found kystique nodules, and in 16 cases, adenomatous nodules. At the patients with nodular goitre the cytology was benign. In a single case there were noticed some cells with suspect features, but the histological exam, achieved after extirpation, showed the benignity of the lesion. In the group with kystique nodules (some of which were haemoragic), FNA also played a therapeutic role, by simple aspiration or by instillation of ethanol. In 18 cases only, the nodules were recommended to be extirpated, but the histological exam proved a benign nature.
**Conclusions:** In our opinion FNA can represent the first step in the diagnosis algorithm, ensuring the possibility to differentiate the benign from the malignant nodules. Thus, a simple, sensitive and specific method is available for the selection of the cases requiring surgical intervention.

## P-614

THYROID CANCER IN PRECARPATHIAN ENDEMIC GOITER REGION

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**Aims:** The research of morphological features of thyroid cancer in Precarpathian endemic goiter region.
**Methods:** The retrospective analisys of archive data and conclusions of histological examinations of thyroid gland from 11001 patients was performed. All these patients were operated on thyroid gland in Lviv Regional Hospital at the period 1923-1998. Quantitative analysis of the material was carried out with the help of personal computer.
**Results:** Thyroid cancer (TC) in 1923-1930 was found in 14.9%, in 1931-1970 in 2.9-5.6% among all patients. In the last 28 years the number of TC increased again by two times and was in 1981-1990 10.6%, in 1997-1998 12.4%. In the years 1923-1930 among the total number of TC papillary carcinoma was found in 28.5%, follicular carcinoma - 7.1%, undifferentiated carcinoma - 57.1%. In the years 1981-1990 papillary carcinoma equaled to 65.0%, follicular carcinoma - 17.2%, undifferentiated carcinoma - 3.9%. In the years 1997-1998 among total numb er of TC papillary carcinoma was found in 78.6%, follicular carcinoma - 8.3%, undifferentiated carcinoma - 2.8%. Medullary carcinoma was found at the first time in 1971-1980, in the years 1997-1998 it increased to 6.9% among total number of TC. Papillary microcarcinoma and oxyphilic cell carcinoma (papillary or follicular) were found only in the years 1971-1998.
**Conclusions:** The increase of number of TC among all operated patients with thyroid pathology and the increase of number of papillary carcinoma among all number of TC were found at the analysing period in Precarpathian endemic goiter region. TC, especially early forms, was found in patients with another thyroid pathology, more frequently with autoimmune thyroiditis, multinodular goiter.

## P-615

**APOPTOSIS AND COLORECTAL CARCINOGENESIS**

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**Aims**: the progression and growth of cancer are regulated by cell proliferation and cell loss, the latter of which is associated with apoptosis, or programmed cell death (PCD). PCD is controlled by inducers (ie p53), and suppressers (ie bcl-2), and the balance between these stimuli determines whether the cell cycle enters mitosis or apoptosis. The relationship between PCD, bcl-2 protein, p53 expression, and cell proliferation (PCNA index) was studied to determine each of their roles in colorectal carcinogenesis.
**Methods**: twenty-six sporadic adenoma specimens subdivided in early and late, 25 FAP adenomas, and 34 colorectal carcinomas were used in this study. PCD was identified and evaluated using the in situ end-labeling method while an immunoperoxidase technique was adopted to detect bcl-2, p53, and PCNA proteins.
**Results**: PCD index was 9.4%±3.3% in sporadic adenomas (1% in early and 14.6% in late), 10.2±3.8% in FAP adenomas, and 6.3%±1.2% in carcinomas. PCNA Index was 39.1±22% in sporadic adenomas (30.5% in early and 46% in late), 36.9±18.1% in FAP adenomas, and 41.5±29.6% in carcinomas. Five sporadic adenomas (3 early and 2 in late) were bcl-2+, vs 12 FAP adenomas and vs 8 carcinomas. Fifteen sporadic adenomas (1 early and 14 late) were p53+ vs 16 FAP adenomas, and 21 carcinomas. None of patients with bcl-2+ carcinoma died during the follow-up whereas only 51% of bcl-2 negative patients survived.
**Conclusions**: during the colorectal carcinogenesis, bcl-2 resulted to be an early event while p53 gene mutation a later event. Moreover, we have demonstrated an imbalance between cell proliferation (increased) and cell death (decreased), probably due to the possible action of bcl-2 in the inhibition of programmed cell death in the colorectal carcinogenesis. More difficult remains to explain the role of bcl-2 in patient survival, even if in other reports its expression resulted to be associated with a more favorable outcome.

## P-616

**LIGHT AND ELECTRON MICROSCOPIC STUDY OF IMMEDIATE AND LATE LEAD-INDUCED HISTOPATHOLOGICAL CHANGES IN RATS (WITH SPECIAL REFERENCE TO THE RETINA)**

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and Dpt. of Anatomy. Kasr el Aini Cairo University**. Egypt.

**Aims:** Since the literature reviewing the lead-induced retinal changes was limited, so the aim of this study was planed to evaluate such changes.
**Methods:** The present study consumed 30 albino rats; 15 newborn and 15 adults. Ten rats from each group were subjected to a daily dose of lead oxide. Light microscopic examination of brain liver, kidney and retina was done. The latter tissue was further examined by electron microscopy.
**Results:** The results of the present research disclosed a severe retardation in growth. There were obvious lesions in brain, liver and kidney of rats subjected to lead. These lesions were more in growing than adult rats. The lead induced retinal changes were more in growing than adult. It affected all the retinal layers specially the retinal pigment epithelium, photoreceptor and ganglion cell layers as well as the blood vessels.
**Conclusion:** Lead toxicity triggers a series of lethal effects that ends in retinal atrophy, as well as severe damage to other vital organs. This should direct the attention of authorities responsible for protection of the environment to put strict measures on industries for prohibition of lead containing compounds.

## P-617

TYROSINE PHOSPHORYLATION AFTER SUPERFICIAL INJURY IN ISOLATED GUINEA PIG GASTRIC EPITHELIUM

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Superficial (microscopic) mucosal injury and subsequent immediate repair is known to be asssociated with induction of proliferation within the proliferative zone of the mucosa. The precise mechanism of this induction is unknown. Aim: To study the phosphotyrosine immunoreactivity of the mucosa (PTYR-IR) after superficial injury as a sign of involvement of tyrosine receptor signalling in the induction of proliferation. Methods: Guinea pig gastric epithelium was separated from the muscularis layer, mounted in Ussing-chamber and injured with 1.25M NaCl. Subsequently, the tissue was perfused (37°C; pH7.0) for 4h and analyzed morphologically. During the perfusion the electric resistance of the tissue was recorded and the tissue was exposed (L/S) either to 200/uM genistein or to 100 nM EGF-TGF , an antagonist or agonist of tyrosine phosphorylation. Proliferative activity (PI) was assessed by immunohistochemistry morphometrically (number of immunopositive cells per 300 cells) after staining of the tissue against Ki-67 nuclear antigen. PTYR-IR was assessed by immunohistochemistry after staining of the tissue with monoclonal phosphotyrosine antibody. Results: Superficial injury induced a significant decrease of PTYR-IR of the tissue. This reduction could not be modulated with exogenous genistein or EGF-TGF . The mean PI of of the injured tissues was 23.0±0.5 and that of uninjured controls 11.8±1.4 after 4h recovery (P<.05). The exposure of the tissue to genistein during the recovery decreased the PI to 2.3±0.5 (P<.05).Both electrophysiologic and morphologic recovery were sensitive to genistein. Conclusions: Superficial mucosal injury results in a significant and independent reduction of phoshotyrosine immunoreactivity (PTYR-IR) of the tissue. Nevertheless, the tyrosine kinase pathway is sensitive to genistein and its inhibition is reflected in the electrophysiologic and morphologic recovery, and, in the PI of the tissues.

## P-618

ORGANOTYPIC SKIN CULTURE AS IN VITRO MODEL FOR SKIN GRAFT-VERSUS-HOST DISEASE

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**Aims**: The aim of this study was to try to reproduce graft-versus-host (GVH) reaction in vitro using organotypic skin cultures as in vitro model.

**Methods**: Epidermal and dermal tissues from dog skin as a source of keratinocytes and fibroblasts were used to reproduce artificial skin (organotypic skin cultures). Additionally, peripheral blood mononuclear cells from the skin donor were used as stimulators in bulk-mixed leukocyte culture (MLC) with responder cells from an unrelated dog leukocyte antigen (DLA) non-identical dog. On day 6, the cell suspension was harvested and used as a source of cytotoxic activated lymphocytes to be injected into organotypic skin cultures. Controls consisted of the same number of autologous lymphocytes added to the organotypic skin cultures and to cultures incubated only with media.

**Results**: Histologically, some of the morphological changes characteristic for skin GVH reaction were locally present at the site of injection of activated lymphocytes, and keratinocytes showed major histocompatibility (MHC) class II antigen membrane expression. This is consistent with the pathologic findings in skin biopsies obtained from patients with clinical skin GVH reaction. Keratinocytes in control organotypic skin cultures did not show any epidermal damage, and failed to show MHC class II antigens expression.

**Conclusion:** This culture system represents a promising in vitro model for studies in marrow transplantation where skin GVH disease develops.

## P-619

PROMOTION OF RAT CARDIAC ALLOGRAFTS SURVIVAL BY INTRATHYMIC INOCULATION OF DRUG DELIVERY SYSTEMS OF IMMUNOMODULATING AGENTS

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**Aims:** Immunosuppressive agents used in organ transplantation, including corticosteroids and cyclosporine, have many sides effects and may alter the physiology of normal and grafted organs. That is why, we developed a method preparation of carboxymethylcellulose lipid microcapsules of FK-506 with an entrapped alloantigens (AAg) from donor cells (DDS-FK-506/AAg).

**Methods:** For the purpose of studying the effects of allograft rejection, two protocols were investigated: a) recipients received intrathymic injection of DDS-FK-506/AAg for 10 days before heterotopic heart transplantation; b) hosts received a similar dose of the drug for 10 days before engraftment, but only by intravenous route. Graft survival increased from about 4 days in b) group animals to 10-12 days in a) group ($P < 0.001$).

**Results:** Histological studies of allografts at day 5 showed that intrathymic injection of DDS-FK-506/AAg resulted in only a mild reduction in cell infiltration, but in a marked decrease in graft edema and interstitial haemorrhage. Hearts explanted as early as 4 days after transplantation in b) group demonstrated lymphocytic infiltrates and evidence of myocyte necrosis.

**Conclution:** We conclude that this model with intrathymic inoculation a single dose of DDS- immunosuppressiv agent concurrently with alloantigen significantly inhibited donor-specific responsiveness to rat cardiac allografts.

## P-620

ANGIOGENIC SWITCH IN A LONGITUDINAL STUDY OF A BRAIN TUMOR MODEL

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Background : it has been suggested that the switch to an angiogenic phenotype can separate two stages in the development : the prevascular phase and the vascular phase. The purpose of the present work is to demonstrate the existence of an angiogenic switch in a longitudinal study of a brain tumor model during tumor growth by means of microvessel density measurements.

*Methods :* the study was performed on 32 rats bearing C6 glioma. At different stages of tumor growth, the histological aspects were described and sections were immunostained for factor VIII-related antigen in order to highlight microvessel endothelial cells. Microvessels were counted at 400 magnification for different areas (central non necrotic area, peripheral area, contralateral grey and white matter area), using image analysis software.

Results : vessel density was significantly higher at the tumor-brain interface than in the center of the tumor or in the contralateral cortex. The vessel density remains stable in the tumor during the first 3 weeks after cell implantation, after which a clear increase of vessel density can be observed.

Conclusions : the present study demonstrates the presence of an angiogenic switch which is concomitant with the development of necrosis and pseudopalisading pattern.

## P-621

MIKROSCOPIC PICTURE OF HEALING PROCESS OF ORAL MUCOSA WOUNDS IN GUINEA PIGS EXPOSED TO TABACCO SMOKE.

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On the basis of clinical observations, it was found that the healing process of postoperative wounds of the mouth in smoking patients was less effective. The aim of our experimental investigation was to check the influence of tobacco smoke on mucosa wounds healing. So the process of mucosa wounds healing in guinea pigs and in guinea pigs constantly exposed to tobacco smoke. The permission to perform the experiment was obtained from Animal Study Bioethical Board, Silesian Medical University. The study was performed on 76 guinea pigs which were divided into 2 control groups (K1 - 4 pigs and K2 - 24 pigs) and 2 study groups, 24 guinea pigs each (D1 and D2). The animals in control groups were not exposed to tobacco smoke (K1 - not wounded, K2 - wounded). Study groups included animals with a cut in mouth mucosa exposed to tobacco smoke (D1 - 20 cigarettes/day, D2 - 40 cigarettes/day). The tobacco smoke exposure time was 3 months altogether, including 3 months after the surgery. The clinical and histopathological evaluation was performed after the 3rd, 7th, 10th, 14th, 18th and 21st day after the surgery (K2, D1, D2). After the animals were sacrificed a samples of healthy tissue were taken (group K1 after the 21st day) and samples of wounded tissue (K2, D1, D2), mouth musoca, liver and kidneys. It was found that healing process in guinea pigs exposed to smoke was increased 3 days, relatively to the amount of smoke received. It was shown that healing process of mucosa of guinea pigs exposed to smoke was accompanied by chronic inflammatory reaction which delayed cicatrization process. It resulted in low quality scar, especially in animals from group D2.

## P-622

### CANINE MAMMARY TUMOURS A MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY: COMPARATIVE ASPECTS WITH HUMAN BREAST TUMOURS

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**Aims:** To use canine mammary tumours as a model to study human breast carcinogenese, we revised histologically 137 cases using the modified diagnostic classification by WHO (1981).

**Methods:** Hematoxilin-eosin staining was used to perform the histological classification and streptavidin-biotin-peroxidase technique for the immunohistochemical study. 22 benign and 33 malignant tumours were selected for immunohistochemical studies, using antibodies for progesterone receptors (PR), Ki-67 (MIB-1), c-erbB2 and p53.

**Results:** Out of 137 cases, 63 (45.9%) were benign tumours and 74 (54%) were malignant tumours. Benign mixed tumour was the most frequent benign tumour (31.9%) and the ductal invasive carcinoma was the most common malignant tumour (14.6%). PR expression was observed in most of the normal and benign canine mammary tissues. However, in malignant tumours the number of cases with PR expression was smaller. In mixed tumours MIB-1 expression was mainly observed in epithelial cells and less frequently in proliferate areas of spindle cells, condroid and bone metaplasia. In malignant tumours, most of the positive cells were present at the periphery of the tumours. P53 expression was seen in less differentiated malignant tumours. C-erbB2 was positive in almost all tumours.

**Conclusions:** Although a difference was observed in the incidence of morphological types between canine and human mammary tumours, a similarity could be observed between histological types and expression of prognostic markers in these tumours. Therefore, this study strengthen the relevency of the canine mammary tumours as a model for the study of human breast carcinogenese.

## P-623

### ROLE OF THE HOST MACROPHAGES IN TUMOUR GROWTH AND METASTASIS IN SYRIAN HAMSTERS: *IN VITRO* STUDIES

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**Aim:** To study a possible role of the host macrophages (Mϕ) in tumour selection and progression a discrete characteristics of Mϕ-selected sarcoma cells, originating from a single spontaneously transformed hamster embryo cell line (STHE strain) and differing in tumorigenic and metastatic activity, were assayed *in vitro*.

**Methods:** The STHE tumour cells studied were established by successive cycles of *in vitro* co-cultivation with resident and LPS-activated Mϕ. Cytotoxic activity (CTA) of activated Mϕ and exogenous hydrogen peroxide ($H_2O_2$) was examined by $^3$H-thymidine release assays; CTA of recombinant TNF-α was assayed by a photometric crystal violet dye cytotoxic technique; gap junctional intercellular communicating capacity was studied by a scrape-loading Lucifer Yellow transfer method in the confluent monolayer cell cultures.

**Results:** We found that (a) in contrast to low-malignant parental cells and STHE cells selected with resident Mϕ, the malignant STHE cell variants *in vitro* selected with LPS-activated Mϕ were all significantly more resistant to CTA of both activated Mϕ and $H_2O_2$; (b) the parental STHE cells and their malignant variants were all uniformly resistant to recombinant TNF-α mediated cytolysis; (c) the malignant STHE cell variants inhibited the ability to communicate with neighboring cells.

**Conclusion:** Collectively these results suggest a selective role for activated macrophages in tumour progression, and it is suggested that it may be connected with reactive oxygen intermediates (mainly, $H_2O_2$) produced by these effector cells. E-mail: *fincross@mailbox.riga.lv*

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## P-624

### ERBB-2 AND NM23 EXPRESSION IN ENDOMETRIAL CARCINOMA

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**Aims:** This study was undertaken to examine the prognostic and possible diagnostic value of erbB-2 and nm23 expression in relation to other clinicopathologic features in patients with endometrial carcinoma.

**Methods:** Expression of the erbB- 2 and the nm23 anti-metastatic gene product was examine by immunohistochemistry in formalin-fixed, paraffin-embedded tissue of 86 endometrial carcinoma.

**Results:** ErbB-2 and nm23 were positive in 64% and 23,3% of cases, respectively. No association was found between these two genes (p=0,063). ErbB-2 was associated only with adenosquamous histological subtype (p=0,044), whereas expression of erbB-2 product was found in 11 of 12 (92%) adenosquamous carcinomas. No association was found between erbB-2 oncogene and histologic grade, depth of myometrial invasion, vascular space invasion and FIGO stage.

Absence of nm23 staining was positively correlated with higher tumour grade (p=0,0065), deeper myometrial invasion (p=0,02) and vascular invasion (p=0,028). No association was found between nm23 immunostaining and histologic subtype or FIGO stage. Both gene products did not significantly correlate with oestrogen and progesterone receptor level.

**Conclusion:** ErbB-2 oncogene product can not be used as prognostic marker in endometrial carcinoma but it might be adopted as diagnostic marker for adenosquamous histologic subtype. Anyway, investigation must be done on larger number of tumours. Since nm23 correlated with tumour grade, depth of myometrial invasion and vascular invasion, loss of its expression in endometrial carcinoma may be helpful in predicting metastases and expansion of disease. Both genes can not be used in predicting survival period.

## P-625

**EFFECTS OF TAMOXIFEN ON STEROID HORMONE RECEPTORS, HORMONE CONCENTRATION AND DNA CONTENT BY FLOW CYTOMETRY IN ENDOMETRIAL CARCINOMA**

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**Aims.** The aim of this study was to find out whether tamoxifen has an effect on hormone steroid receptors, hormone concentration, DNA content and proliferative activity in endometrial cancer and to correlate the tamoxifen induced changes with clinical stage, tumor differentiation, depth of invasion and histologic type.

**Methods.** Thirty postmenopausal women with endometrial carcinoma were treated with 30 mg of tamoxifen daily for 7-10 days after the curretage. Steroid hormone receptors (ER and PR), levels of FSH, LH, prolactin, estradiol, progesterone, testosterone, DHEAS, SHBG, and DNA ploidy and proliferative activity were determined before and after the therapy. The patients were also divided into favorable and unfavorable prognosis groups according to classical histological parameters.

**Results.** After the treatment, there was a net increase in the PR and SHBG, and a significant decrease in the ER. The increase in PR and decrease in ER occurred in the patient group with favorable prognosis regarding histologic type, degree of differentiation and clinical stage, but also in the unfavorable prognosis group regarding the depth of myometrial invasion. Statistically significant decrease in the FSH concentration was observed in the groups with favorable prognosis regarding histologic type, depth of myometrial invasion, and grade of differentiation. Concentration of SHBG was significantly increased in groups with favorable prognosis if histologic type and grade of differentiation were taken into account.

**Conclusions.** We conclude that tamoxifen therapy can induce progesterone receptor synthesis even in tumors with low initial progesterone receptor levels, making such tumors potentially responsive to additional hormonal therapy with progesterone.

## P-626

**Adenomyosis: frequency and association with pato-anatomic changes.**

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**Aims:** Adenomyosis refers to the presence of islands of endometrial glands and stroma within the myometrium with or without hyperplasia of the myometrium.

**Methods:** To evaluate the prevalence and associated pato-anatomical changes, the clinical records and hysterectomy specimens during a 2 year period (549 patients) were retrieved. Data were collected on indication for the hysterectomy, presence of dyspareuni, menstrual pain, chronic pain, age, parity, and former transcervical procedures. All histologic diagnosis were reviewed.

**Results:** Depending on the distances of the adenomyotic islands from the endometrium the occurence of adenomyosis varied between 10%-18%. We defined adenomyosis as islands of glands and stroma more than 3 mm from the endometrium, together with myometrial hyperplasia; 68 (12%) women fulfilled these criteria.
The data analysis showed significant association between the presence of endometrial hyperplasia and adenomyosis, but there was no association with endometriosis, endosalpingiosis, leiomyomas, polyps or endometrial carcinoma. Furthermore there was no significant association with dyspareuni, menstrual pain, chronic pain or indication for hysterectomy and adenomyosis.

**Conclusion:** The results might indicate that adenomyosis could be associated with estrogene and progesterone receptor status.

## P-627

**Immunohistochemical localization of metallothionein in the human endometrial lesions**

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**Introduction:** The aim of the present study was to investigate the role of Metallothioneins (MT) in endometrial lesions, in correlation with the expression of estrogene (ER) and progesterone (PgR) receptor content, p53, bcl-2 and proliferation indices.

**Methods**: 65 cases of endometrial carcinomas and 34 cases of hyperplasias with or without atypia, were studied immunohistochemically using a monoclonal mouse antibody (E9) against a conserved epitope of I and II isoforms

**Results:** MT expression (>5% of neoplastic cells) was observed in 6.2% of carcinomas, a focal MT positivity was detected in 16.9% of carcinomas while in 76.9% of carcinomas completely lacked MT expression. In the group of hyperplastic lesions MT expression was only detected in small foci in 3.3% of the cases and especially in the atypical hyperplastic lesions. A statistically significant difference of MT expression was observed between carcinomas and the group of hyperplasias ($p=0.0014$). MT showed statistically significant tendency far higher expression in the cases of FIGO stage IC than in those of FIGO IA,B stage ($p=0.03$). Carcinomas with squamous metaplasia showed higher MT expression ($p=0.005$) than these without squamous metaplasia. A statistically significant inverse association between MT expression with estrogen (ER) receptor content($p=0.0025$) and progesterone (PR) receptor ($p=0.0016$) content was observed in all cases. No correlation between MT expression with the grade of differentiation, p53, bcl-2 and the proliferation indices (PCNA, MIB1) was found.

**Conclusion:** These data suggest that MT expression is an early event in endometrial carcinogenesis, which maybe is regulated by estrogen and progesterone and it could be used as an additional biological marker indicating aggressive endometrial lesions.

## P-628

**MORPHOLOGIC VARIANTS OF UTERINE SARCOMAS**

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***Aim: to determine histologic variants and frequency of uterine sarcomas on operative material.***

**Methods: We studied clinics, diagnostics and histologic structure of uterine sarcomas. After general and gynecologic examination patients under went panhysterectomy. Histologic analysis was performed at the level of light microscopy by histologic and histochemical methods.**

**Results: Within the period of 11 years (1988-1998) 5674 women having uterine neoplasia were operated. Uterine sarcomas were diagnosed in 131 (2,3%) patients, uterine cancer – in 1824 (32,1%) patients. Uterine sarcomas constituted 6,7% of all malignant tumours. The age of the patients varied between 30 and 70 years. More than 70% of uterine sarcomas were revealed in the age group 50-69. Histologic types of sarcomas were the following: endometrial stromal sarcoma – 52, leiomyosarcoma – 43, carcinosarcoma – 20, mesodermal mixed tumour – 15, malignant hemangiopericytoma –1. Postoperative radiotherapy was performed.**

**Conclusion: Clinical and histologic study of uterine sarcomas has been performed.**

## P-629

**BRENNER TUMOR: HISTOLOGIC, HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL INVESTIGATION**

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**Aims:** The modern classification of ovarian tumors based on histogenetic principles is clinically important in the evaluation of prognosis and differential therapy. Since the histogenesis of Brenner tumor (BT) has been linked with the urothelial nature, the purpose of this study was to compare the histochemical and immunohistochemical patterns of BT with transitional bladder cells.

**Material and Methods:** Out of the total of 1130 ovarian tumors diagnosed over the period between 1989 and 1998, there were only 7 BT, 6 benign and 1 proliferating. In addition to the hematoxylin and eosin, periodic acid-Schif, mucicarmine and alcian blue were utilized. Selected sections were immunostained for keratin, carcinoembryonic antigen, epithelial membrane antigen, chromogranin and neuron-specific enolase. The same methods were utilized for selected bladder tumors of the transitional cell type, grade I - II. **Results:** Cells of BT and bladder epithelium revealed the presence of glycogen in all cellular layers, and an alcianophilic surface mucous coat. Immunohistochemically, carcinoembryonic antigen, epithelial membrane antigen, keratin reaction, chromogranin and neuron-specific enolase reactivity were found in BT and urothelium.

**Conclusion:** The common histochemical and antigenic pattern of BT cells and urothelium point to their common origin, which is an indirect confirmation of the hypothesis that BT cells derive directly from the müllerian system of the female peritoneum, which, due to the relationship of the gonadal ridge to the mesonephros, preserves the ability to undergo transitional cell differentiation.

## P-630

ENDOMETRIAL CHANGES IN TAMOXIFEN-TREATED PATIENTS

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**Aim.** Antiestrogenic effect of tamoxifen (TAM) treatment of hormonal dependent breast cancer significantly increases the survival rate. This therapy progress may be complicated with proliferate endometrial stimulation inclusive malignant transformation. The TX similarity to the diethystilbestrol is discussed as a probably causative factor. We describe six patients with endometrial abnormalities associated with prolonged TAM breast cancer therapy.

**Method.** A search of the files at Univ. Dept. of Obst. and Gynecol., Hradec Králové, from 1996 to 1997 identified 93 patients treated with TX for hormonal dependent breast cancer. In twenty of them, the vaginal ultrasound encountered the endometrial abnormalities. Six women were selected from the mentioned set because of vaginal ultrasound and/or morphological examination were performed before the onset of TAM treatment, i.e., the diagnosed changes may be noted as a very probably side effect of the TAM therapy.

**Results.** Six patients aged between 43 and 63 years (mean, 59 years) were treated with TAM (20 mg daily dosage) in time period between 9 and 36 months (mean, 28,5 months). Four of them were presented with hyperplastic endometrial polyp with focal atypical hyperplasia in one lesion. In one woman, the endometrial well differentiated mucousal carcinoma was diagnosed after 27 months of TAM treatment. In sixth postmenopausal patient, the uterine adenomyosis was diagnosed.

**Conclusion.** Endometrial changes as a possible side effect of TAM treatment are usually formed of hyperplastic polyps. Periodical gynecologic assessment should be a part of prevention of more serious lesions such as atypical hyperplasia or malignant transformation. As a rare complication of TAM therapy, the uterine adenomyosis was described in one postmenopausal patient.

## P-631

**P53 AND BCL-2 EXPRESSION AND CHEMORESISTANCE IN OVARIAN CARCINOMAS.**

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**Aims:** We look for a possible relationship between the p53 and bcl-2 proteins expression and the response to chemotherapy in ovarian carcinomas.

**Methods:** Formalin-fixed paraffin-embedded tissue sections obtained from 62 ovarian carcinomas of different histologic types (31 serous, 11 endometrioid, 4 mucinous, 2 clear cell, 3 undifferentiated and 11 compound) and FIGO stages (I, 11 cases; II, 9 cases; III, 30 cases and IV, 8 cases) were studied. The expression of p53 and bcl-2 proteins was determined by immunohistochemical staining, using Mab anti-p53 (DO-7) and anti-bcl-2 (cl.124) (DAKO). The specimens were graded by semiquantitative method. All patients selected had received first-line, platinum-based combination or mono-chemotherapy.

**Results:** The patients examined included 6 cases with absent response (progressive disease), 16 cases with partial and 40 cases with complete clinical response to chemotherapy. P53 expression: 33/60 (55%) cases showed positive staining for p53 and 27 were negative. Bcl-2 expression: 39/62 (63%) cases stained positive for bcl-2 and 23 were negative. Correlation between p53 and bcl-2 expression and the response to chemotherapy: The patients were distinct in two main groups, including respectively those with absent and partial response (PR) or those with complete response (CR) to therapy. Eleven (55%) of the 20 patients with PR and 22 (55%) of the 40 patients with CR were p53 positive. Fourteen (64%) of the 22 patients with PR and 25 (63%) of the 40 patients with CR were bcl-2 positive.

**Conclusions:** No association between p53 and bcl-2 expression and response to chemotherapy was found.

## P-632

**ARE STEROID RECEPTORS USEFUL IN DISTINGUISHING ATYPICAL HYPERPLASIA FROM WELL DIFFERENTIATED ENDOMETRIAL ADENOCARCINOMA ?**

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**Aims**: To evaluate if the immunohistochemical investigation of estrogen and progesteron receptors (ER, PR) is useful in distinguishing atypical hyperplasia (AH) from well differentiated endometrial adenocarcinoma (AC).

**Methods**:histerectomy specimens were immunohistochemicaly investigated for ER and PR epithelial positivity on paraffin embedded tissues. The morphological diagnosis was as follows: 10 cases of AH alone, 67 cases of well differentiated endometrial adenocarcinoma from which 25 cases were associated with AH, and 20 cases with other degrees of endometrial hyperplasias. The percentage of positive epithelial cells and the staining intensity were appreciated on a scale with 3 levels.

**Results:** In EH without atypias there is an uniform positivity for both receptors and the staining intensity is high.There is a decreasing of both ER and PR from EH, to AH and AC. In AH and AC there is a very important inter-case variability of ER and PR positivity. Some cases were high positive for one or both receptors while others had a low positivity. The staining intensity was also variable, in general moderate.

**Conclusions** : There is a decrease of ER and PR positive epithelial cells from endometrial hyperplasias without atypias to atypical hyperplasia and well-differentiated endometrial adenocarcinoma. Between AH and G 1 AC the differences are not statistically significant. This feature and in plus the great inter-case variability of the ER and PR positivity sustain that immunohistochemical investigation of steroid receptors is not useful in the differential diagnosis of atypical hyperplasia and well differentiated endometrial adenocarcinoma but may be of great value for the therapeutic decision in both lesions.

## P-633

**GOSEKI GRADING AND GASTRIC CANCER**

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**Aims.** Goseky et al. have described a novel grading system based on architectural findings and functional property of the cells to add to TNM staging system in the ability to predict outcome. They differentiated 4 groups of GC: tubules well differentiated, intracellular mucus poor (group I); tubules well differentiated, intracellular mucus rich (group II); tubules poorly differentiated, intracellular mucus poor (group III); tubules poorly differentiated, intracellular mucus rich (group IV). Our aim was to apply this histological grading to our case histories of GC to verify its prognostic value.

**Methods**: new sections from 80 specimens of Advanced GC were stained with Alcian/PAS to assess for tubular differentiation and intracytoplasmic mucin content. The Goseki grading was carried out as a separate exercise "blind" to the results of Lauren's classification, WHO grading system, TNM system, and patient outcome.

**Results**: the smallest category was Goseki grade II (13%) vs grade III (26%), grade IV (29%), and grade I (32%). There was a significant correlation between Goseki grade I-II tumours and Lauren intestinal type and well- or moderately differentiated tumours; between Goseki grades III-IV tumours and Lauren diffuse type tumours and poorly differentiated tumours. As regards mucin content, Goseki grades II-IV tumours were statistically associated with Lauren diffuse type tumours. Grade III/IV tumours had a greater tendency towards lymph node involvement with the highest proportion of N2 (62%) ($p<0.05$). Sixty percent of patients with III TNM stage and Goseki grades I-III (mucin poor) tumours survived during follow-up vs 11% of patients at same stage with cancers mucin rich patterns.

**Conclusions**: we have shown that Goseki system can provide a more accurate prognosis in patients with GC when added to the TNM staging system alone. Particularly, its use may be of help to modulate the adjuvant treatment after surgical resection for patients with III TNM stage.

## P-634

**HISTOLOGICAL AND CLINICAL FEATURES OF RECTAL NEUROENDOCRINE TUMORS : ABOUT 12 CASES.**

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Rectal neuroendocrine tumors represent 12,6 % of gut neuroendocrine tumors. Cellular differentiation is often a missing datum on histological reports. However, it has clinical relevance because chemotherapy depends on tumor differentiation (i-e 5 FU-Streptozotocine for well differentiated tumors and VP16-Cisplatin for their undifferentiated counterparts).

The aim of this study was to emphasize histologic and clinical features of rectal neuroendocrine tumors in a retrospective study. Twelve cases, collected from the files of our Institute, were available with following data: age, sex, first symptoms, tumor size, histology, treatment and evolution. Among these 12 cases, 9 were women, 3 were men ; mean age was 52 (26-76). First symptoms were : transit dysfunction, abdominal and pelvic pain, rectal bleedings and painful hepatomegaly. Tumor size varied from 10mm to 48 mm. Surgical treatment consist of endoscopic resection in 7 cases, recto-sigmoidectomy in 2 cases and perineal and abdominal amputation in 1 case. Adjuvant or first chemotherapy was done in 5 cases. Histological analysis of these case, previously classified as neuroendocrine tumors, recognized 9 well differentiated cases (with carcinoid or atypic carcinoid features) and 3 undifferentiated cases (small cell type).

**Conclusion**: among rectal neuroendocrine tumors, which are rare, undifferentiated types need to be distinguish, because they require specific chemotherapy.

## P-635

RESULTS OF MULTIFACTORIAL STUDY OF CHRONIC GASTRIC ULCER MARGIN

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**Aims:** The aim of this study was the evaluation of regenerative, disregenerative, displastic and metaplastic processes and Helicobacter infection in the chronic gastric ulcer margin.

**Methods:** It was studied the operative and bioptic material (500 cases) in the Department of Pathomorphology of the Republican Central Clinical Hospital of Georgia (1195-98). Paraffin sections were processed through the general histological, special histological, histochemical and morphometric methods. The quantitative data was statistically processed.

**Results:** According to the well known 3 degrees of displasia, the results of investigation showed the important peculiarities in structure of layers in epithelium, shape of epitheliocytes, shape of nuclei, distribution of chromatin, forms of mitosis and atypical mitosis, nucleus-cytoplasm index, relationship of epitheliocytes with basement membrane, goblet cells, shape, stroma and ratio of glands, quantity of mucous secretion, cells of APUD system and distribution of quantity of Helicobacter.

**Conclusion:** Based on the quantitative and qualitative analysis of study data there were determined 5 degrees of displasia of chronic gastric ulcer margin, which will be helpful in differential diagnosis of I-II and II-III displasias.

## P-636

**ENTEROCHROMAFFIN-LIKE CELLS IN PATIENTS WITH HELICOBACTER GASTRITIS BEFORE AND AFTER ERADICATION THERAPY**

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**Aims:** Helicobacter pylori infection contributes to hypergastrinemia which is known to have trophic stimulus on enterochromaffin-like (ECL) cells. In H. Pylori gastritis both hyperplastic and pseudohyperplastic changes occur in ECL-cells. In this study, our aim was to compare gastrin producing (G) cells and ECL-cells in biopsies maintained before and after eradication of H. pylori in order to assess the exact nature of ECL-cells, whether they are truely hyperplastic or just cell clusters as a result of glandular atropy.

**Methods:** Thirty patients with Helicobacter gastritis are included in the study. In each case, 2 antral and 2 corpus biopsies prior to 2-weeks of triple therapy (amoxicillin + clarithromicin + omeprazole) and 6-8 weeks after the therapy are evaluated. Activity of Helicobacter gastritis, degree of atrophy, and type of metaplasia are recorded for each biopsy. Immunohistochemical expression of both chromogranin-A and gastrin was assesed semiquantitatively.

**Results:** The activity of Helicobacter gastritis, the degree of atrophy and type of intestinal metaplasia did not correlate with the degree of chromogranin A expression in the oxyntic mucosa. Chromogranin A expression in oxyntic mucosa did not differ between biopsies taken before and after the eradication of H. pylori. Immunoexpression of gastrin in antral mucosa was significantly related to the degree of chromogranin A expression in biopsies maintained prior to therapy ($p< 0.05$), altough this could not be demonstrated in posttreatment biopsies.

**Conclusions:** We concluded that ECL-cell proliferations seen in H. gastritis are mostly due to hypergastrinemia and that they are not reversible after H. pylori eradication therapy at least in the first few months.

## P-637

### A RISK OF GASTRIC CANCER IN CHORNOBYL CATASTROPHY VICTIMS

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**Aims:** A relationship between pathology of stomach and duodenum in persons irradiated because of the Chornobyl catastrophy and Helicobacter pylori (HP) infection was investigated.
**Methods:** 522 biopsies of gastric mucosa and 249 biopsies of duodenal mucosa from 259 patients with chronic gastritis and duodenal ulcer (160 – liquidators; 63 – inhabitants of radioactively polluted territories; 36 – nosologic control) were studied by methods of histochemistry (including lectin binding analysis), autoradiography and statistics.
**Results:** We have revealed that dependence of chronic gastritis from the HP infection is stronger in exposed persons (99 % of liquidators and 100 % of inhabitants contrary to 85 % in control group). Changes in lectin conjugation with surface epithelium have detected a decreased L-fucosae binding. Thus, it reflected a reduction of viscosity and hydrophobic properties of mucous secret and testified an imperfection of cytoprotection. The presence of radionuclides was found in gastric and duodenal mucosa in inhabitants of contaminated territories. Statistical analysis of indices of epithelium regeneration pathology (hyperplasia, papillary changes, mitotic regime and dysplasia) reflected the highest degree of dysregenerative changes in liquidators as compared to inhabitants and nosologic control patients.
**Conclusions:** Taking into account a neoplasmogenous role of ionizing radiation, precancerous status of chronic gastritis, association og gastric cancer and gastric MALT-lymphomas with HP infection and their growth in liquidators of the Chornobyl accident we qualify exposed persons (especially liquidatirs) as a risk group for gastric cancer originating.

## P-638

### CUANTIFICATION OF CELL PARAMETERS IN HELICOBACTER PYLORI-POSITIVE GASTRIC ULCER

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**Aims.** The aim of this study was to assess the quantitative parameters of epithelial cells in gastric ulcer with Helicobacter Pylori (HP) infection, and to estimate quantitative criteria, which could be used as guidelines for prognosis and treatment.
**Methods.** We have studied 65 patients of both sexes, which were operated for gastric ulcer. The operational fragment from corporal and antral portion were processed through paraffin-technique and stained with H&E with prolonged hematoxylin staining period, Giemsa modified and Warthin-Starry methods. The quantitative two phase, nuclear volume measurements and proliferative activity assessment were made on the representative sections, using a professional digitizing interactive program. In each case a hundred cells were measured and statistical analized.
**Results.** The gastric ulcer was macro- and microscopically diagnosed. We found chronic active gastritis in the pilorıc antrum (62%) and body of the stomach (30%). The presence of HP is asociated with intestinal metaplasia: incomplete (65 %) or complete (25 %). In HP-positive chronic gastritis and intestinal metaplasia the nuclear volume and nuclear area are greater than in other pathological states associated with gastric ulcer. The area difference between cell and nucleus area is smaller in same affections. The hiperproliferative activity was present in the inflammatory processes studied, but unrelated to intestinal metaplasia.
**Conclusions.** Our results suggest that nuclear parameters are essential prognostic indicators and may be used in early detection of precancerous states of gastric mucosa asociated with HP infection.

## P-639

### MESENCHYMAL (STROMAL) TUMORS OF THE GASTROINTESTINAL TRACT: ENDOSCOPIC BIOPSY DIAGNOSTIC, MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS

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**Aims:** To study the incidence and peculiarities of gastrointestinal tract (GIT) mesenchymal tumors in order to estimate the diagnostic obstacles and their morphogenesis.
**Methods:** Fifteen GIT mesenchymal tumors from endoscopic biopsies and subsequent resection materials were analyzed. Paraffin sections were stained after HE, Mallory and Gomori. Immunohistochemical procedures were performed using primary antibodies against vimentin, cytokeratin, desmin, S-100 protein and LSAB kit (all from DAKO).
**Results:** GIT mesenchymal tumors represented 1,55% of all GIT tumors in our endoscopic biopsy records, most of them are benign (86,70% vs 13,30%). They were localized predominantly in the stomach (66,67%) and endoscopic diagnosis was possible if the mucous membrane was ulcerated. The morphological pattern and immunohistochemical verification (intermediate filament markers and S-100 protein) determines most of them as "generic" type stromal tumors of GIT.
**Conclusions:** These results confirm that the endoscopic biopsy technique is a reliable method for the diagnostics and characterization of these rare tumors.

## P-640

### THE CORRELATION BETWEEN THE INTESTINAL METAPLASIA TYPES I & II AND PCNA/ Kİ 67 PROLIFERATIVE INDEX

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There are numerous reports about the different malignant potential of intestinal metaplasia (IM) subtypes. The aim of this study was to investigate the immunreactivity coexistence of PCNA / Kİ 67 which are widely used proliferative markers, on the intestinal metaplasia types I & II.

Thirty two gastric biopsies were used in this study. Differentiation of IM types I & II was made according to the criteria attributed by Filipe & Jass*

To identify the IM type I & II, Alcian blue pH 2.5/ periodic acid Schiff (AB/ PAS) stain was used. For the demonstration of cell proliferation, proliferating cell nuclear antigen (PCNA) and Kİ 67 were used.

Twenty three cases were stained with AB/ PAS and classified as a Type II IM (71.8 %).Kİ 67 immunreactivity was not seen in any of cases. PCNA positive proliferative index was found in eight of cases( 25%).Spearman and Pearson correlation tests were used on this results ($p > 0.05$).

Our results showed that there is no correlation between the type I&II IM and the PCNA proliferative index.

[*: Filipe MI, Jazz JR: Intestinal Metaplasia Subtypes and Cancer Risk. In Gastric Carcinoma (eds:Filipe MI, Jazz JR) pp. 97-105; Churchill Livingstone, Edinburg; 1988.]

## P-641

APOPTOSIS AND EPITHELIAL PROLIFERATION IN CHRONIC ACTIVE GASTRITIS AND HELICOBACTER PYLORI INFECTION

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**Aims**: To investigate the pathogenic role of Helicobacter pylori (HP), gastric mucosal apoptosis, foveolar leukocyte infiltration and epithelial proliferation were assessed in this study of chronic gastritis.

**Methods**: Gastric antral and corporeal mucosal biopsy samples were collected endoscopically from symptomatic patients: 7 cases of normal mucosal histology, 8 cases of HP negative and 21 cases of HP positive chronic gastritis, respectively. HP infection was confirmed using the modified Giemsa stain as well as by immunohistochemical reactions and its severity was graded according to the Genta classification. Apoptosis was measured by staining with the TUNEL-assay and the characteristic cellular morphology. Epithelial cells, cells in mitosis and leukocytes were counted in the epithelium and the upper, mid, and lower thirds of the antral foveolae were analysed separately. Stromal apoptosis was scored by means/microscopic fields. Differences of the intensity of apoptosis, mitosis, leukocyte infiltration and of the numbers of epithelial cells were statistically analysed by ANOVA using the SAS System.

**Results**: Epithelial cell apoptotic and mitotic index as well as leukocyte and foveolar epithelial cell numbers were increased in HP negative chronic gastritis. In HP positive cases there was a more intense leukocyte infiltration and a higher incidence of apoptosis then in HP negative chronic gastritis and the stromal apoptosis was also more frequent. The different sites of the antral foveolae showed no variations in the cellular indices, and the corporeal mucosal changes were similar. The severity of the infection did not correlate with any of the variables observed.

**Conclusions**: HP localised on the mucosal surface iniciate a higher incidence of mucosal apoptosis than chronic inflammation of other origine by inducing a more intense epithelial leucocyte infiltration.

## P-642

**Immunohistochemical localization of metallothionein in human colorectal neoplasms in correlation to cathepsin D, CD44, p53, pRb, bcl-2, c-erbB-2, EGFR and proliferation indices.**

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**Introduction:** The purpose of the present study was to investigate the role of Metallothionein (MT) in colorectal tumours in correlation with the expression of cathepsin D (CD), CD44, p53, Rb, bcl-2, c-erbB-2, EGFR, proliferation indices (Ki-67, PCNA) and with conventional clinicopathological variables.

**Methods:** The immunohistochemical expression of MT in 23 cases of colorectal adenomas and in 94 adenocarcinomas was examined by the avidin-biotin peroxidase (ABC) method using the monoclonal mouse antibody E9, on formalin-fixed, paraffin-embedded tissue.

**Results:** Positive MT expression (>5% of neoplastic cells) was observed in 30.4% of adenomas and 25.5% of adenocarcinomas, while 8.7% of adenomas and 14.9% carcinomas showed a focal MT positivity. In contrast 60.9% of adenomas and 59.6% of carcinomas almost completely lacked MT expression. In the series of adenocarcinomas, MT expression was correlated with CD of neoplastic cells ($p=0.05$) and c-erbB-2 protein expression ($p=0.05$). An inverse relationship was demonstrated between MT expression and MIB-1 ($p=0.003$). There was no statistically significant difference of MT expression between adenocarcinomas and adenomas as well as with the other parameters examined.

**Conclusions:** These data suggest, that MT does not seem to indicate an aggressive biological behaviour in colorectal adenocarcinomas. However, it could be a potential prognostic factor, especially in correlation with other known prognostic markers. However, further studies are required, however to establish the value of MT as prognostic indicator in colorectal tumours.

## P-643

**GASTROINTESTINAL STROMAL TUMORS**

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**Aims:** morphological and immunohistochemical study of 12 benign and malignant gastrointestinal tumors, localized in the stomach, small and large bowel.

**Methods**: routine histological and immunohistochemical techniques for cytokeratin, vimentin, desmin, actin, S100-protein, Ki67, p53, bc12.

**Results**: This paper will attempt our experience with gastrointestinal stromal tumors, based on an analysis of 12 cases ( 6 benign and 6 malignant ). The benign tumors showed leiomyomatous respectively epitheloid leiomyomatous differentiation ( 4 respectively 2 cases ), localized in the stomach and 2 stromal sarcomas with an incomplete neural differentiation of the gut. From the immunohistochemical reactions the most constant stain for these tumors is vimentin. The tumors with myoid differentiation showed muscle-specific protein reactivity. S-100 protein expression has been observed in the sarcomas with neural differentiation.

**Conclusions**: the gastrointestinal stromal tumors represent a heterogeneous group of lesions, with a controversial histogenesis, showing a complete or incomplete myoid, neural, ganglionic or mixed features of differentiation.

## P-644

**TUFTING ENTEROPATHY CAUSING SEVERE INTRACTABLE DIARRHOEA AND FAILURE TO THRIVE IN AN INFANT**

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Tufting enteropathy (TE) is a newly recocognised clinicopathological entity characterised by partial villous atrophy, crypt hyperplasia, chronic inflammatory infiltrate of the lamina propria and epithelial tufts composed of closely packed teardrop-shaped enterocytes in jejunal mucosa. The aetiology of TE is unknown. An abnormal epithelial cell–extracellular matrix interaction has been proposed as the most likely pathogenic mechanism.

A six-month-old female infant had persistent diarrhoea and severe failure to thrive. Histopathology of jejunal samples showed subtotal villous atrophy, hyperplastic crypts, marked and diffuse mononuclear infiltrate in the lamina propria. Surface enterocytes were pseudostritified/crowded creating numerous epithelial tufts consisting of oval-shaped enterocytes with preserved microvilli and lack of cytoplasmic inclusions. Near to the tip of almost all villi small groups of enterocytes showed marked cytoplasmic vacuolation. Transmission EM showed decreased supranuclear cytoplasm with rounded apical membrane, shorter brush border but preserved cytoplasmic organelles. The intensity and extent of light microscopic changes in three serial jejunal biopsy samples were variable and showed some histological improvement with corticosteroid therapy.

TE is a rare cause of protracted diarrhoea and failure to thrive in infants and has characteristic histopathological features. Accurate diagnosis is important for clinical management, prognostic and genetic reasons as an autosomal dominant mode of inheritence has been recognised.

## P-645

ANGIOGENESIS IN GASTRIC CANCER

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**Aims**: to compare the biology of gastric cancers in their early and advanced stages. As a basis for comparison we evaluated the extent of neovascularisation in tumor.

**Methods**: the microvessel quantitation in gastric cancer was analysed in a study group that included 20 patients with early and 29 patients with advanced gastric carcinoma. Small blood vessels were visualized by staining endothelial cells for von Willebrand Factor (DAKO) using a standard immunoperoxidase ABC-technique on parafin-embedded sections. The three most vascular areas ("hot spots") were identified in each slide. Microvessels were counted at magnification x200. The average count from the three "hot spots" and the highest single field count were recorded for subsequent statistical analysis.

**Results**: early gastric cancer demonstrated significantly higher microvessel count than advanced cancer (p=0,0088). Also, younger patients showed significantly higher average counts than older patients (p=0,029). There were no significant differences between parameters of neovascularisation and sex or histological type of tumor.

**Conclusions**: this preliminar investigation indicates the biological differences between early and advanced gastric cancer

## P-646

**MICROSATELLITE INSTABILITY AND p53 MUTATIONS IN SPORADIC RIGHT COLON CANCER**

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**Aims:** We studied colon cancer in the attempt to determine the prognostic significance of different molecular alterations and their correlation to the clinicopathological variants of colon tumors, as well as to their location in right or left colon.

**Methods:** We studied 125 cases of colon cancer (50 in right colon and 75 in left colon), analyzing: (a) the *p53 gene*, p53 protein expression; (b) bcl-2 protein; (c) microsatellite instability; and (d) repair genes *hMLH1* and *hMSH2* in those cases in which microsatellite instability was detected.

**Results:** The overall analysis demonstrated the following results: (a) the incidence of *p53 gene* mutations was 40%; (b) overexpression of the p53 protein occurred in 58% of cases; (c) the rate of microsatellite instability was 26% in right colon and 14.6% in left colon; in right colon, there was a correlation between the presence of microsatellite instability and tumor size; (d) the presence of microsatellite instability and *p53 gene* mutations in right colon was associated with lymph node metastases; and (e) bcl-2 protein overexpression was associated with Duke's stage B.

**Conclusion:** Microsatellite instability is clearly associated with right colon cancer and there is evidence that it may be triggered during the growth and development of these lesions. The development of left and right colon tumors involves differing carcinogenic mechanisms, possibly associated with different clinical implications and clinicopathological features.

## P-647

REVEALING HELICOBACTER PYLORI AT DISEASES OF THE UPPER DEPARTMENT OF GASTROINTESTINAL TRACT AT THE LIQUIDATORS OF CHERNOBYL DISASTER

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The purpose of the present work was revealing Helicobacter pylori (HP) at liquidators of Chernobyl disaster. They possibly have reduced cell and humoral immunity necessary for elimination HP, and the intake of radionuclides was an additional source of regenerative possibility disturbances at gastrointestinal mucosa. Histological slides were painted with azure-eosine for morphological detection of HP. It was found, that from 1206 liquidators of the first year after disaster (1050 men and 156 women) 500 (41.5%) had chronic diseases of a stomach and duodenum (428 men and 72 women). Chronic gastritis was revealed at 266 of 500 (53.2%), chronic gastroduodenitis at 106 (21.6%), peptic ulcer of a stomach at 20 (4.0%), duodenum at 96 (19.2%), stomach and duodenum at 7 (1.4%), polyp of a stomach at 2 (0.4%), the resection of a stomach was made at 1 (0.2%). Gastric mucosa on HP was examined at 112 of 500 liquidators with diseases of the upper gastrointestinal department, from them 94 were with chronic gastritis, 10 with peptic ulcer of a stomach and 8 with ulcer of duodenum. HP was revealed at 54.5% of the patients, including 52% of the patients with chronic gastritis, at 80% of the patients with peptic ulcer of a stomach and 50% of the patients with duodenal ulcer. It is necessary to mark, that the patients with chronic gastritis, HP carriers, at 36 of 49 (73.5%) had morphological indications of chronic active gastritis, while in group of the patients, where HP was not revealed, predominated chronic gastritis with weak activity (29 of 45, 64.4%). Thus, among 1206 liquidators of Chernobyl disaster the high level of inflammatory diseases of the upper department of gastrointestinal tract (41.5%) was revealed. At 54.5% from 112 inspected HP was revealed, which promotes activation of gastritis and development of peptic ulcer of stomach and duodenum.

## P-648

PROTOZOAN INFECTION OF THE UPPER INTESTINAL TRACT IN IMMUNOCOMPROMISED PATIENTS WITH STOOL NEGATIVE TESTS HISTOLOGICAL AND ELECTRON MICROSCOPY EVALUATION OF BIOPTIC TISSUES.

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**Aims**: We describe the usefulness of the histological examination of enteric biopsies from AIDS patients with chronic diarrhea and negative microbiological tests, in order to evaluate the sensitivity of gastrointestinal bioptic approach for protozoan identification.

**Methods**: 67 oesophagogastroduodenal biopsies were examined. In 46/67 cases (group A) stools were negative for enteric bacteria, mycobacteria, C.difficile toxin, Rotavirus, and parassites. The remaining 21 cases (group B, 19 with stools positive for Cryptosporidium parvum, CP and 2 for Giardia lamblia, GL) were added because the therapeutic approach was unsuccessful and double infection was suspected. Histological examination and electron microscopy (EM) were performed.

**Results:** In 24/67 cases, a protozoan infection was diagnosed: in group A. Enterocytozoon bieneusi (EB) in 5 cases, CP in 3, Isospora-like sp. (IL) in 3, GL in 1, Leishmania in 1 oesophageal fragment; in group B, CP was observed in 11 cases, two of whom were coinfected with mycobacteria and adenovirus. Semi- and thin sections were resolutive for definitive diagnosis in 12/24 positive cases: 5 EB, 3 CP -one in intracytoplasmic parasitophoruos vacuoles at gastric biopsy-, 2 IL -one with parassites also in macrophages of the lamina propria and in the lumen of vessels-, one GL and one Leishmania.

**Conclusions:** Biopsy is a useful method for diagnosing upper enteric diseases in AIDS patients with chronic diarrhea and a negative stool examination. Moreover, semithin sections study increases the sensitivity of histological examination, EM also reveals atypical localizations of CP and the dissemination of IL; and allows the identification of microsporidia species, something that is necessary to ensure correct therapeutic strategies.

## P-649

**Is p53 protein involved in metastasis? An immunohistochemical study of p53 expression in colorectal carcinomas and corresponding metastases.**

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**Aim.** To investigate the involvement of p53 in metastasis in colorectal carcinomas.
**Patients and methods.** 24 cases of colorectal cancers and corresponding metastases were studied. Out of the patients in the study group, 20% were aged less than 50 yr. Samples were stained by hematoxilin-eosin-safran, PAS and Alcian-Blue. p53 expression was evaluated by a three-step immunohistochemical method.
**Results.** All of the cases included in our study group were adenocarcinomas. Survival ranged from 1 to 5 yr, 3 of the patients being in complete remission. p53 was overexpressed in 17 out of the 24 cases. We did not notice a significant difference in the p53 expression in metastases vs primary colorectal cancers. In patients aged less than 50 yr., more than 50% of the cells expressed p53. Two cases displaying a cytopasmic staining did not show a different evolution as compared to the remaining patients, whereas in three cases, p53 expression markedly decreased in metastasis compared to primary cancers (the patients with significantly longer survival). p53 expression was frequently more intense in the most invasive areas of tumors and around necrotic areas.
**Conclusions: 1.** p53 is not directly involved in the metastatic process but rather plays a role in local tumor invasion and cell differentiation. **2.** In young people, the tumors showed a lower differentiation and a higher proportion of p53 positive cells, both features being probably related with a negative prognosis in such cases. **3.** A prognostic role for p53 is supported by the observation that in those cases in which p53 expression decreased progressively in evolution, the survival was longer. **4.** p53 might be involved in chemoresistance, based on clinical evolution and increased expression in hypoxic cells (alleged to became chemoresistant).

## P-650

**Distribution of Neuropeptides in Congenital Innervation Defects of the Distal Colon**

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**Aims:** Congenital colorectal innervation defects based on disturbance of migration of neuroblasts from the myenteric plexus into the submucosal layer, are very common findings in infants. Different entities like Hirschsprung's disease (HD), neuronal intestinal dysplasia (NID) A and B, and hypoganglionosis are defined as defects in autonomic neurogenesis of parasympathetic or sympathetic origin. Neuropeptides associated with the non-adrenergic non-cholinergic (NANC) system are known to have physiological effects on motility and smooth muscle contraction in the gut.
**Methods:** In the present study, 37 distal colorectal mucosa biopsies from 17 infants were investigated using immuno- and concventional enzyme-histochemical (AChE, LDH, SDH) methods. Specimens always included the submucosal layer and were taken from 0.5 to 3 cm above the dentate line. Five children were diagnosed with HD, 4 with NID B and hypo-ganglionosis and 8 did not show visible alterations. Localization and distribution of peptidergic nerve fibers were examined using antibodies against vasoactive intestinal polypeptide (VIP), peptide histidine methionine (PHM), pituitary adenylate cyclase activating peptide (PACAP), neuropeptide tyrosine (NPY), C-flanking peptide of NPY (CPON), sub-stance P (SP) and calcitonin gene-related peptide (CGRP).
**Results:** Compared to normal tissue, the number of nerve fibers containing VIP, PHM and PACAP, mainly coexpressed in the parasympathetic system, were clearly reduced in cases of HD in the submucosal as well as within the mucosal layer. Endocrine cells containing VIP and PHM have been detected within the glandular cells. Semi-quantitative scoring showed no for between neuropeptides associated with the sympathetic system (NPY and CPON) in HD and NID B in the submucosal layer, but an increase of NPY and CPON containing nerve fibers within the lamina propria mucosa has been observed. SP and CGRP expressed in sensory nerve fibers could not be detected. **Conclusions:** Alterations of peptidergic innervation in congenital coloretal innervation defects are far from clear and might become a diagnostic cri-teria in addition to the widely used enzymhistochemistry-based diagnostic system.

## P-651

**MORPHOMETRIC FEATURES OF HIRSCHSPRUNG'S DISEASE AND ALLIED DISORDERS**

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There are many clinical conditions that resemble Hirschsprung's disease(HD) despite the presence of ganglia cells on rectal biopsy. Histopathological diagnosis is established by application of standard histological techniques enzyme and immunohistochemical methods. The enzyme and immunohistochemical methods for the small and medium laboratories are still expensive and complicated which cause problems in the histological diagnosis, whereas the standard histological techniques very often lead to confusion.

**The aim** of this paper is to compare the number and spatial distribution of the ganglia knots and ganglia cells using modern morphometric methods in normal subjects and in patients with clinical condition which is, or resembles Hirschsprung's disease.

**Material:** As a learning set were used samples of the rectum of cadavers who died in the perinatal period(10), then cadavers in the adult age(10) and biopsies from patients with clinical diagnosis HD (30).

**Methods:** Standard histological stains: HE, Van Gieson, Azan-Malory. For visualization and quantification of the ganglia and nerve knots, the following immunohistochemical markers were used: neurospecific enolase (NSE), synaptophysin (SY), PGP 9.5, imege analyzing software Lucia M, commercial image editors and standard statistical methods.

**Results and Conclusions:** From the morphological point of view, congenital aganglionosis, hypoganglionosis and normal ganglia could be observed as morphological continuum defined with the number of the ganglia and ganglia cells per area. The precise morphometrical measurements have shown to be useful in acquiring experience for quick semiquantitative assessment of the presence and maturity of the ganglia and ganglia knots. For certain cases of neural intestinal dysplasia, an additional enzyme and immunohistochemical visualization is necessary. The mentioned morphological entities appear either solitary or combined in solitary or mosaic distribution. Van Gieson's staining and Azan-Malory out of the standard, empirical histological stainings, has shown to be useful in enlightening certain structures at the rectal wall.

## P-652

**ANGIODYSPLASIA OF SMALL BOWEL, SPECIALLY SUB-SEROSAL, WITH ARTERIOVENOUS SHUNTS AND INTERSTITIAL ELASTOSIS; PERITONITIS CAUSED BY RUPTURE OF DYSPLASTIC VESSELS AND HEMOPERITONEUM.**

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We present a case report of a 74 year old man, affected by angiodysplasia of the duodenum, which became worse in peritonitis and hemoperitoneum. After he had undergone two surgical interventions, it was 14 days since he had died. Grossly, the segment of stomach was 6 cm long along the small curve, continuing to a segment of duodenum 3 cm long. Its serosa had the features of sero-fibrinous peritonitis, while, its gastric and duodenal mucosa didn't show any ulcerative lesions. Microscopically, duodenal sub-serosa presented a serious irregular increase of blood and lymphatic vessels with arterovenous shunts. It sometimes appeared these vessels were filled with blood and showed a nodular arrangement. Moreover, there was an evident sub-serosal component, characterised by an elastotic nature, aranged in islets and interstitial spreading among the glands too. First of all, the importance of this case is due to the extreme rarity of the angiodysplasia of the duodenum, specially when it becomes worse in peritonitis and hemoperitoneum, this case having been described only one time till now, then to the localisation mainly sub-serosal and lastly to the presence of the elastotic component, which we have first described in literature.

## P-653

GASTRIC CARCINOMA AND ASSOCIATED CHANGES IN ENDOSCOPIC BIOPSIES

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Gastric carcinomas are relatively frequent in Kosova.As far as the histological aspect is concerned there were found mostly adenocarcinomas, but their histological classification is sometimes difficult becouse of the complexity of the normal and metaplastic gastric mucosal architecture which results in associated changes. Gastric carcinoma is found often with precancerous lesions. The aim of this research was to represent the most frequent precancerous lesions which could be found in association with gastric carcinomas in endoscopic biopsies. There were analyzed 1211 endoscopic biopsies of the gastric mucosa which were sent in the Institute of Pathology of Faculty of Medicine in Pristina from the Endoscopic ambulance of Internal Clinic during the period of January 1990 to December 1997. We have applied routine H&E method of staining as well as other histochemical staining methods (PAS, AB-PAS). Gastric carcinoma was found in 213 (17.5%) cases out of 1211 analyzed endoscopic biopsies. Males were more attacked (in 59.7%) than females (40.3%). We have found most cases of carcinomas in the sixth decade of life. Histologically the most frequent was intestinal type 64.3% according to Lauren, while diffuse type was in 26.7% of cases. The signet ring cell carcinoma was found in 5.1% of cases, whereas in 3.7% of cases we couldn't differentiate the histological type.The associated lesions found as solitary changes or united with gastric carcinoma were severe epithelial dysplasia in 95.7% of cases and intestinal metaplasia with chronic gastritis.We have found mostly the III-type of intestinal metaplasia in 68.5% of cases while the typesI/II were rare.
Chronic atrophic gastritis was detected in 31.9% of cases, rare as a solitary lesion but often in association with intestinal metaplasia.
Our data indicate that gastric carcinoma is rare detected alone, but always in association with epithelial dysplasia as well as with intestinal metaplasia and chronic atrophic gastritis.

## P-654

ESOPHAGOGASTRIC VARICES - IS THERE A RELATIONSHIP BETWEEN THEIR RUPTURE AND VESSELS OF THE LIGAMENTUM FALCIFORME HEPATIS OR CHANGES OF THE SEASONS?

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**Aims:** To see whether two unrelated factors, namely 1./ the number and size of the vessels of the ligamentum falciforme hepatis (LFH) and/or 2./ temporal variation in the incidence of lethal ruptures can help defining a group of patients with portal hypertension with a higher risk for variceal rupture (VR).
**Methods:** LFH was removed from 48 autopsied bodies, and the size of the umbilical vein as well as size and density of paraumbilical veins were graded from 0 (non patent) to 4 (large and/or numerous). Association between overall vessel diameter and signs of portal hypertension (splenomegaly and ascites) or VR was checked with the $\chi^2$ test. For data related to the time distribution of VRs a 3 year retrospective autopsy study was done, and the relative seasonal frequency of VR was analyzed.
**Results:** Dilated vessels in the LFH were strongly associated with signs of portal hypertension and the presence of esophageal varices. We also found a weak association ($p = 0,0216$) between narrow and less numerous (grade 0-2) vessels and VR. Lethal VRs were more common in summer than winter, and had a medium frequency in autumn and spring, but no significant difference could be found between the seasons.
**Conclusions:** An increased flow (that can be detected by Doppler ultrasound) and larger overall diameter of paraumbilical (and umbilical) veins seem to lower the risk of VR. Seasonality, on the other hand, seems a weak predictor of VR.

## P-655

**PROGNOSTIC SIGNIFICANCE OF LYMPHATIC AND BLOOD VESSEL INVASION BY TUMOUR CELLS IN COLORECTAL CARCINOMA**

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**Aim:** This study was undertaken to examine the prognostic value of lymphatic and blood vessel invasion by tumour cells in colorectal carcinoma.
**Method:** In this study 100 patients with colorectal carcinoma were examined. Vessel invasion by tumour cells were examined in formalin fixed, paraffin embedded tissue using standard hemalaun-eosin method, Verhoeff method and immunohistochemistry (anti-human Von Willebrandt factor, DAKO, EPOS).
**Results:** Vessel invasion by tumour cells was positively correlated with higher tumour grade (p=0,0001), Dukes stage (p=0,0001), appearance of metastatic disease in 5-year period (p=0,0001), and lower 5-year survival (p=0,0001). Lower 5-year survival rate was observed in patients with vascular invasion considering correction of the stage too (Dukes B: p=0,018; Dukes C: p= 0,0002). However, when we compared 5-year survival period in patients with Dukes stage B and Dukes stage C when blood vessel invasion were confirmed we did not find any statistically difference (p=0,62).
**Conclusion:** Lymphatic and blood vessel invasion may be independent prognostic criteria in patients with colorectal carcinoma. Moreover it seems that using this parameter it is possible to separate subgroup of the patients with colorectal carcinoma in Dukes B stage with bad prognosis who need postoperative adjuvant therapy.

## P-656

**IMMUNOHISTOCHEMICAL EXPRESSION OF FATTY ACID SYNTHASE (FAS), APOPTOTIC-REGULATING GENES, PROLIFERATING FACTORS AND RAS PROTEIN PRODUCT IN COLORECTAL ADENOMAS, CARCINOMAS AND ADJACENT NON NEOPLASTIC MUCOSA.**

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The normal mucosa-adenoma-carcinoma sequence in colon pathology provides an attractive model of tumor progression (TP). To establish the strength of oncogenes , tumor suppressor genes and proliferative markers we used immunohistochemistry to evaluate p53, bcl-2, c-myc, p21-ras, ki67 expression together with fatty acid synthase (FAS), in normal, dysplastic and neoplastic specimen. Moreover these data and clinicopathological features were associated with overall survival (OS). Archive material from 100 adenomas and 100 adenocarcinomas (ADC) including adjacent to tumor non-neoplastic mucosa (ANNM) from patients with a five year follow-up period were studied. FAS was associated with adenocarcinoma (P=0,0001); p53 protein with high-grade dysplasia adenoma (AHGD), adenocarcinoma (P=0,0001), and tumor size (P=0.003). Bcl-2 was associated with ANNM (P=0,02). C-myc with ANNM (P=0,001), tumor size and nodal involvement (P=0.006). P21-ras was associated with AHGD (P=0.0001) and ANNM (P=0.01). Ki67 with adenocarcinoma, AHGD and TS (P=0.0001). Univariate analysis on neoplastic tissue revealed histological grade, tumor size, nodal involvement, p21-ras and p53 to be reliable markers of OS; p21-ras, p53 and c-myc were reliable markers when evaluated on ANNM. Multivariate analysis revealed TS, nodal involvement and p21-ras to be independent prognosticators of OS on adenocarcinoma; p21-ras and c-myc on ANNM. We suggest that the evaluation in concert of clinicopathological data and immunohistochemical markers on both normal an abnormal colon tissue may give important messages about tumor progression.

## P-657

THE RELATIONSHIP BETWEEN STROMA'S AMOUNT OF CANCER OF STOMACH WITH AMOUNT AND COMPOUND OF MUCIN , PRODUCED BY TUMOURAL CELLS.

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**Aims:** To reveal the relationship between the stroma's amount of cancer of stomach and amount of mucin, produced by tumoural cells; compound of stroma and mucin.

**Methods:** In 5 cases the amount and compound of mucin were estimated by PAS-reaction, Alcianblau staining with pH 2,5 and 1,0, and used lectins, marked by peroxidase: WGA (Wheat germ agglutinin), PNA (Peanut agglutinin), SBA (Soybean agglutinin), HPA (Helix pomatia agglutinin), LCA (Lens culinaris agglutinin), LAL (Laburnum anagyroides lectin), SNL (Sambucus nigra lectin).

**Results:** In the case of cancer of stomach with a large amount of stroma (scirrhous-21 cases) more often was found the marked secretion of PAS-positive mucin (76% cases), unsulfated (48%) and sulfated mucin (24%), in 20 cases of moderate amount of stroma (25%, 35% and 15% accord.) and among 14 cases with a small amount of stroma (29%, 28% and 7% cases accord.). With a large amount of stroma the tumoural cells often contain the receptions by SNL, in a large amount by WGA and do not contain receptions by PNA, SBA. With a moderate or small amount of stroma in tumour their cells contain a small amount of receptions by WGA. It often contains the receptions by PNA and SBA and does not contain the receptions by SNL.

The receptions by HPA are found with the same frequency (60%) in the groups with the different amount of stroma. The receptions by other lectins are revealed in isolated instances and in a small amount.

**Conclusion:** It was revealed the relationship between the amount of stroma in the cancer of stomach and amount of mucin, produced by tumoural cells, the presence in the tumoural cells the receptions by WGA, PNA, SBA and SNL.

## P-658

**PRIMITIVE INDIFFERENTIATED CARCINOMA OF PAROTID GLAND : A CASE REPORT**

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Indifferentiated carcinoma of salivary glands are divided in three types: the lyn thaepithelial carcinoma (bearing a stricking ressemblance with UCNT) ; the indifferentiated large cell carcinoma (without neuroendocrine differentiation) and small cell carcinoma (SCC) (with neuroendocrine differentiation). SCC are rare tumors of the major salivary glands (<1 %). Up to date, approximately 45 cases have been reported. The literature review showed that this tumor has a sex ratio M/F of 1.5:1, occurs in middle aged patients (mean : 50-60 years) ans is located in the parotid gland (83 %).

We report the case of a 67-year-old female patient with a painless swelling of the left parotid gland. No other tumor was detected elsewhere. Superficial parotidectomy was performed. The tumor was grossly circumscribed and measured 17x11x5mm. Histologically, this inhabitual indifferentiated carcinoma consisted of small to medium cells, with scanty large cells and focal squamous differentiation and a strinking lymphoid stroma. Immunohistochemically, tumor cells expressed epithelial and neuroendocrine markers (NSE, chromogranin and synaptophysin). Despite its peculiar cytology and stroma, we think that this tumor should be considered as a variant of SCC. The patient refused chemotherapy. Radiotherapy alone was performed.

Even if better than in other sites, the prognosis of SCC is poor : survival at 1, 3 and 5 years is respectively of 77,7 %, 40,7 % and 29 %.

## P-659

PRECANCEROUS LESIONS OF THE LARYNX - ANALYSIS OF MONO- AND POLYCLONALITY OF THE CELLS WITH HIGH VALUE OF PROLIFERATIVE INDEX (p53, PCNA).

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**The aim of the study** was to evaluate the relationship of p53 and PCNA content in the laryngeal epithelium with a degree of its dysplasia.

**Material and method.** 45 patients with chronic laryngitis (mean age 57.7 +/-6.2 years) who underwent a control biopsy of the larynx were divided into two groups: 1st group with epithelial dysplasia (n=36: grade I=19, grade II=13, grade III=2 and grade IV-preinvasive carcinoma=2) and 2nd group with epithelial hyperplasia acanthotica (n=9). After routine histopathological analysis of mucosal specimens, the tissue was studied with monoclonal antibodies p53 and PCNA (DAKO) using the Avidin-Biotin- Complex method.

**Results.** Table 1 - Mean values of p53 and PCNA in cases studied.

| | p53 (%) | PCNA (%) |
|---|---|---|
| d I - grade I dysplasia | 4.45 +/-3.19 | 10.04 +/-6.09 |
| d II - grade II dysplasia | 15.5 +/-7.37 | 7.00 +/-6.10 |
| d III+IV - grade III+IV dysplasia | 12.45 +/-8.51 | 15.84 +/-8.7 |
| group 1 - Dysplasia I+II+III+IV | 8.91 +/-7.43 | 9.42 +/-6.71 |
| group 2 - Hyperplasia acanthotica | 3.67 +/-3.4 | 7.39 +/-3.63 |

Statistical analysis revealed significant correlation of p53 content with the degree of dysplasia (r=0.59,p=0.0012) and following significant differences only in p53 content: gr.2 vs gr.1(p=0.007), gr.2 vs dII(p=0.005), gr.2 vs dIII+IV(p=0.02), dI vs dII(p=0.03), dI vs dIII+IV(p=0.03).

**Conclusions:** 1) p53 index can be an additional factor defininig laryngeal dysplasia associated with chronic laryngitis. p53 content correlates with proliferation activity of epithelial cells and divides dysplasia into grades. 2) PCNA content in laryngeal epithelium does not correlate with the intensity of dysplasia associated with chronic laryngitis.

## P-660

EXPRESSION OF EGF AND PDGF IN PRIMARY LARYNX SQUAMOSUS CELL CARCINOMA

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**Aims:** We are looking for clinical benefit by compearing expression of Epidermal Growth Factor (EGF) and Platelet Derived Growth Factor (PDGF) in biological materials (tumor tissue, serum, laringolavat) primary larynx squamosus cell carcinoma patients, to macroscopic characteristics and degree of tumor differentiation.

**Methods:** Study is prospective investigation in two groups of patients: 1. Churgical treated patients with diagnosis of primary larynx squamosus cell carcinoma (n-64), 2. Patients with no malignant diseases of larynx (n-20). We noted local characteristics of tumor: 1. Localisation, 2. Exstension, 3. Degree of cell differentiation, 4. Tumor size. Surgical procedures: 1. Laryngectomia totalis, 2. Laryngopharyngectomia, 3. Laryngectomia supraglottica, 4. . Laryngectomia partialis verticalis, 5. Chordectomia simplex, 6. Chordectomia distensa, 7. Dissectio colli. EGF and PDGF concentracion is measured in: 1. tumor tissue, 2. Serum and laryngolavat 7, 14, 21 days after operation. We used IRMA method including primary and secondary specific radio market antibody.

**Results:** From I to IV stage concentration of EGF and PDGF is in increasing. Exspression of actual growth factors is higher in tumors over 2 cm, and it is also in positive correlation to degree of cell differentiation. EGF and PDGF concentration in serum and in laryngolavat is in decreasing 7, 14, 21 days after radical enucleation of tumor.

**Conclusions:** Clinical benefit of results is specifical following up in prognosis of laryngeal cancer affection patients.

## P-661

CHRONIC SCLEROSING SIALADENITIS WITH AMYLOID DEPOSITION - A SIX WEARS FOLLOW UP STUDY

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A rare case of chronic sclerosing sialadenitis (CSS) occurring in a 56-year old man was reported. Bilateral submandibular gland swelling with increased salivation after denture setup was a main presenting symptom. Scintigraphy with 74 M Bq (2 m Ci) Tc-99-pertechnetate showed enlarge-ment of both submandibular, sublingual glands and left parotid gland with higher than normal uptake of the radionuclide. Biopsy of surgically removed submandibular and sublingual glands showed signs of CSS at stage III-IV. Amyloid deposition in small vessels' walls and in interstitial connective tissue, positive to lambda-light chain, was detected. Cellular infiltration was composed predominantly of B-lymphocytes and plasmocytes with numerous Russels' bodies. About two years later the minor salivary glands of buccal mucosa were affected. Because of the lips' swelling and an increased salivation twofold operative removal of the minor salivary glands was required. Histology was the same as in SMGs. The treatment with nonsteroid antiinflammatory drugs (aspirin, voltaren) had only transitory effect only. Transition of CSS in generalized autoimmune sialadenitis with unusual local amyloid deposition was suggested. No progression to MALT-lymphoma was established during the six-year follow up observation of the patient.

## P-662

**PERICARDIAL EXTRAMEDULLARY HEMATOPOIESIS IN CHRONIC MYELOID LEUKEMIA Ph(-) AND CHRONIC MYELOMONOCYTIC LEUKEMIA**

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Pericardial extramedullary hematopoiesis seems to be extremely rare. We present two patients with CML Ph(-) and CMML who developed cardiac tamponade due to pericardial effusion caused by extramedullary hematopoiesis. The clinical presentation and course following these particular complications clearly indicate a progression of disease from a chronic to an accelerated or aggressive phase.

Pericardial biopsies revealed a multifunctional cellular infiltration of hematopoietic cells carrying the morphology of immature and mature myeloid cells and rare megakaryocytes particularly in the case of CML Ph(-)

Histochemical and immunohistochemical studies of the material showed positivity for chloroacetate esterase, lysozyme/myeloperoxidase pointing to myelocytes and also for glycoprotein/factor VIII pointing to megakaryocytes. Glycophorin A and C for erythroblasts was negative.

The patients' peripheral blood during their present admission was typical for CML and CMML in a chronic phase. The bone marrow biopsies performed at that time were also consistent with the diagnosis. In the follow up of our cases, clinical deterioration of the disease accompanying the development of pericardial effusion was noted.

**Conclusion**: Histologically confirmed extramedullary hematopoiesis involving the pericardium seems to be very rare, being the presenting symptom of acceleration or aggressiveness. Although the pathophysiology of extramedullary hematopoiesis remains uncertain, it rather represents a feature of acceleration of the disease where the malignancy evolves into a more aggressive process with increased 'metastatic' potential and capacity to invade organs not usually involved.

## P-663

IMMUNOPHENOTYPING OF NONTUMORAL CELLULAR POPULATIONS IN MALIGNANT LYMPHOMAS

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**Aims**:A very heterogeneous population of nontumoral cells exists in the nonHodgkin's malignant lymphomas (nHML) and Hodgkin's disease (HD); like a remaining lymphoid parenchyma, or being tumor associated. Identifying immunohistochemically (IHC) these types of cells (T or B lymphocytes, dendritic cells, histiocytes, macrophages) we tried to correlate their occurrence with the type of ML.

**Methods** : We used formalin fixed, paraffin embedded material of 49 nHML and 18 HD cases. A IHC ABC method was performed, using monoclonal antibodies (Mab),for T and B cells (UCHL1,L26), CD 68, S-100 protein (Dako,Denmark) and TBO1 for NK cells (a gift from Prof. F. Malavasi, University of Turin, Italy).

**Results** : We found the T cells sparse in all 9 small B cell nHML and frequent in the 4 centroblastic centrocitic ones; the major part of T cells was TB01 positive. In 7 out from 22 large B cell nHML, T cells were very numerous and associated with S-100 positive reticular dendritic cells and CD 68 positive cells in high number. In the 14 T cell nHML, the associated cells were not found significatively increased. In HD, only the presence of TB01 positive cells was correlated with the histologic lymphocyte predominance type

Conclusions. The nontumoral cells in ML are remaining small lymphocytes, very numerous in some cases.TB01, a novel Mab, is demonstrated very useful in identification of the NK cells in lymphocyte predomination type of HD and in centroblastic-centrocitic ML.

Aknowledgement. We are grateful to Prof. G. Bussolati and Dr. D. Novero from University of Turin, Italy, for their kindly offered help in performing the IHC.

## P-664

PRIMARY B-CELL LYMPHOMAS OF THE LUNG AND LYMPHOID INTERSTITIAL PNEUMONIA: morphological, immunohistochemical and molecular study.

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Primary B-cell lymphomas of the lung (PBLL) are rare tumors which can pose diagnostic problems with reactive conditions, i.e. lymphoid interstitial pneumonia (LIP). In addition, it is not clear if there is any relationship between both types of disorders. The aims of this study were: 1) to delineate morphological, immunophenotypic and molecular features of PBLL 2) to determine useful features for differential diagnosiswith LIP; 3) to investigate the frequency of microsatellite instability (MI) in LIP and PBLL.

Formalin-fixed, paraffin-embedded samples from 25 PBLL and 9 LIP (5/9 were from patients that later developed PBLL) were retrieved from the files of several spanish hospitals. Morphology, immunophenotype (CD20, CD3, CD43, bcl-2, CD21, LMP) and genetic molecular features (EBER, and PCR studies for IgH gene rearrangement, t(14;18) and MI) were studied.

Twenty lymphomas were of low-grade MALT type, and 5 were large B-cell lymphomas (3 exhibiting a low-grade MALT type component). Histological growth pattern of PBLL was mainly diffuse with spread to interalveolar septa and bronchovascular bundles, often imparting a vaguely nodular appearance Cytologically, low-grade lymphomas were composed more frequently by small lymphocyte with interspersed scattered large B-cells. Lymphoepithelial lesions were not restricted to MALT-type lymphomas Angioinvasion was present in all large B-cell lymphomas and in only 3 low-grade. All cases expressed CD20 and often CD43; LMP and EBER staining were uniformly negative. IgH gene rearrangements were monoclonal in all but one case of PBLL and polyclonal in every case of LIP. The t(14;18) was never detected. MI was detected in 2/8 low-grade MALT lymphomas and 1/1 large B-cell lymphoma, and no case (0/7) of LIP.

In summary, PBLL usually exhibit histological features common to other MALT type lymphomas, with some particularities concerning pattern of growth.. IgH gene rearrangement studies are very useful in differential diagnosis with LIP. Although studied in a limited number of cases, MI is not detected in LIP and appears to be present in only a minority of MALT type lymphomas. MI seems not play a significant role in pathogenesis of PBLL, but more extensive studies are needed.

# P-665

## EXPERIENCE OF BONE MARROW TREPANOBIOPSY IN UDMURTIA

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This paper is objected to the clinical and anatomic analysis of bone marrow (BM) trepanobiopsy in patients at the Udmurt hematology center during 1993-1998, 207 samples of BM from patients aged 16-72 (average 48.8) were studied. The table summarizes the BM pathology data.

Bone marrow pathology

| M Pathology | Years | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | |
| Hypoplasia and aplasia | 9 | 4 | 11 | 9 | 9 | 10 | 52 |
| Ostcomyclodysplasia | 3 | 6 | 7 | 2 | 14 | 19 | 51 |
| Tumors | 6 | 5 | 3 | 0 | 1 | 1 | 16 |
| Other diseases | 2 | 0 | 0 | 1 | 0 | 0 | 3 |
| Normal BM | 20 | 4 | 12 | 2 | 9 | 8 | 55 |
| Low quality of biopsy specimens | 9 | 3 | 3 | 1 | 0 | 5 | 21 |
| Total | 49 | 27 | 36 | 15 | 35 | 45 | 207 |

Hypoplasia and aplasia rank first in BM pathology, which predominate in patients under 50. Osteomyelodysplasia appears a frequent pathology more often observed in patients over 50. This pathology rate has been lately increasing. Tumor impairments are rarely observed since the ones do not require morphological investigation of BM. Erytremia is not a frequent pathology in Udmurtia. The decrease of observing of normal BM indicates the correct selection of patients for this procedure. Our data prove this method highly informative to verify clinical diagnosis.

# P-666

## THE EXPRESSION OF p53 PROTEIN IN NON HODGKIN'S LYMPHOMAS (NHL) - IMMUNOHISTOCHEMICAL ANALYSIS BY LSAB+ TECHNIQUE ON 35 CASES

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The tumor supressor gene product p53 is present in a variety of cells. Expression of this protein has been detected recently in NHL-s, but the relationship between p53 expression and the prognosis is still unclear as well as there is high variation in the reported incidence of p53 expression.

**Aims:** The aim of this study was to estimate the incidence of p53 expression detected by DAKO monoclonal antibody p53 clone DO-7, which reacts with wild type and mutant type of this protein in a group of agressive and very agressive NHL-s.

**Methods:**Thirty five cases with diagnosed agressive and very agressive NHL at the Institute of pathology were included in this study. Thy were classified according to REAL classification. Paraffin sections were used for histochemical and immunohistochemical staining for lymphocyte markers. P53 protein clone DO-7 was detected on paraffin sections by LSAB+ technique after antigen retrieval by microwave heating. Control slides from neoplastic prostatic tissue were used.

**Results:** Positive neoplastic cells were found in 13 (37,14%) cases, which could be divided into two groups according to the pattern of reactivity: 7 cases showed clear positive signal of random cells, not exceeding more than 5% per section, and the rest showed weak diffuse nuclear staining. One case showed cytoplasmatic positivity.

**Conclusion:** These results show that p53 protein is abnormally expressed in a substantial proportion of NHL-s. However the molecular basis of this expression remains to be elucidated. In the absence of molecular cytogenetic technique this could be done by using of different clones of p 53 antibodies able to differentiate wild and mutant form of this protein.

It remains to explore the prognostic significance of p53 expression in patients with NHL-s.

# P-667

## EXPLOITING THE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) - VEGF RECEPTOR INTERACTION FOR TUMOR ANGIOGENESIS STAINING

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**Aims:** Recent advantages in molecular biology have proven a crucial role in angiogenesis for the VEGF/VEGF receptor interaction. Flk-1 (fetal liver kinase-1) is one of the VEGF receptors that has been cloned and characterized, and is shown to exist in a soluble form (sFlk-1) through alternative splicing that can bind VEGF with equal affinity when compared to the native form. Since there is a high concentration of extracellular matrix bound VEGF in most tumors, we are working towards exploiting this highly specific interaction to stain for angiogenesis.

**Method and Materials:** Using standard molecular biological and biochemical techniques, we have obtained and produced a recombinant form of the sFlk-1, called sFlk-1-AP, which has an attached reporter alkaline phosphatase enzyme to faciliate protein detection. Supernants of transiently transfected NIH-293 cells were concentrated, purified and then analyzed.

**Results:** Conditions for sFlk-1-AP concentration and purification have been optimized and single bands of 200 kD protein with alkaline phosphatase activity were obtained. Quantification of the purified and concentrated fusion protein showed final yield of 300 µg/ml. Specific binding of the sFlk-1-AP to VEGF was demonstrated in vitro on sections of hypervascular tumors, with activity competitively displaced by soluble VEGF. Homing of this highly specific molecule to tumor neovasculature was investigated by in vitro staining.

**Conclusions:** A highly specific interaction between the fusion protein sFlk-1-AP and VEGF has been demonstrated in vitro. Specific staining of tumor neovasculature could have widespread applications in tumor detection and characterization.

# P-668

## IMMUNOHISTOCHEMICAL STUDY ON EXPRESSION OF E-CADHERIN AND P53 PROTEIN IN BREAST CARCINOMAS. AN IMPLICATION FOR PROGNOSIS VALUE

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Introduction: Both E-cadherin and p53 protein are invasion suppresor genes products. Our study try to determine if the correlation between than can have prognostic value.

Methods: Tissue material from 35 breast lesions (10 benigns and 25 malignant) of untreated patients was included in this study. We have made parallel samples for paraffin embedded blocks which were stained with H.E. For immunohistochemistry 5 µm thick cryostat serial sections were made for both E-caderin and p53. We used MoAb anti L-Cam, from Boehringer – Mannheim for E-cadherin staining and MoAb DO7, from Dako for p53.

Results: E-cadherin was expressed in all cases of benign and malignant lesions with different grades of intensity. We obtained intensive reaction at benign lesions and low reaction to undiferentiated malignant tumors. p53 was expressed in 65% of all cases. We had a positive correlation between p53 expression and tumor size.

Conclusions: We found a good correlation between high expression of E-cadherin and low grade of malignancy. Also the overexpression of p53 can be associated with poor prognosis. The correlation between the expression of the to invasion suppresion genes products can contribute at the quantification of the prognosis in breast carcinomas.

## P-669

COMBINED IMMUNOHISTOCHEMICAL EVOLUATION OF TUMOUR MARKERS AT PRECANCER DISEASES AND GASTRIC CANCER

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At development of metaplastic and dysplastic changes in gastric mucosa antigens were revealed, that indicated on embryanic reversion of mucosa and different directions in differentiation of progenitor cells.

Gastric cancer takes one of the first places in oncological morbidity structure in economically developed countries. In this connection it is very actual the development of new methods and criterions in early diagnostics of precancer diseases and gastric cancer.

**Aims:** The purpose of study was comporative analysis of carcinoembryonic antigen, pepsinogen C, beta-1-meconial antigen, trophoblastic beta-1-globuline tissue expression at their combined evaluation in gastric bioptates for early cancer and precancer diseases of stomach.

**Methods:** It was studied 86 cases of gastric polyps with different histological forms, 48 cases of cancer (adenocarcinomas of different differentiation degree, undifferentiated cancer). It was used undirect immunoperoxidase method.

**Results:** It was found, that at increasing gravity of pathological process (degree of dysplasia and intestinal metaplasia) in gastric, polyps was more high quantity and intensity of expression of above mentigned oncological markers. In difference from polyps at gastric cancer it was found more high intensity of antigen tissue expression, their coexpression, their revealing in the most structural elements of mucosa, including basal ports of epithelial cells and stroma.

**Conclusions:** Combined immunohistochemical evaluation of these markers may be used as additive method for accurate diagnostics of precancer diseases and gastric cancer.

## P-670

**EXPRESSION OF ANGIOTENSIN AT1 RECEPTOR IN ADRENAL GLAND STUDIED BY IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDISATION. A COMPARISON WITH HYPERPLASIA AND NEOPLASIA.**

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Aims: the anatomical distribution of angiotensin AT1 and AT2 receptor is related to organs or tissues involved in blood pressure regulation of fluid-electrolyte balance. It is found in the adrenal, vascular smooth muscle, kidney and heart. We compared the expression of the AT1 receptor in normal adrenal tissue and hyperplasia. We also investigated the receptor in neoplastic conditions.

Methods: 38 adrenal specimens: normal adrenal gland (n=8), cortical hyperplasia (n=9), cortical adenoma (n=2), cortical carcinoma (n=6); medullary hyperplasia (n=9) and pheochromocytoma (n=4) were investigated. Paraffin sections were incubated with rabbit polyclonal antibody against human angiotensin II AT1 receptor (Santacruz Biotechnology). The sections were treated with biotinylated anti-rabbit antibodies (followed by incubation with streptovidin-peroxidase conjugate). The peroxidase reaction was developed with AEC. The ISH study was carried out with the Angiotensin II AT1 probe. After hybridisation, an anti-FITC antibody (Biogenex, San Ramon, CA) was applied.

Results: the normal adrenal gland revealed immunohistochemically a heterogeneous staining of the different cortical layers and strong staining of the medulla. In cortical hyperplasia the AT1 receptor staining is diffusely positive, whereas expression is absent in cortical adenoma and focally positive in carcinoma (15-49%). Medullary hyperplasia and the pheochromocytoma are strongly positive in a homogeneous way. ISH shows a diffuse cytoplasmic staining in normal adrenal cortex and in a sparse subcapsular layer in cortical hyperplasia. In cortical adenoma and carcinoma a nuclear staining is found respectively in 40% and in 25%. The ISH findings in normal, hyperplastic and tumoral medullary tissue reveal a low nuclear and cytoplasmic staining between 10-30%

## P-671

**STROMAL RESPONSE IN MAMMARY CARCINOMA AND PREMALIGNANT BREAST LESIONS**

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**Introduction:** The authors' propose is to study the expession of myofibroblastic stromal response to breast cancer and premalignant breast lesions.

**Material and method:** We studied 24 cases of breast lesions without treatment The IHC study was performed by specific antibodies against myofibroblastic antigenes: α-smooth actin (A), vimentin (V) and desmin (D); factor VIII against endothelial cells and of course we had evaluated the hormonal status by identifiying hormonal receptors. The immunostaining was evaluated by LSAB method.

**Results:** Invasive carcinoma expressed actin in 12 cases, among them in 8 cases the myofibroblastic cells, V in 4 cases and non case expressed D.In situ carcinoma expressed actin in 2 cases but for myoepithelial cells and vessels, non case for V or D.The only case which combined the lobular invasive carcinoma with fibrocystic desease, expressed actin for vessels and myoepithelial cells and D in 1 case.Fibrocystic disease expressed A in 8 cases for myofibroblastic cells, V in 5 cases and D in 2 cases. Actin was expressed in all cases for vessels and in particular for capilary vessels, belonging in our opinion to neoangiogenesis phenomena. We recorded 6 cases with A positive for neoangiogenesis vessels. In all cases synchronuos stromal reaction was recorded.

**Conclusions:** Neoangiogenesis vessels recorded in our cases, according to the literature suggest that fibroblasts, myofibroblasts, vascular smooth muscle cells, pericytes and other specialized mesenchymal cells with various myoid features, represent cellular isoforms of a common ancestor cells. The degree of differentiation toward the smooth muscle phenotype would depend on microenvironmental factors. The particular aspect recorded by our study is the express of myofibroblastic markers in fibrocystic disease, where maybe like in wounds cases we assist to a garnulation like tissue heal or a quasineoplastic proliferative conditions.

## P-672

THE INFLUENCE OF IONIZING IRRADIATION ON THE ULTRASTRUCTURE OF THE FETOPLACENTAL COMPLEX

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**Aims:** To determine the morphological changes in placenta, organs of fetus and newborn from the families exposed by radiation after Chernobyl accident.

**Methods:** There were examined 52 women (placentas and thyroids of stillborn and dead neonates) that have been divided into four groups on the basis of received radiation dose or place of residence at the time of or after Chernobyl accident 1 group (control) - women with physiologic pregnancy course with absence of radionuclides incorporation; 2 group - with radionuclides content in placenta 0,5-1,0 Bq/kg; 3 group - 1,0-2,8 Bq/kg; 4 group - 2,8-4,8 Bq/kg. The samples were stained with hemotoxilin-eosin, van Gieson staining with picrofuxin, immunohistochemical staining for PCNA, CEA and TUNELL (for apoptosis).

**Results:** The expression of PCNA in nucleas of cytotrophoblast and syncytiotrophoblast in 4-th group were noted in the majority of cases (62%). The positive CEA reaction was found in enlarged nucleas of cytotrophoblast and syncytiotrophoblast and in endothelial cells nucleas of fetal vessels, villous stroma fibroblasts. There was positive TUNELL reaction for apoptosis in majority of placentas from 2, 3, 4 groups in endothelial cells of small fetal vessels and villous stroma. We also noted positive CEA and PCNA reaction in thyroid glands from 4-th group.

**Conclusions:** There is possibility of radiation apoptosis in placenta. The changes in placenta and thyroids can be evaluated as early manifestation of transplacental oncogenesis.

## P-673

**COMPARATIVE INVESTIGATION OF DETECTION OF MYCOBACTERIUM TUBERCULOSIS IN PARAFFIN EMBEDDED TISSUE : A COMPARISON BETWEEN ZIEHL-NEELSEN STAIN, IMMUNOHISTOCHEMISTRY AND PCR**

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Aims: the light microscopical diagnosis of tuberculosis in lymph nodes is nearly always made on the presence of necrotising granulomas undergoing central caseosis surrounded by Langhans histiocytes and epithelioid histiocytes. This suggestive diagnosis is rarely confirmed by the finding of tubercle bacillae by Ziehl-Neelsen staining. The final diagnosis depends mainly on a positive culture(lasting 6 weeks). An objective criterium, immediately available, is necessary for pathology practice.
Methods: 36 lymph nodes from 26 patients were investigated. 4 µ consecutive sections were taken from the 36 cases and stained for HE and Ziehl-Neelsen staining. Immunohistochemistry was carried out using a monoclonal antibody against Mycobacterium spp (Dako, Belgium). 15 µ sections were also cut and the DNA extracted and analysed using PCR. The primers used were P2 against all bacteria and 10.7 MB UZ1, specific for Mycobacterium spp.
Results : all lymph nodes show necrotising lymphadenitis with caseosis. Ziehl-Neelsen staining was positive in 1/36 cases. Immunohistochemistry revealed positive bacteria in 3/36 cases with positive staining of bacilli and granular material. The PCR technique was positive in 12 / 36 cases.
Conclusion: the use of the Ziehl-Neelsen staining for the detection of the sparse acid fast bacillae in light microscopy is time consuming and questionable since the granular material also stains. The higher yield of the monoclonal antibody against Mycobacterium spp staining positive bacillae and granular material proves the value of this technique. The positive PCR reaction in 12/36 cases confirms the higher sensitivity of the PCR technique with objective proof of the infection.

## P-674

ROLE OF GROUP II PLA2 IN INFLAMMATORY CHANGES IN *S. aureus* INFECTION

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**Aims:** Group II phospholipase A2 (PLA2) is an acute phase protein that has marked bactericidal properties. Aims of the present study were to define the role and sources of group II PLA2 in purulent inflammation.
**Methods.** Live *Staphylococcus aureus* bacteria were injected into the peritoneal cavity of transgenic mice expressing human group II PLA2 and of their PLA2 deficient littermates. Hematological and histological changes were studied after the administration of *S. aureus*. The expression of group II PLA2 in peritoneal tissues was studied by mRNA *in situ* hybridization.
**Results:** Transgenic mice showed subacute inflammation, proliferation of fibroblasts and abscesses in the peritoneum one week after i.p. administration of *S. aureus*, whereas PLA2 deficient mice showed minor inflammatory changes only. The number of peripheral blood polymorphonuclear cells was markedly elevated in transgenic mice, but not in PLA2 deficient mice. Administration of bacteria markedly increased the expression of group II PLA2 in proliferating fibroblasts of peritoneal abscesses.
**Conclusions:** The results suggest an important role for group II PLA2 in inflammatory response and formation of abscesses in *S. aureus* infection.

## P-675

BRAIN LESIONS DUE TO HERPES VIRUSES IN PATIENTS DIED FROM AIDS

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**Aims:** Lesions due to Herpes simplex virus {HSV} 1, 2 types, Cytomegalovirus {CMV}, Epstein-Barr virus { EBV} belong to the most frequent and severe complications of AIDS. Succesful treatment of the patients is impossible without evaluation of their role in single cases.
**Methods:** On the autopsic material of St.Petersburg ( 40 cases) we succeeded to study microscopical changes in brain caused by different pathogens. Immunohistochemical (IHC) investigation on the paraffin slices (BIOGENEX) was provided. Histological and IHC data was compared with duration of AIDS and the treatment of patients with zovirax.
**Results:** The histological appearance of lesions caused by different herpes viruses were moderate and similar in all infections, they consisted in enlargement and hyperchromatosis of nuclei, mostly localized in small groups. The typical picture of necrotic herpes encephalitis was not observed. Clinical and laboratory data made possible diagnose meningoencephalitis due to HSV and CMV in majority of cases, but the antiviral therapy with zovirax differed strongly in single cases. In majority of cases the degree of lesions on autopsic material was moderate, only in 1 case the lesions due to HSV played the leading role in the lethal outcome.

| virus | diagnosed in clinics | IHC posit. |
|---|---|---|
| HSV | 9 | 8 |
| CMV | 10 | 3 |
| EBV | 0 | 1 |

**Conclusions:** The lesions by HSV, CMV, EBV can be observed practically in all lethal cases of AIDS but their pathogenic role depends on duration of the disease and tactics of antiviral therapy and differs strongly.

## P-676

**OPPORTUNISTIC PULMONARY INFECTIONS IN PATIENTS WITH AIDS**

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Opportunistic pulmonary infections occur frequently as a complication in patients with AIDS.
**Aims:** The aim of the evaluation was to determine the frequency of certain opportunistic pulmonary infections and observe their histological characteristics.
**Methods:** This paper is based on the histopathological analysis of ninety post mortem lung biopsies in AIDS patients where the respiratory infection was the terminal outcome of the disease. Microscopic evaluation of lesions on lungs was done on paraffin-embedded sections after standard histopathological and histochemical methods. Immunohistochemical analyses concerning monoclonal antibodies have been done through APAAP method.
**Results:** Bacterial infections (35.56%) were represented by fibrinose suppurative bronchopneumonia, necrotizing pneumonia, or abscess formation. Pneumocystis carinii pneumonia was nearly the most frequent pulmonary complication (26.67%). The foamy oesinophilic content was found in intra-alveolar cavities, as well as fibrin alveolar epithelial cells, alveolar macrophages, and vegetative or cystic forms of Pneumocystis carinii. The interstitial inflammatory infiltrate was most often scant, and consisted mainly of lymphocytes and rare plasma cells. Pulmonary tuberculosis and atypical microbacterial infections (20.00%) were characterized by granulomatose changes with extensive tissue necrosis and occurrence of caverns. Cryptococcal pneumonia (10.00%) showed heterogeneous histological results and represented a part of a disseminated mycosis. Cytomegaloviral pneumonitis (7.78%) was associated with other lesions on lungs. Characteristic cytomegaloviral inclusions were present in intra-alveolar epithelial cells.
**Conclusions:** Opportunistic infections result in heterogeneous pathohistological lesions on lungs, and therefore require application of specific pathohistological techniques, as well as correlation with other clinical diagnostic procedures.

## P-677

**HLA-CLASS II ANTIGENS EXPRESSION IN RENAL CELL CARCINOMA**

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**Aims**: Correlation between presence of HLA-class II antigens in renal cell carcinoma (RCC) and its clinical and morphological characteristics.

**Methods**: Cryostat sections of 37 RCCs (25 clear cell, 10 granular and 2 chromophobe) were studied with indirect immunoperoxidase method applying monoclonal antibodies (MoAb) to HLA-DR (Tü36), -DP (Fa) and -DQ (Tü22) antigens. Tumor-infiltrating mononuclear cells were analyzed also, using anti-CD14, -CD3, -CD4 and -CD8 MoAb. Number of positive cells was estimated semiquantitatively and correlated with both clinical (patients age and sex, tumor size and TNM status) and morphological (citology, histology and tumor grade) characteristics of RCC.

**Results**: All RCCs expressed HLA-DR antigens, 92% HLA-DQ and 73% HLA-DP. Level of expression was higher in granular than in clear cell type, but no correlation with tumor grade could be established. Cases with more pronounced local spread (T3,T4) and distant metastases (M1) showed diffuse presence of all class II antigens. Those tumors had, also, greater average diameter than those with focal HLA-class II antigens expression. Higher level of class II antigens was accompanied by an increase in T lymphocyte/monocyte and CD4/CD8 ratio.

**Conclusions**: Higher level of aberrant HLA-class II antigens in RCC was associated with parameters (greater size, T3,T4 and M1 status) which implies more aggressive tumor behavior, possibly because, despite capability of antigen presentation, HLA-class II molecules expression on tumor cells induce anergy rather than activation of T cells, due to a lack of co-stimulatory molecules (e.g. B7).

## P-678

**NOTES ON MESANGIAL MATRIX IN MEMBRANO-PROLIFERATIVE GLOMERULONEPHRITIS**

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**Aims**: Investigation of the membrano-proliferative glomerulonephritis from the point of view of the structure induced by proteoglycans in the mesangial matrix.

**Methods**: The specimens resulted from the renal biopsy with a TRU-CUT needle were analyzed. The following staining techniques were used: Haematoxylin-Eosin, Szekely trichrome, PAS, toluidine blue, alcian blue with critical electrolyte concentration. Direct immunofluorescence relied on anti-human total Ig, IgG, IgA, IgM, labeled with fluorescein isothiocianat.

**Results:** A number of 20 cases of glomerular diseases were diagnosed as membrano-proliferative glomerulonephritis according to the correlation noticed between the morphological lesions and the characteristics of the immunofluorescent extensive deposits. Subsequently, the proliferation degree of the mesangial matrix was evaluated together with the changes of the proteoglycans occurred in structure. There was observed the presence of the glycosaminoglycans of heparan- sulphate- or chondroitin-sulphate-type. The increase and the damage of the mesangial matrix was firstly correlated with the appearance of the heparan sulphate solely, and, secondly, with the appearance of both heparan and chondroitin sulphate. The morphological and immunofluorescent aspects supported the clinical course, including a possible evolution toward the glomerulosclerosis.

**Conclusions:** Remodeling of the extracellular, mesangial matrix is strongly connected to the transformation of glomerular diseases into glomerulosclerosis and chronic renal failure. In this context, the estimation of the proliferation degree and, consequently, the changes in the structure of the mesangial matrix represent key elements for the common practice in clinical nephrology. Moreover, the quantification of such changes can serve as an indicator for the prognosis.

## P-679

**THE IMPORTANCE OF RENAL HISTOPATHOLOGY FOR THE THERAPEUTIC EFFICACY OF CAPTOPRIL AND INDOMETHACIN IN CONGENITAL NEPHROTIC SYNDROME**

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The therapeutic response to captopril and indomethacin in relation to the renal histopathologic findings was examined in eight children (four males) with congenital nephrotic syndrome (CNS). Median age at diagnosis was 2.5 weeks. Renal histology was available in all and revealed CNS of the Finnish type (CNF) in six children and diffuse mesangial sclerosis (DMS) in one child. The eighth child who was diagnosed at the age of 3 weeks had histological changes that were not typical of either Finnish type or DMS type. His kidney shows partial failure of both glomerular and tubular development. There was only minor tubular dilatation and this was generally associated with hyaline casts. The main histological features found in CNF children were cystic dilatation of proximal tubules, sclerosis andfibrosisofglomeruli, tubular atrophy and interstitial fibrosis. Combined treatment with captopril and indomethacin was commenced at median age of 2.3 months. Following this treatment a good clinical and laboratory response was noted only in the patient with DMS histology. This improvement was maintained for 29.7 months. Unfortunatelly this patient developed E.coli peritonitis and septicaemia and died of septic shock aged 41 months. The remaining patients showed no clinical improvement after treatment. For this reason they underwenta unilateral nephrectomy in the first 6 months of life. After 7-72 (median 39) months follow-up six of them are alive.

In conclusion, the response to treatment with captopril and indomethacin in children with CNS appears to be related to the renal histology. This finding demonstrate the importance of renal biopsy before a therapeutic trial with combined captopril and indomethacin treatment is initiated. On the basis of this limited experience, more similar studies are necessary to further confirm this finding.

## P-680

**CROMOPHOBE RENAL CARCINOMA: MORPHOLOGICAL, IMMUNOHISTOCHEMICAL AND FLOWCYTOMETRIC INVESTIGATION**

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**Introduction:**We present the case of a 67 years old man hospitalized for lombar pain, nausea and vomiting. Echographical examination with Doppler and N.M.R. demonstrated a hypervascular left renal mass with no evidence of renal artery and cave vein involvement. Adenopathy was absent. The patient underwent a left radical nephrectomy. The macroscopically examination showed a well circumscribed and endocapsulated tumor located in the upper pole, soft, yellow to tan-beige on cut surface.

**Material and methods:** Fragments of the tumor were fixed in formaldehyde 10%, included in paraffin and the sections were stained with HE,VG; immunohistochemical with cytokeratine,EMA,VIM and histoenzymatic with colloidal iron (Hale's stain). Single tumor cells suspensions were obtained by enzymatic digestion and used for flowcytometric evaluation of apoptosis by means of propidium iodide staining

**Results:** The histological examination revealed proliferation of large tumor cells with abundant cytoplasm and distinct cell borders arranged in large sheets separated by delicate fibrous septa. Nuclei were round, central with conspicous nucleoli. Most tumor cells had eosinophilic and finely granular cytoplasm. Other cells showed perinuclear haloes and some cells were large with abundant, reticulated and translucent cytoplasm. Immunohistochemical analysis: the cytokeratin profile ($AE_1$, $AE_3$) was positive, EMA positive, VIM negative. The tumor cells stained positively for Hale's colloidal iron.

**Conclusions:** All these findings plead for the diagnosis of chromophobe renal cell carcinoma. The percentage of apoptotic cells was 72%. The activation of apoptosis seems to be p.53 independent, as revealed by immunohistochemistry and only slightly Fas dependent.

## P-681

**ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF HEPATIC SINUSOIDS IN CHRONIC VIRAL HEPATITIS**

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**Change of liver sinusoids is one of manifestations of chronic hepatitis. In present work, we studied particularity of hepatic sinusoidal wall sturucture in the patients with chronic active B (CAHB) and delta (CAHD) hepatitis, using electron microscopic and immunohistochemical methods. For ultrastructural study liver biopsies of the patients with CAHB (12 cases) and CAHD (16 cases) were fixed in 2,5 % glutaraldehyde and postfixed with 1% $OsO_4$. In the serial cryostat sections distribution of the main extracellular matrix components: types I, III, IV, V collagen, fibronectin and laminin were detected by immunofluorescent technique.**

**Electron microscopy revealed appearance of the basement membrane around the sinusoidal wall in the liver of the patients with chronic viral hepatitis. Ultrastructural changes were also accompanied by perisinusoidal fibrosis.**

**In immunofluorescent study increasing amount of collagen types I, III, IV, V and fibronectin in the sinusoidal wall was observed. A prominent peculiarity of CAHB and CAHD was the presence in high content of laminin around the sinusoids. Continuos linear deposition of collagen and laminin clearly underlined enlarged sinusoidal borders. In the liver of the patients with CAHD the changes described above were more aggressive.**

**Our results collectively demonstrate change of hepatic sinusoidal wall structure in chronic viral hepatitis, resulting in liver dysfunction.**

## P-682

**CHRONIC VIRAL HEPATITIS. HISTOLOGICAL AND IMMUNOHISTOCHEMICAL PROFILES IN PATIENTS WITH ONE OR DUAL HEPATITIS VIRUS INFECTIONS.**

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**Aims :** The study was aimed to evaluated some histological and immunohistochemical (IHC) features of 88 chronic viral hepatitis carriers.

**Methods :** We analysed liver biopsy specimens of these patients with confirmed clinical, virological and serological tests. Histological findings were scored according to ISHAK system . IHC-ABC method for identifying surface and core antigens(HBsAg, HBcAg) on tissue samples, was performed.

**Results :** Among our cases, 39% were B virus chronic hepatitis(HBV), 49% were C virus chronic hepatitis (HCV)and 9% have dual virus infections (HBV + HCV). The histology score show the presence of moderate and severe chronic hepatitis in:25% HBV patients, 30% HCV patients and 33 % in dual infected patients. For moderate and severe fibrosis the percentage was 36% in HBV patients, in 51% HCV patients and 67% in HBV + HCV patients.The IHC technique identified HBs Ag in more than 60% of liver biopsies from serological positive HBV patients, but the HBcAg only in 30% of them. For HBV + HCV biopsies, HBsAg was positive in 50%and HBcAg in 25%.

**Conclusions :** In patients with chronic HCV and HBC + HCV the staging and grading score was higher then in HBV patients. The IHC tests confirm only partially the viral etiology. However the presence of the HBcAg is significant for viral replication.

## P-683

HEPATIC EPITHELIOID HEMANGIOENDOTHELIOMA. CLINICO-PATHOLOGIC DIMENSIONS.

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**Aim:** Epithelioid hemangioendothelioma (EHE) is an uncommon vascular neoplasm with distinctive histopathologic appearance and highly unpredictable clinical course. Beside occurring mostly in the soft tissue, lung, and bone, the liver may also be involved. Controversial interpretations of this rare clinicopathologic entity are reflected in frequent misdiagnosis.

**Material, methods:** Two cases of hepatic EHE, a 47-year-old woman and a 50-year-old man were retrieved from the series of 56 patients surgically treated for primary liver neoplasia. The patients presented with upper abdominal discomfort. Imaging procedures revealed multiple 3cm large lesions in the liver. In the search for a particular neoplastic profile that might predict the tumorous behaviour a detailed pathohistological analysis was done. Immunohistochemical panel consisted of antibodies to epithelial, hematopoietic, and mesenchymal determinants including cytokeratins, CD 32, CD 34, CD 68, F VIII, S100 and vimentin, so as PCNA, MIB 1, bcl-2, and p53. Liver resection (the female) and transplantation (the man) was followed by a 5- yrs healthy period.

**Results:** The tumours were characterised by clusters of plump epithelioid cells with vacuoles, projecting into tiny vascular spaces, and dendritic cells, all embedded in fibrous stroma. Mitosis were rare. In the both types of tumorous cells, endothelial markers (F VIII, CD 34) were intensively positive, whereas CD1a, CD 68 and S100 protein were completely negative. The coexpression of CD34 and F VIII on a subset of the tumorous cells, so as diffuse infiltration of F VIII positive dendrocytes was observed. Proliferative index comprised 20% of tumorous cells.

**Conclusion:** Correct diagnosis of hepatic EHE requires immunophenotypic characterisation of the tumorous cells that intermingle with macrophages and dendrocytes probably presenting reactive elements. Clinical outcome might be influenced with the grade of tumorous cellularity whereas mitotic counts, nuclear atypias, and the proliferative index do not seem to be prognostically significant.

## P-684

LIVER STORAGE OF HYDROXYETHYLSTARCH

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Hydroxyethylstarch (HEA) is a widely used plasma expander. Hitherto no hepatic side effect has been reported.

From september 1997 to april 1998, a liver biopsy was performed in 7 patients for unexplained refractory ascites (4 cases) or anicteric cholestasis (3 cases) after iterative intravenous infusions of HEA (mw 200 000). HEA infusions were administered for 1.5 to 10 months (cumulative doses 690 to 3990 g) for large volume paracentesis in cirrhotic patients abstinent for alcohol (3 cases) , chronic haemodialysis (1 case), plasma exchanges (2 cases) and arterial hypotension (1 case).

Diffuse microvacuolization and hyperplasia of CD68 positive Kupffer cells with focal sinusoidal obstruction were observed in all post-infusions biopsies but absent in the pre-infusion biopsy obtained from 1 patient. Microvacuoles appeared empty on HES, they were negative for Oil-Red-O and contained few positive granules with PAS and argentic staining. Cytoplasmic microvacuoles with peripheral PAS enhancement were focally noted in hepatocytes of 4 cases. For 5 patients, other lesions reflected the underlying liver disease (cirrhosis = 3, chronic hepatitis = 1, sarcoidosis = 1). Four patients died from sepsis (3 cases) or hepatic failure (1 case).

These data suggest that HEA could accumulate in liver as it has been described in skin and nerves; this storage could be responsible for onset or worsening of portal hypertension and hepatic dysfunction.

## P-685

ACHIEVEMENT OF LIVER IRON CLEARANCE IN EX-THALASSEMIC PATIENTS AFTER BONE-MARROW TRANSPLANTATION (BMT): A CLINICAL-PATHOLOGICAL STUDY.

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**AIMS:** Histomorphologic and clinical study on 66 thalassemic patients who showed either clearance or a very significant decrease in liver iron overload after BMT. These modifications are compared with associated lesions such as hepatitis and fibrosis.

**METHODS:** Liver biopsy has been performed on thalassemic patients prior to BMT since 1984 and at regular intervals thereafter. Among patients cured by successful allogenic BMT, 41 patients (mean age 16+/-2.9 years) were subjected to a program of regular phlebotomy 9.5+/-1.5 years after BMT (6 ml/Kg blood withdrawal at 14-days interval for a mean period of 35+/-18 months), 18 patients (mean age 18 years) received desferrioxamine therapy and 7 young patients without any therapy (mean age 5 years), consented to follow-up by sequential biopsies. The liver iron overload was histologically graded in parenchymal and mesenchymal sites (according to previously published study), whereas hepatitis grading and staging were evaluated according to Ishak and Coll. Liver iron concentration (LIC) was assayed by atomic absorption spectrophotometry and expressed as mg/g dry weight.

**RESULTS:** Complete liver iron clearance (mean LIC 0.9+/-0.4) was observed in the 41 patients who underwent the phlebotomy program. Among 18 patients treated by chelation therapy, 2 showed complete iron clearance as desferrioxamine was withdrawn in the remaining 16 patients when a very mild emosiderosis was obtained (mean LIC 2+/-0.5). Seven young patients reached spontaneous iron clearance (mean LIC 1.5+/-0,5). Chronic hepatitis improved significantly in the last biopsy, whereas fibrosis showed a mild decrease in many cases.

**CONCLUSIONS:** This study demonstrates that complete reversibility of liver iron overload in ex-thalassemic patients is possible. The associated improvement of chronic hepatitis may correlate to hypothesis that liver iron burden acts as a damaging cofactor in hepatitis course.

## P-686

ARE BILE DUCT LESIONS IN CHRONIC HEPATITIS C ASSOCIATED WITH SERUM HEPATITIS C RNA LEVELS OR VIRAL GENOTYPES ?

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**Aims:** Bile duct lesions are frequently observed in patients with chronic hepatitis C (CHC). The aim of this study was to determine whether bile duct lesions in patients with CHC might be associated with hepatitis C virus (HCV) genotypes and serum HCV RNA levels.

**Methods:** 100 liver biopsy specimens from 100 patients (M :W, 59 :41) with CHC were studied. The histological appearance of bile duct lesions for each biopsy were graded according the METAVIR group (a panel of 10 French pathologists) with some modifications as follows : 0 = no lesion ; 1 (mild or moderate) = lymphocyte or plasma cell infiltrate, variation in nuclear staining, epithelial cell vacuolization, stratification and loss of polarity of epithelial cells or combination of these ; 3 (severe) = necrosis and/or bile duct destruction. Infiltration of the biliary epithelia without epithelial cell lesions was not considered as a bile duct damage. Histological evaluation was performed without knowledge of the clinical, biochemical and virological data. Serum HCV RNA levels was determined at the same time the biopsy was performed, with Quantiplex 2.0 branched DNA test (Chiron). HCV genotyping was performed using the Inno-LiPA HCV (Innogenetics) second generation assay. Patients were infected by the following genotypes : 1 (n=52), 3 (n=32), other than 1 and 3 (n=11) and indeterminate (n=5).

**Results:** Bile duct damage was observed in 71% of the patients. Bile duct lesions were mild or moderate and severe in 49% and 22% of the cases, respectively. The frequency and the severity of the lesions were not associated with serum HCV RNA levels (frequency, p=0,61, severity, p=0,22) and genotypes (frequency, p=0,65 ; severity, p=0,35).

**Conclusion :** These results confirm that bile duct lesions are frequently observed in patients with CHC but are not associated with serum HCV RNA levels and genotypes.

## P-687

**PEROXISOME PROLIFERATOR ACTIVATING RECEPTOR gamma EXPRESSION IN THE LIVER OF CARBON TETRACHLORIDE INTOXICATED RATS**

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**Aims:** Peroxisome Proliferator Activating Receptor gamma (PPAR gamma) is a ligand-dependent transcription factor important in adipocyte differentiation and glucose homeostasis. The intrahepatic distribution of PPAR gamma was examined in a model of liver injury and regeneration induced by carbon tetrachloride ($CCl_4$) administration in rats.

**Methods:** Male Wistar rats were administered intraperitoneally with 1 ml $CCl_4$/Kg of body weight. Toxicological end points and markers of hepatocellular regeneration were assessed at various time points (0, 12, 24, 36, 48, 60 and 72 h) post-$CCl_4$ injection. The enzymatic activities of aspartate and alanine aminotransferases in serum and liver histological findings were used to estimate $CCl_4$-induced hepatotoxicity. The rate of [$H^3$] thymidine incorporation into hepatic DNA, the enzymatic activity of liver thymidine kinase activity and the assessement of mitotic index in hepatocytes were used as indices of regeneration. PPAR gamma was detected immunohistochemically in paraffin embedded liver sections.

**Results:** $CCl_4$ administration caused liver injury, followed by hepatocel lular proliferation, which presented peak at 48 h post-treatment. Mild PPAR gamma immunoreactivity was prominent in centrilobular hepatocytes 12 h post-$CCl_4$ administration. At 24 h post-toxin administration intense PPAR gamma expression was found in centrilobular hepatocytes and foam cells, while at 36 h intense staining was found in hepatocytes in the vicinity of inflammatory sites. At further time points examined, moderate PPAR gamma expression was noted in proliferating hepatocytes and intense in isolated ones in the vicinity of inflammatory infiltrations.

**Conclusion:** Our study describes the hepatic expression of PPAR gamma, implicating its contribution in toxin-induced injury and regeneration.

## P-688

MINOCYCLINE-INDUCED SYSTEMIC GRANULOMATOUS REACTION

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We report for the first time a systemic granulomatous reaction including acute granulomatous hepatitis related to minocycline.

A previously healthy 23-year-old women was admitted for fever, erythrodermia and lymphadenopathy. For the preceding 4 weeks, she had received minocycline (200mg/day) for facial acne. White blood cell count was 13X10 9/L with hypereosinophilia, increased T cell count and atypical lymphocytes. Biochemical liver tests revealed AST and ALT increased up to 15N and 7N respectively. As a lymphoma was suspected, cervical lymph node, liver, bone marrow and skin biopsies were performed. Cervical lymph node biopsy showed expansion of the subcapsular paracortical areas with hyperplasia of langherans cells and no lymphoma. Liver biopsy revealed acute granulomatous hepatitis with mixed periportal and lobular necrosis. The marked inflammatory infiltrates included CD8+ lymphocytes and plasmocytes. Bone marrow biopsy showed non necrotizing epithelioid granulomas. Skin biopsy revealed lymphocyte perivascular infiltrates, exocytosis and rare eosinophils. Diagnoses of tuberculosis and viral hepatitis were excluded. Cessation of minocycline was followed by an improvement of symptoms and biological tests. Although fever, eosinophilia, lymphadenopathy and hepatitis have been previously described, minocycline has not been reported to induce granulomatous hepatitis or systemic granulomas. These histologic features may help early recognition of minocycline side effects related to T cell activation.

## P-689

DNA PLOIDY PATTERNS IN LUNG CANCER AND THEIR PROGNOSTIC VALUE

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**Introduction:** DNA ploidy histogram interpretation is one of the most important sources of variation in DNA image cytometry. The prognostic value of ploidy status in different histologic types of lung cancer is uncertain.
**Aim:** To assess the ploidy status in lung cancer and its relationships with histologic type and survival rate.
**Methods:** DNA content was measured by image analysis (CAS-200 system) in formalin-fixed paraffin embedded lung carcinoma samples from 51 autopsy cases. Mean survival rate for each histologic type of lung carcinoma was compared with DNA ploidy type.
**Results:** Most small-cell-lung cancers (SCLC) showed hypoploid, or hypodiploid DNA histograms with DI of the first peak 0,5-0,9. In this group the mean survival (2,66 months) was shorter than in SCLC with aneuploid histograms and DI above 1,0 (6,83 months). Non-small-cell-lung cancers (NSCLC) showed very heterogeneous DNA ploidy patterns. The mean DI in NSCLC was two to four times higher than in SCLC. In adenocarcinoma and giant-cell carcinoma, where nearly all histograms were aneuploid the mean survival was 3,25 and 2,60 months respectively. In squamous-cell lung carcinomas which were mostly hyperdiploid or tetraploid the mean survival was 4,61 months.
**Conclusion:** Hypoploidy in SCLC is a distinct DNA content abnormality, which is a negative prognostic factor. No conclusive correlation could be found between DNA ploidy type, histology and survival in NSCLC.

## P-690

**PULMONARY INFECTION BY *DIROFILARIA IMMITIS* MIMICKING LUNG CANCER. A CASE REPORT**

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**Clinical history and methods.** A 56-year-old woman, was admitted to the hospital due to trauma of the knee. A routine chest x-ray examination disclosed a solitary, radiodense nodule in the right upper lobe of the lung. Chest computed tomography showed a subpleural coin lesion, without calcification or cavitation, of about 1.5 cm in diameter. Physical examination, bronchoscopy, brochoalveolar lavage as well as laboratory data were normal. In suspicious of malignancy, a surgical approach with intraoperative examination of the lesion was performed.
**Results.** On cut surface, the specimen showed a pink, roundish nodule, demarcated to the pulmonary parenchima. Frozen sections showed a granulomatous reaction with a central area of necrosis and inflammatory cells. No neoplasia was found and therefore only a partial resection of the pulmonary upper lobe was performed. Light microscopic examination disclosed the presence of cross-sections of coiled degenerate worms, ranged from 70 to 250 mμ in diameter in the lumen of a medium-sized artery, within the area of coagulative necrosis. They were poorly preserved, but the presence of a smooth cuticle, muscle fibres and fragments of intestine allowed the identification of the worms as *Dirofilaria immitis*. At the edge of the necrosis, macrophages, lymphocytes, rare eosinophils and fibroblasts were found. Two small-sized arteries were thrombized. A chronic inflammation was found in the alveolar septa of residual lung parenchima. The patient was discharged without any therapy and at long term follow-up she is live and well.
**Conclusions.** Dirofilaria infection has a worldwide distribution, but the pulmonary localization of *Dirofilaria immitis* is quite rare. However, the parassitic origin of a pulmonary solitary coin lesion should be considered as a possible differential diagnosis of lung neoplasia.

## P-691

MIB-1 PROLIFERATION INDEX CORRELATES WITH SURVIVAL IN PLEURAL MALIGNANT MESOTHELIOMA

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**Aims:** The aim of our study was to establish whether cell proliferation index, assessed by the monoclonal antibody MIB-1, would correlate with survival in patients with pleural malignant mesothelioma (MM).
**Methods:** Seven cases of MM with long-term survival (LTS) (≥ 3 years from diagnosis) were retrieved from the files of our Institute. Each LTS case was coupled with 3 control cases with short-term survival (STS) (< 3 years from diagnosis) according to the following criteria: age (± 3 years), sex, histologic subtype. Immunohistochemical studies with the monoclonal antibody MIB-1 were performed on representative tissue sections from each case. A labeling index (LI) was determined randomly counting 1,000 tumor cells and expressing the results as a percentage of positive cells. The relationship between LI and survival in each pair case-control was assayed by the Wilcoxon signed rank test.
**Results:** MIB-1 immunostaining revealed nuclear staining in each case. Heterogeneity in the distribution of MIB-1 positive cells was observed. MIB-1 LIs varied from 1% to 8.8% (mean value: 5.2%; median value: 5.8%) in the LTS cases while in the control cases MIB-1 LIs varied from 5% to 86% (mean value: 27.8%; median value: 17.4%). Statistical analysis showed a significant difference between LTS cases and STS control cases ($p$=0.02).
**Conclusions:** Our results indicate that the differences in biological behavior of MM in long-term and short-term survivors may be explained in part by differences in tumor growth fraction and that MIB-1 proliferation index could represent an important prognostic parameter for this tumor.

## P-692

**THE PULMONARY-RENAL SYNDROME AMONG CHERNOBYL NUCLEAR ACCIDENT LIQUIDATORS**

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**Aim**: The aim of the paper is to study pulmonary and renal pathologies among died Chernobyl nuclear accident liquidators.
**Materials and Method**: The organs of 38 died Chernobyl nuclear accident liquidators, who worked in Chernobyl 30-km zone in 1986-87 and died in1993-98,were studied.Their average age was 42.8 years. The irradiation dosage was between 0,92 and 25 rad.
The pieces were embedded in paraffin. Hystological speciment were stained with HE, PAS, Van Gieson, partially by alcyan and tolidin blue under different pH, and impregnated with silver by Foot.
**Results**: In all cases the distinctive pulmonary and renal changes called pulmonary-renal syndrome were observed.
The macrophage agglomerations loaded with brown dust were detected in lungs, interstitial tissue, under pleura, around vessels and bronchi besides antracosis. The agglomerations were accompanied with interstitial fibrosis, panacinar emphysema, deforming bronchitis and peribronchial lymphoid tissue atrophy. On the background the symptoms of viral and bacterial infections manifested and led to death in 26.3% (10 cases). Renal changes of "sclerosing glomerulonephrosis" type characteristic of radiation lesions (Mostofi, 1972) were detected in all cases. Also there were medullar sclerotic changes, glomerulosclerosis and canaliculus epithelium atrophy, often accompanied by arterial hypertension.
**Conclusion**: Taking into account the fact that Chernobyl dust contains fissionable isotopes (Reva et al 1998) we consider the incorporated radionuclides endogenous action to be more affective to reported pulmonary, vascular and renal changes than exogenous irradiation.

## P-693

SIMIAN VIRUS 40 (SV-40)-LIKE DNA SEQUENCES AND SURVIVAL PROBABILITIES IN PLEURAL MALIGNANT MESOTHELIOMA (MM).

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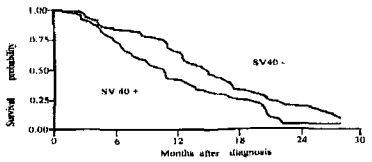
**Aims:** To determine if the presence of SV 40-like DNA sequences in human pleural MM tissue affects survival probabilities of patients.

**Methods:** DNA was extracted from paraffin sections from MM tissue samples of 83 patients and amplified by the polymerase chain reaction using two separate primers pairs, namely PYV.for/PYV.rev and SV.for3/SV.rev.

**Results:** SV 40-like DNA sequences were detected in 50 of the 83 MM samples examined (60.2%) with either of two SV 40 primer sets. Of the 50 SV-40 +ve patients.one-year K-M survival was 44.0% (95%CI: 30.1-57.1), whereas in the remaining 33 SV-40 –ve cases one-year K-M survival was 65.8% (95%CI: 46.7-79.4). There was a trend toward better one-year survival probabilities in patients with SV-40 –ve MM (logrank test: 2.83; p = 0.093).




**Conclusions:** These data provide further evidence for a role of SV 40 in the biology of human pleural MM.

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## P-694

**PROLIFERATE ACTIVITY IN PULMONARY CARCINOIDS**

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**Aim.** On the basis of known Ki-67 dependence on tumor malignancy, we compared this marker expression quantitatively in pulmonary typical and atypical carcinoid tumors and attempted to predict their biological behavior especially in cases associated with tumorous lymphadenopathy, satellites, and carcinoid tumorlets.

**Method.** Using material from surgically treated patients, we examined 54 cases of carcinoids divided into five groups. 1) Forty-two typical carcinoids (TC), 2) Twelve atypical carcinoids (AC) diagnosed according to modified Arrigoni's criteria (Trawis et al., 1998), 3) Thirty-two TC without metastases, satellites, and tumorlets (M,S,T), 4) Eight AC without M,S,T, and 5) Fourteen TC and AC associated with M,S,T. Groups 3, 4, and 5 were formed of tumors selected from group 1 and 2.

The proliferate activity was evaluated by Ki-67 (MIB-1, Immunotech France, 1:25). Its nuclear labeling was counted in more than 50 HPF and calculated as a number of positive nuclei in 10 HPF.

The Fisher exact test was used for statistical analysis.

**Results.** The Ki-67 nuclear expression was found in 19 (45%) out of 42 TC and in 9 (75%) out of 12 AC. In set of TC without metastases (M,S,T), the Ki-67 positive labeling was found in 14 (44%) out of 32 cases (group III) and in six (75%) out of eight AC (group IV). In all TC and AC tumors with M,S,T (group V), the Ki-67 expression was encountered in 8 (57%) out of 14 cases. However, using the Fisher exact test for statistical analysis, there was no significant difference between all examined groups.

**Conclusion.** No statistical significant difference was found in Ki-67 expression in pulmonary typical and atypical carcinoids. It appears to be a factor which can not be used for tumor prognosis prediction or adjuvant therapy indication in surgically treated patients.

## P-695

MORPHOLOGY OF THE LUNG CANCER IN PERSONS LIVED IN THE RADIACTIVELY POLLUTED SEMIPALATINSK TERRITORIES OF KAZAKHSTAN

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**Aim** of the study was to investigate the morphological features of the lung cancer in persons lived in the radioactively poluted Semipalatinsk territories of Kazakhstan.

**Methods.**Clinicomorphological analysis of 17 lung carcinomas of patients who were exposed for a long time to the radiation in the region of Semipalatinsk atomic firing ground befour 1993 year. The material consisted of 15 surgical and 2 endoscopic bronchobiopsy cases was studied at the light, electron microscopy and immunohistochemical level. There were 7 peripheral and 10 central carcinomas.

**Results.** Carcinoma was represented by various histological types: small cell (8 cases), squamous cell (5 cases), large cell (2cases) and adenocarcinoma (2 cases). Peculiar dust deposits were found in the tumours of different localisation and histological type. The dust particles accumulated not only in macrophages and areas of fibrotic tissue, but could be seen in single tumour cells as well. There was not any correlation with the smoking background. The peripheral cancer "in the scar" was diagnosed in 5 cases. Lung carcinomas had low proliferate activity according to the low expression of Ki-67 and bcl-2.

**Conclusion.** The lung cancer in persons lived in the radioactively poluted Semipalatinsk territories of Kazakhstan are represented by different types of tumour with prominent dust contaminants and low proliferate activity.

## P-696

MALIGNANT PLEURAL MESOTHELIOMA: EPIDEMIOLOGY, DIFFERENTIAL DIAGNOSIS AND PROGNOSIS.

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Pleural mesothelioma is a cancer with a poor prognosis. The association with asbestos exposure is specific. Recently, in South Italy, a marked increase of the disease has been registered. A number of 125 diffuse pleural mesotheliomas diagnosed between 1989 and 1998 at the Institute of Pathology, were selected and histologically subtyped according to WHO criteria. Occupational and non-occupational asbestos exposure was detected by National Mesothelioma Register. The age distribution showed a peak between 60 and 70 years of age. The tumour was prevalent in men (90 cases). Effective exposure to asbestos was identified in 52,8% of patients; in 12% was not occupational (domestic, environmental, hobby-spare time) and in 35,2% of cases the source was unknown. The follow-up evaluated until 28 February 1999 was available for 107 cases. Seven patients are still alive. The median survival time was 11 months. The three-year survival rate was poor. Histological subtyping of tumour showed 84 (67.2%) cases of epithelioid-type, 33 (26.4%) mixed and 8 (6.4%) spindle cell-type. No significant correlation between survival and histological types (epithelioid and mixed subtypes) was found. The epithelioid type was more frequent among cases with occupational exposure while mixed and spindle-cell subtypes resulted more frequent in non-occupational exposure cases. Moreover, paraffin embedded blocks of 54 cases of mesothelioma and 30 cases of metastatic pleural tumours were immunostained for calretinin (calcium binding protein), E-cadherin (homotypic adhesion protein), keratins, CEA, HBME 1, EMA, and vimentin. E-cadherin best discriminate, in our study, between mesothelioma (epithelioid and mixed subtypes) and metastatic adenocarcinima, resulting diffusely positive in all cases of adenocarcinoma, whereas only few cases of mesothelioma demonstrated focal and weak reactivity. Calretinin was strong positive in MM (76%). Our results suggest that the high mortality rate indicates that no progress toward early diagnosis has been made and the histological diagnosis is still very difficult.

## P-697

TRANSFORMING GROWTH FACTOR β1 EXPRESSION IN BRONCHOPULMONARY CARCINOIDS

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**Aims**: Comparative evaluation of Transforming Growth Factor β-1 (TGFβ) expression with angiogenesis, metastases and tumour size in carcinoids.

**Methods**: TGFβ (antibody TGF-b1, Serotec) and angiogenesis (antibody CD34, Daco) were detected immunohistochemically using the avidin-biotin-peroxidase complex technique in 48 resected bronchopulmonary carcinoids. Tumours were staged according to the TNM classification and determined histologically as typical carcinoids (TC) - 35, and atypical (AC) - 15 cases, in accordance with criteria for neuroendocrine tumours (Travis, 1997). TGFβ immunoreactivity was graded as: not detectable = 0; <25% = 1; 25%-74% = 2; >75% = 3 of the stroma and tumour cells staining separately. Microvessel density was evaluated according to Weidner's method.

**Results**: TGFβ expression did not indicate the histologic type nor the presence or absence of lymph node metastases. The size of carcinoids was related to their histologic types, the diameter TC<AC (p.=0.047) but not related to TGFβ expression. Tumour angiogenesis was related to the histologic type (p.=0.01) but not to TGFβ expression. Tumour angiogenesis was related to the histologic type (p.=0.01) but not to TGFβ expression.

**Conclusion**: TGFβ expression was heterogeneous with accumulation of extracellular matrix, and without any indication for the extend of tumour disease.

## P-698

**ALU-VPA/MYCL1 IS INFORMATIVE MARKER OF MICROSATELLITE INSTABILITY IN COLORECTAL CANCER.**

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**Aims:** Short arm of chromosome 1 is often deleted in human colorectal cancer (CRC) and its deletion is associated with poor prognosis. We tested status and possible clinical significance of locus Alu-VpA/MycL1 (1p34.3) witch allows to detect loss of heterozygosity (LOH) and also microsatellite instability (MSI) in CRC.

**Methods:** We studied Alu-VpA/MycL1 by hybridization to locus-specific probe of PCR products blotted from sequencing gels. 50 primary colorectal carcinomas from adult patients were tested and results were compared with clinico-pathological features of tumors.

**Results:** 46 patients (92%) were heterozygous, 6 tumors (14% from informative) showed certain loss of heterozygosity (LOH) and 11 (22% from total) showed MSI+ of Alu-VpA/MycL1. LOH of the locus correlates with perceptible mucus content ($R_{Sp}$ = 0,35; $p$ = 0,057), and in some extent with lower patient age ($R_{Sp}$ = 0,30; $p$ = 0,1). MSI of Alu-VpA/MycL1 correlates with proximal tumor localization ($R_{Sp}$ = 0,30; $p$ = 0,028), presence of metastases ($R_{Sp}$ = 0,28; $p$ = 0,055) and near-diploid caryotype ($R_{Sp}$ = 0,46; $p$ = 0,071), that is characteristic for tumors with MSI or replication errors positive (rer+) phenotype.

**Conclusions:** Testing of this locus apparently is not very informative in terms of LOH but may be useful for MSI state settlement of CRC in addition to five loci proposed by 1998 NCI Workshop on Microsatellite Instability[1]

**References:** Borland CR et al, Cancer Res. 58:5248-57, 1998.

## P-699

**PCR FOR 17P LOCUS MAY BE SIGNIFICANT IN METASTATIC COLORECTAL CANCER PROGNOSIS.**

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**Aims:** Short arm of chromosome 17 is one of the most often deleted genome part (up to 95% cases) in human colorectal cancer and the deletion is associated with poor prognosis. 17p allelic imbalance was shown to be an independent prognostic parameter in CRC patients with potentially curative resected distant metastases. So we to wanted to estimate possible clinical use of 17p loss analysis by PCR on YNZ22 locus (D17S30, 17p13.3).

**Methods:** We studied YNZ22 locus by PCR in primary colorectal carcinomas of 49 adult patients and compared results with wide range of clinico-pathological features of tumors.

**Results:** Distribution of YNZ22 alleles in cancer patients was very close to that in healthy europeoids population. 40 patients were heterozygous (informative), and 16 tumors (40% of informative) showed loss of heterozygosity (LOH) of YNZ22. All these 16 tumors had also LOH of p53 gene (17p13.1). Frequency of YNZ22 LOH in men was twice as high as in women ($R_{Sp}$ = 0,33; p = 0,037). In studied population PCR-proved LOH of YNZ22 correlates with moderate or low differentiation grade ($R_{Sp}$ = 0,69; $p$ = 0,001), formidable mucus content ($R_{Sp}$ = 0,32; $p$ = 0,054) and with metastatic potential ($R_{Sp}$ = 0,28; $p$ = 0,086). 71% (5/7) of tumors with one or more metastases, and 100% (3/3) of tumors with metastases in pericolic fat were stated as YNZ22 LOH. 43% (3/7) of metastases-positive tumors with YNZ22 LOH had metastases in pericolic fat.

**Conclusions:** though prognosis for patients with metastases and PCR-proved YNZ22 LOH needs more wide investigation, simple one step PCR on YNZ22 locus seems to be be useful as analysis for 17p losses of in CRC patients with potentially curative resected metastases.

## P-700

**ASSESSMENT OF RET/PTC-1 EXPRESSION IN PAPILLARY THYROID CARCINOMA USING TAQMAN RT-PCR.**

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**Aim:** Papillary thyroid carcinoma (PTC) has a wide spectrum of biological behaviour. While the majority of cases behave in a clinically indolent fashion, some are highly aggressive. At the genetic level a specific activated form of c-*ret* has been found in a minority of papillary carcinomas. In PTC, c-*ret* is activated when somatic rearrangements occurring within intron 11 juxtapose the intracellular domain of RET to the amino-terminal portion of different donor genes. The clinical implications of c-*ret* activation remain controversial. The aim of this study was to evaluate the expression of *ret*/PTC-1 transcripts in a series of PTC.

**Methods:** Fifty-eight formalin fixed paraffin embedded samples of thyroid carcinoma (PTC and Anaplastic thyroid carcinoma (ATC)) were analysed for ret/PTC-1 expression using 5' Nuclease Assay (TaqMan RT-PCR). RNA from the TPC-1 cell line was included as a positive control for c-*ret* activation.

**Results:** Thirty three percent of all cases (PTC +ATC) were found to express the chimeric RNA characteristic of ret/PTC-1. A striking feature among the ret/PTC-1 positive PTC cases (twelve of fifty) was the background of chronic thyroiditis that was observed in the majority (58%).

**Conclusions:** It has been suggested that some thyroid cancers may induce an immunological response similar to that seen in thyroiditis because the antigenic profile of the cells has been altered in the process of neoplastic transformation. The exact role of oncogene activation in autoimmune disease remains unclear and a direct causative link between thyroid cancer and Hashimoto's disease has not yet been established. Thus, further investigation at the molecular level may elucidate not only the genetic basis for neoplastic transformation but also the induction of non-neoplastic thyroiditis.

## P-701

GANGLIOGLIOMA OF A TEMPORAL LOBE: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF 12 CASES

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**Aims:** Here, we investigated 12 surgical biopsy cases of temporal lobe ganglioglioma. These tumours represent the most common neoplasm in the patient with chronic, medically intractable temporal lobe epilepsy.

**Methods:** the speciments were stained with H&E, Gomori reticulin, Cresyl violet, Palmgren for axons and immunostained with Synaptophysin, Neurofilament Protein, GFAP and Ki-67.

**Results:** The male:female ratio was 1.8:1 with the median age at diagnosis 18 years. Epilepsy was a presenting clinical symptom. The tumours were composed of admixed neoplastic ganglion and glial (astrocytic) cells. Ganglion cells were dysmorphic, mostly large, often binucleated with visible Nissle substance and axonal processes. The astrocytic component was pilocytic (WHO grade I, 6 cases), fibrillary (WHO grade II, 3 cases), anaplastic (anaplastic ganglioglioma WHO grade III, 2 cases,) and non-neoplastic (gangliocytoma WHO grade I, 1 case). Desmoplasia, calcifications, microcystic changes and perivascular lymphocytes showed considerable variations. The immunostains demonstrate the expression of synaptophysin and neurofilament protein in ganglion cells while the astrocytic component was strongly GFAP-positive. Nuclear labeling for Ki-67 was observed exclusively in the astrocytic component with a relatively low (less than 2%) labeling indices even in the cases of anaplastic ganglioglioma.

**Conclusions:** The results suggests that the neoplastic astrocytic component in ganglioglioma is usually less aggressive than in ordinary astrocytoma. These can help in elucidation of the well known facts concerning the inconsistent correlation between the outcome and anaplasia in ganglioglioma as well as the clinical stability of these tumours for many years.

## P-702

S PHASE FRACTION and DNA PLOIDY IN CYTOSPIN PREPARATIONS of DIFFUSE ASTROCYTOMAS: COMPARISON of COMPUTERIZED IMAGE ANALYSIS FINDINGS with FLOW CYTOMETRY

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**Aim:** To investigate prognostic significance of flow cytometry (FC) and image analysis (IA) in tissues from 29 diffuse astrocytomas.

**Methods:** The cell cycle stage distribution ( percentage of cells in G0/G1, S and G2/M phases) were calculated using Coulter M plus program for flow cytometry. Cytocentrifuge slides of deparaffinized nuclear suspensions were stained by Feulgen pararosanilin technique for image analysis . The cell cycle distribution was analyzed using the Multicycle software program.

**Results:** We found statistically significant difference in percentage of cells in S phase between flow cytometry and image analysis in grade 2 and grade 4 groups. But there was no significant difference in grade 3 group while comparing two techniques. Neither techniques were found to be significant for distinguishing different grades e.g., grade 2 versus grade 4, grade 2 versus grade 3, and grade 3 versus grade 4.

**Conclusion:** FC demonstrated a direct proportion between number of aneuploidy and histologic grade, i.e., higher the aneuploidy, higher the grade, wherein similar were obtained in image analysis.

## P-703

**GENETIC ALTERNATIONS ON CHROMOSOME 11 AND CHROMOSOME 17-p53 GENES IN MALIGNANT ASTROCYTOMAS**

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**Aims**: We investigated the deletion loci on chromosome 11 & chromosome 17p-p53 genes in malignant astrocytoma specimens of human brain..

**Methods**: We analysed 42 astrocytomas (28 high grade III & IV, and 14 low grades I & II) for loss of heterozygosity **(LOH)** and microsatellite instability **(MI)** using microsatellite sequences on chromosome 11p, 11q and 17p as polymorphic markers.

**Results**: LOH on chromosome 11 were found in 18 of 28 high grade (64.28%), but only one of 14 low grade (7.14%). LOH on chromosome 17p-p53 gene were detected in 10 of 28 high grade (35.71%) and only one of 14 low grade (7.14%), respectively. In addition, the results of MI study also revealed the similar findings 25% (grade III) and 28.57% (grade IV) showed MI changes on chromosome 11 and 17-p53 genes, but 0% (grade I) & 7.14% (grade II) were identified, respectively. The highest frequencies of LOH of chromosome 11 were found at the two foci D11S929 (located at 11p14) (50%) and D11S912 (located at 11q24-25)(46.42%).

## P-704

THE COMPUTER AUTOMATIC ANALYSIS OF THE MORPHOLOGICAL IMAGE OF A DEGREE OF PRION ENCEPHALOPATHY SEVERITY.

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Aims: By morphometrical methods to study character of structural damages of a brain at various types of prion encephalopathy and to determine possibility of usage of the data, obtained through automatic image analysis, in the express-diagnostics of biopsies material.

Methods: We investigated 7 sectional observations. The age of the patients changed from 19 to 67 years. In 2 cases is a "classical" family form of illness Creutzfeldt-Jacob disease (CJD), in 2 cases - sporadic form CJD, in 2 - new "atypical form" CJD and in 1 - amyotrophic leucospongiosis. The tissue specimens process with toluidine blue, Congo red, are impregnated by silver and immunohistochemistry for prion plaques. Light and polarizing microscopy had used. The computer analysis of the morphological image was carried out on universal microscope Hund H 500 with television system connected to the personal computer IBM PC Pentium by the special computer program "Cruiz" (the program is developed by Donetsk Institute of Artificial Intelligence).

Results: The morphological picture of structural changes of a brain in our observations practically was similar, described in the literature at CJD and included: diffuse or focal (most sharply expressed at amyotrophic leucospongiosis) spongiosis, fallout of neurones, activation of a fibrillar astroglia: a hyperplasia and hypertrophy of astrocytes. The specific volume of a gradient of spongiosis obtained through the automatic computer analysis of the morphological image has shown, that the most expressed changes are watched in occipital, temporal and frontal lobes. The specific volume of a gradient a spongiosa, which mirroring a degree of lost of neurones, in these departments of a brain 6,9734±0,2653; 6,8901±0,3466 and 4,3742±0,1195 accordingly.

Conclusions: our results may be utilised in the express-diagnostics on biopsies material of definition of a degree of morphological damages of a brain at CJD.

## P-705

INTRACRANIAL MENINGIOMAS HISTOLOGICAL GRADE AND RECURRENCE IN PATIENTS OF DIFFERENT GENDER AND AGE

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**Aims**: To study the interrelation of meningiomas tumor grade and recurrence considering age and gender.

**Methods**: Utilizing the WHO Classification of Tumors (1993) the tumor grade and recurrence were studied in 134 patients aged between 19-72.

**Results**: Female/male ratio was 1.9:1. 51.5% (19 males, 50 females) patients were with benign, 22.4% (11 males, 19 females) with atypical and 26.1% (17 males, 18 females) - with anaplastic meningiomas.

The number of repeated referrals to our Institute among all studied cases was 46 (25 males, 21 females). Relapse of benign meningiomas was 15 (6 males and 9 females), atypical -15 (7 males, 8 females) and anaplastic -16 (12 males and 4 females).

In 60.4% (81) meningiomas were registered in the 40-60 years-age groups. Among them 48 with benign, 17 with atypical and 16 with anaplastic meningiomas. Most common the recurrence was observed in middle-aged (40-50 years) patients: 14 of 46. Though meningiomas more often develop in women, they often recur in men (53% against 24% in women). This tendency is consistent in age groups up to 50 years, however, with marked male bias in patients ages 19 to 29.

**Conclusions**: The results suggested the existence of a close tie between the development of meningioma, its recurrence and the gender and age of the patient.

## P-706

**PROLIFERATING MARKERS (PCNA and Ki-67), p53 and bcl-2 IN CONGENITAL TUMOURS**

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**Aims:** 1. to analyse the expression of proliferating markers (PCNA, Ki-67), bcl-2 and p53 in 10 patients with benign tumours, and 10 patients with malignant tumours, and 2. to analyse the relationship between the expression of these markers and clinical parameters including age, sex, tumour size and maturity, disease course and outcome.

**Methods:** We analysed patients with congenital tumours treated at the Children's Hospital, from 1985 to 1996. Histopathologic analysis of tumour tissues was performed at the Department of Pathology, School of Medicine, University of Zagreb. Congenital malignant and benign tumours of various histologic types, which had been diagnosed in newborns and infants up to 3 months of age, were analysed. The study performed immunohistochemistry on paraffin embedded archival material using primary antibodies (DAKO). The alkaline phosphatase/antialkaline phosphatase (APAAP) method was used. Microwave pre-treatment was performed to improve immunostaining.

**Results:** Slight expression of proliferating markers, particularly PCNA, and p53 was observed in benign congenital tumours. None of the examined benign tumours showed expression of bcl-2 oncoprotein. In the majority of malignant tumours, especially rhabdomyosarcomas, there was slight to moderate expression of investigated markers. However, there were no statistically significant differences in the expression of examined markers between the groups of benign and malignant tumours; PCNA (p=0.88), Ki-67 (p=0.86), p53 (p=0.90) and bcl-2 (p-0.057).

**Conclusions:** Our results suggest that the examined markers are present in congenital tumours. They probably play a role in the development of these tumours. Further studies on larger groups of patients are necessary to find out whether these tumours are the result of a flaw in development or oncogenesis and which oncogenes are included in the process.

## P-707

EVALUATION OF EXPRESSION OF CHOSEN PROTEIN CONNECTED WITH APOPTOSIS AND PROLIFERATING ANTIGENS IN NEUROBLASTOMA GROUP TUMORS IN CHILDREN

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**Aims:** To evaluate an expression of the products of p53, mdm2, waf1, bcl2, rb1 genes and proliferating antigens (Ki-67 and PCNA) in groups of Neuroblastoma with different stage of maturation and PNET group.

**Methods:** Our material consisted of 63 cases of neuroectodermal tumors divided into subgroups characterized by the different grade of the morphological maturation. We performed immunohistochemical research with monoclonal antibodies against P53 (clone DO7), MDM-2, WAF1, BCL-2, RB1, as well as Ki-67 and PCNA antigens.

**Results:** There were statistically important differences between PCNA, Ki-67 and P53 indices dependent on the grade of maturation of investigated tumors. In PNET subgroup we seen the highest value of proliferating markers indices. We also showed correlation between PCNA and Ki-67, PCNA and P53, PCNA and RB1, PCNA and MDM2, Ki-67 and P53, Ki-67 and RB1, P53 and RB1, P53 and MDM2, P53 and WAF1, as well as RB1 and MDM2 expression. There were no correlation between BCL2 expression and other evaluated proteins in our group of tumors.

**Conclusions:** The higher value of P53, Ki-67 and PCNA indices are strongly correlated with morphological features of malignancy of the tumors. We postulate possible correlation between expression of proteins connected with apoptosis and proliferative activity in Neuroblastoma group tumors.

## P-708

PRELIMINARY COMPARATIVE STUDY OF THE AMPLIFICATION OF N-MYC BY FISH IN NEUROBLASTOMA FIXED IN FORMALIN AND IN BOUIN'S FIXATIVE.

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**Aims**: The amplification of the gene N-myc is a major factor prognosis in localized neuroblastomas in child of less than 3 years. It was been generally researched by PCR, Soutern Blot or FISH (Fluorescent In Situ Hybridization). The aim of our study is to verify if this amplification can be determined from histological samples of neuroblastoma fixed in Bouin's fixative or in formalin.

**Méthods**: For 4 cases of neuroblastoma, we can dispose of samples fixed in Bouin's fixative or in formalin. In two cases, an amplification of N-myc was found by technique of reference (case 1: 100 copies of the gene and case 2: 10 to 25 copies), the two others had no amplification. The samples fixed in Bouin's fixative were pretreated in a $NH_4OH$ solution (0.04%) during five minutes in room temperature and not the samples fixed in formalin. FISH has been performed with a probe N-myc (LSI N-myc (2p23-p24) spectrum orange, Vysis, Woodcreek, USA) directly marked by the spectrum orange fluorophore, followed by nuclear counterstain by DAPI.

**Results**: In samples of neuroblastoma with N-myc amplification, some areas showed many nuclei exhibiting multiple N-myc signal in FISH with the two fixative. An evaluation of the number of the copies of the gene N-myc was not possible in case 1, but possible in case 2. In the area studied, the distribution of the nuclei with N-myc amplifiaction was heterogeneous, with some nuclei exhibiting signals and others not. 2 cases of neuroblastomes without amplification of N-myc were negative in FISH with the two fixative.

**Conclusions**: FISH applied on fixed samples in formalin or in Bouin's fixative seems be able to allow the research of N-myc amplification in neuroblastomas.

## P-709

IDIOPATHIC FETAL GROWTH RESTRICTION AT 20-22 WEEKS OF GESTATION

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**The aim** of the present study was to identify which organs are principally affected in cases of fetal growth restriction (FGR) at 20-22 weeks of gestation.

**Methods:**8 cases with fetal growth restriction (FGR) and high (0.41±0.04) placenta/fetal weight index (PFI) were compared with 10 controls (PFI= 0.26±0.01) in cases of induced abortions for socio-economic reasons at 20-22 weeks of gestation. Weights of placenta (PW) and fetus (FW), fetus length (FL), head (Ch), chest (Cch), abdominal (Ca) circumferences, fetal kidneys (KW), pancreas (Pn W), liver (LW) and heart (HW) as well as some indices were recorded.

**Results:** FGR group had smaller parameters of FW (390±2.0 vs 610±2.6 g, p<0.001), FL (26±0,9 vs 30±0,6 cm, p<0.001), Cch (15±0.6 vs 18±0.4 cm, p<0.001), Ch (18±0.04 vs 22±0.4 cm, p<0.001), Ca (13±0.4 vs 15±0.3 cm, p<0.001). All FGR cases had decreased LW (16±0.2 vs 29±0.2 g, p<0.001); PnW (0.2±0.01 vs 0.4±0.07 g, p<0.001); KW (4±0.3 vs 6±0.2 g, p<0.001), HW (2.1±0.2 vs 3.4±0.2 g, p<0.001). PW was unchanged (160±12 vs 160±8 g, p>0.05).

**Conclusions:** these results suggest that the discrepancy between fetal and placental growth at 20-22 weeks of gestation is associated with fetal kidneys, pancreas, heart and liver growth restriction.

## P-710

NEURONAL INTESTINAL DYSPLASIA AS A CAUSE OF PRIMARY CONSTIPATION IN CHILDREN

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**Aims**: Since there is some different data in literature concerning on frequency of neuronal intestinal dysplasia (NID) we performed this study to estimate the presence of this neuronal intestinal disorder in our material of 339 biopsy cases with clinical symptoms of primary constipation.

**Methods**: Biopsies of colon (suction or full thickness) were examined by histological and histochemical (acetylcholinesterase – AchE, lactate dehidrogenase – LDH and succinate dehydrogenase – SDH) methods of staining.

**Results:** From 339 biopsy cases with symptoms of primary constipation in children, we diagnosed isolated form of NID type B in 76 (22,7%) cases, age in ranged from 3 days to 12 years with male:female ratio, 3:1. In additional 23 (6,8%) cases NID B was associated with distal aganglionosis. In all cases, NID B was characterized by the presence of giant ganglia which were 2-3 times larger than normal, containing more than 7 LDH positive nerve cells. Beside that, nerve fibers were present in submucosa with ganglion cells localized inside or around nerve bundles. In some cases we found heterotopic ganglion cells in the mucosa, muscularis propria and circular or longitudinal muscle layer. Elevated AchE activity in lamina propria mucosae and/or muscularis mucosae was found only in children younger than 24 months of age.

**Conclusions:** According to our findings isolated NID type B as neuronal intestinal disorder, is the cause of primary constipation in children in 22,7%. This is in contrast to the some literature data (Meier-Ruge 1992) were the number of cases with NID type B is much more higher (40,6%).

## P-711

**ACTIVATING MUTATIONS AND NUCLEAR ACCUMULATION OF β-CATENIN IN A MAJORITY OF HEPATOBLASTOMA.**

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**Aims:** β-catenin is a multifunctional protein involved in adherens junctions and in transduction of the Wnt signal. The recent finding of frequent β-catenin mutation in several cancers, including hepatocellular carcinoma, has highlighted the important role of this protein in oncogenesis.

**Methods:** We have investigated the status and expression of β-catenin in hepatoblastoma from french and italian patients.

**Results:** Genetic alterations in one β-catenin allele were found in tumors from 12/18 patients (67%), including mostly sporadic cases and one familial Beckwith-Wiedemann syndrome, but not in a FAP case. These alterations consisted of interstitial deletions (7 tumors), or missense mutations (5 tumors) in the GSK-3β phosphorylation/ubiquitination motif which plays a crucial role in β-catenin stability. Immunostaining of neoplastic cells with anti β-catenin antibody showed accumulation and delocalization of the protein from cytoplasm to nucleus in all tumor components (fetal, embryonal, macrotrabecular, squamous and teratomatous), with more intense staining at the invasion front and in less differentiated cells, and occasional staining of isolated cells scattered through the tumors. Search for truncating mutations in the APC tumor suppressor gene in four tumors harboring wild-type β-catenin scored negative. We also investigated the transactivational activity of β-catenin mutants D32N, G34E, T41A, and Δ14-45 in a Tcf reporter gene assay in 293 cells. All mutants drastically activated the reporter gene (ca 100 folds). Interestingly, there were about 4 times as active as the wt protein, demonstrating the dominant functional effect of these mutations.

**Conclusion:** Our results indicate that genetic alterations resulting in activation of the Wnt/β-catenin signaling pathway play a crucial role in malignant transformation of immature hepatic cells.

## P-712

IMMUNOHISTOCHEMISTRY OR HISTOENZYMOLOGY FOR ACETYLCHOLINESTERASE RECTAL SUCTION BIOPSY DIAGNOSIS OF HIRSCHSPRUNG'S DISEASE ?

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**Aims:**We report here a comparative study of immunohistochemical and histochemical techniques for the evaluation of acetylcholinesterase activity in superficial rectal biopsies in order to assess the diagnostic utility of the former.

**Methods**: Blocks of frozen biopsies, taken during a period of 29 months from 40 patients including 19 cases of Hirschsprung's disease, were cut and histoenzymological (modified Karnovsky and Roots method) and immunohistochemical (three layer peroxidase method) staining for acetylcholinesterase were done, which were examined by two observers independently.

**Results:** We found the immunohistochemical technique to be highly specific having a strong predictive value with no false positive diagnosis in our series. Moreover, it is, though requiring frozen sections, cheaper than the conventional histoenzymological technique and does not involve use of toxic chemicals. However it has a relatively lower sensitivity, with the positive diagnostic yield of 83.3% for the cases of Hirschsprung's disease above one month and 76.9% for those below one month of age.

**Conclusions**: We believe that the apparent hyperplasia with thickening of cholinergic nerve fibres observed in superficial rectal biopsies reflects increased amount of acetylcholinesterase which could be the result of acquired non-cholinergic to cholinergic transition, rather than a true hyperplasia of nerves; this explains the parallelism with the histochemical and biochemical results on one hand and the failure of other immunohistochemical approaches studied so far focussing on mere better visualisation of ganglion cells and nerve fibres on the other hand.

## P-713

**The intra-and peritumoral vascularisation of skin melanoma does not correlate to the metastatic phenotype**

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The correlation between the prognosis and the vascularity of skin melanoma is still contradictory. This can be explained by the different methodologies used to detect tumor vasculature. Almost all of the studies use the principle of selecting the vascular hot sports. We believe that it should be applied only to solid tumors of large size (i.e.centimeters). Skin melanoma is usually diagnosed at a log smaller size (a few mm) where there is no need to select areas, since the entire tumor can be sampled for a representative study. Therefore, we have selected 36 primary human skin melanoma cases with a minimum of 48 month follow-up period (max. 168 months). Since the individual biological behavior of the tumors does not necessarily follow the initial clinical stage based on the thickness of primary tumor, we have also classified them according to the actual type of progression during the study period as non-, lymp-node-, and organ-metastatic forms irrespective of the thickness of primary tumor. Vascularity was determined in paraffin sections of the primary tumors using immunohistochemical double labeling of blood vessels for CD31 and laminin. Peri-and intratumoral microvessel density, vessel perimeter and diameters of vessel-free intratumoral areas were determined by computer assisted image analysis using QU-2 Olympus image analysis software. Instead of using the selection of vascular "hot spots" in or around the tumours, we have systematically measured intra-and peritumoral microvessel densities. In case of each tumor section 5-10 randomly selected 300x400 μm fields were analysed. In all the categories applied (according to tumor thickness or clinical outcome), microvessel densities were 4-6 times higher in the peritumoral zones compared to the intratumoral ones and there was no difference in this respect between the various categories. The comparison of the intratumoral microvessel densities in respect of tumor thickness or clinical outcome indicated no statistically significant differences between the various categories.

## P-714

QUANTITATIVE PARAMETERS IN PREDICTING RECURRENCE OF BASAL CELL CARCINOMA OF THE CHEEK REGION

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**Aims.** Basal cell carcinomas (BBC) have often a benign evolution, but in some cases recur after treatment and produce local invasion or metastase. In this study we proposed to investigate quantitatively the BBC of the cheek region and to discuss the importance of these parameters for diagnosis of aggressive form of BBC and apreciate the prognosis.

**Methods.** We used 26 fragments with BBC obtained from the cheek regions and follow up the cases. From 6 patients were obtained tumor's relapses (four with two relapses) which can estimate the evolution and prognosis. The pieces were processed through paraffin-technique and stained with H&E. We were utilized an interactive digitizing video overlay system (PRODIT 5.2) with standard, nuclear volume and two-phase (phase one = cell, phase two = nucleus) measurements. In each case we measured fifty cells. We calculated the nuclear and cellular parameters and the results were statisticaly processed. We estimate the form and sense of correlations when they exist.

**Results.** In case of relapses, the asymetric positive variations of areas kept during the evolution, more accented in the last relapse, at fourteen months after primary tumor ablation. There is an explosive increase of the parameters, similar to the primitive tumor appearance, but at a superior quantitative level. In the primary tumor that gives relapses, therefore with high-risk, the cellular area is smaller than in case of tumors without relapses, with low-risk. The nucleus has an approximate identical area and volume, but the cancerous cells have a greater variability of the form-factor parameters.

**Conclusions.** The obtained results indicate that among the studied parameters, the cellular and nuclear areas could be proposed to evaluate the prospective outcome of each patient and the recurrence appearance.

## P-715

LATENT PSORIATIC ARTHRITIS: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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The aim of this work is to investigate morphological substrate of latent psoriatic arthritis.

Material and methods. Material of research was the section cases of 17 persons (15 men and 2 women) with clinical not complicated forms of psoriasis. Duration of disease was from 3 till 14 years. Are investigated specimens of skin, synovium and surrounding tissues of knee and akle joints with light, immunohistochemical and polarizing microscopy. The computer analysis of the morphological image was carried out on universal microscope Hund H 500 with television system connected to the personal computer IBM PC Pentium.

Results. Is established, that, despite lacking clinical manifestations of an arthritis, at all persons with Psoriasis Vulgaris both during an aggravation of disease and in a period of remission, take place attributes of local disorganization of a connective tissue, alterative-proliferative capillaritis, accompanying by reactions of hypersensitivity both immediate and delayed types. In infiltrates dominate CD4-positive T-lymphocytes. In synovium we found the picture of small-sized focal proliferative synoviitis. The character of a morphological picture not in all cases correlates with duration of disease and frequency of relapses.

Conclusion. This findings is proof that changes in psoriasis has systematic character. This feature should be taken into account at treatment of the patients.

## P-716

THE PHYLLODES TUMOR OF THE PROSTATE: MULLERIAN ORIGIN?

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**Aims:** The phyllodes tumor of the prostate is an unusual lesion. Less than 30 cases have been reported in the literature so far. We report one case in a 47-year-old man and discuss its histogenesis.

**Case report:** A 47 year-old man consulted for dysuria evolving for many years. The patient had presented 12 years before for the same obstructive symptoms. A prostate biopsy analyzed at that time only showed fibrosis. The pelvic CT scan in 1998 showed a 4 cm mass located on the left posterolateral side of the prostate. Tumorectomy was performed and there was no recurrence 10 months after surgery.

**Results:** Histologically the tumor was composed of a glandular component with leaf-like architecture and a moderately cellular stromal component, with discrete nuclear atypia and no mitosis. The glands were lined by 2 cellular layers: a superficial cylindrical layer and a cuboidal basal layer. Some glands were lined by cylindrical mucinous cells. There was no normal prostatic parenchyma. Epithelial cells were immunoreactive for KL1 but were not immunoreactive for PSA and PAP. Basal cells were marked with CK903 antibody. Stromal cells were immunoreactive for vimentin and some of them for CD34.

**Comments:** The phyllodes tumor of the prostate has recently been included in the spectrum of prostatic stromal proliferation of uncertain malignant potential (PSPUMP). This tumor grows slowly and recurrences are frequent. Its surgical total removal is necessary because malignant transformation to high-grade sarcoma and distant metastases have been reported. Its histogenesis is still unknown, but most of the authors think that the prostatic phyllodes tumor derives from urogenital sinus. In our case the development of the tumor at the posterior side of the prostate, the lack of PSA immunoreactivity and the presence of « endocervical-like » mucinous glands could suggest that it derives from embryonic mullerian remnants in the prostatic utricle.

## P-717

**COMPARATIVE IMMUNOCYTOCHEMICAL ASSESSMENT OF ISOLATED CARCINOMA CELLS IN LYMPH NODES AND BONE MARROW OF PATIENTS WITH CLINICALLY LOCALIZED PROSTATE CANCER.**

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**Aims:** After radical prostatectomy for clinically localized prostate cancer, biochemical progression is seen in up to 40 % of the patients due to persistent local and/or systemic remnants. Isolated disseminated carcinoma cells, undetectable by current staging methods, are of special interest as potential precursors of subsequent overt metastases.

**Methods:** In the present study immunohistochemistry (IHC) was performed to evaluate simultaneously the frequency of occult carcinoma cells in lymph nodes (LNs) and bone marrow (BM) obtained from the iliac crests of 45 patients with untreated stage T1-3 pN0M0 prostatic carcinoma. IHC using monoclonal antibodies (MAbs) against epithelial cytokeratins was performed on 521 paraffin embedded LNs histopathologically classified as tumour-free ($pN_0$), as well as on BM cytospin preparations. To confirm the prostatic origin of positive cells in LNs, additional IHC for prostate specific antigen (PSA) and epithelial glycoproteins were performed.

**Results:** In total, isolated tumour cells in LNs and/or BM were detected in 17 (37.8%) of the 45 patients. Parameters such as tumour stage, grade and volume of the primary tumour as well as blood serum PSA levels could not detect patients harboring disseminated single tumour cells in LNs or BM. Following a median observation time of 24.9 months, no significant correlation between IHC-positivity and PSA progression as a measure of early relapse was observed.

**Conclusions:** Although the overall incidence of occult tumour cell spread corresponds to similar incidence of relapses after radical prostatectomy as reported by others, the fate of these cells needs to be evaluated in longer follow up studies.

## P-718

**Loss of RBM expression as a sensitive marker of intratubular male germ cell neoplasm.**

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Introduction: RBM protein (Acronyn of RNA binding motif) is encoded by the azoospermia factor-b (AZF-b) region of the human Y chromosome. The protein is nuclear and expressed exclusively in the male germ cell lineage: spermatogonia, spermatocytes and round spermatides. In maturation arrests of the spematogenesis process, RBM is expressed up to and including the last stage of germ cell development.

Aim: The goal of the present study is to analyze the expression of RBM gene in germ cell tumors and in the seminiferous tubules in the vicinity of those neoplasms in order to identify the possible presence of intratubular germ cell tumors.

Methods: Sections from eighteen testicular germ cell tumors were stained with anti-RBM antibody using the avidin-biotin method.

Results: All the germ cell tumors were completely immunonegative for RBM protein. Distal tubules exhibiting spermatogenesis, were immuno-positive for RBM. Defined areas of intratubular germ cell neoplasia also showed loss of RBM expression. Proximal tubules with spermatocyte arrest, which were expected to express RBM, were protein negative. This unique observation enables to redefine them as intratubular germ cell neoplasia.

Conclusions: RBM is a novel marker with obligatory expression in male germ cell lineage. Malignant germ cell tumors and intratubular germ cell neoplasm lose the expression of the protein. The lack of RBM expression may serve as a new diagnostic tool of pre-invasive malignancy of the testis.

## P-719

**LYMPHOEPITHELIOMA-LIKE CARCINOMA OF THE URINARY BLADDER- Report of a case and review of the literature**

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**Aims:** A case of Lymphoepithelioma-like Carcinoma (LELC) of the urinary bladder is presented to call attention to the importance of differentiating it from malignant lymphoma or from severe chronic cystitis.

**Methods:** A 61- year-old male patient presented with hematuria. H&E stained slides of the transurethral resection specimen of the bladder tumour were examined. Immunohistochemical studies included cytokeratin (MNF 116), leukocyte common antigen (LCA), CD 20 ( pan B), UCHL-1 (panT) and prostate specific antigen (PSA).

**Results:** The tumour was characterized by nests and sheets of undifferentiated malignant cells arranged in syncytia admixed with an intense lymphoid infiltrate.The tumour cells were large with scant, ill-defined cytoplasm, large vesicular nuclei with prominent nucleoli and numerous mitoses. Necrosis was also present. The prominent lymphoid reaction consisted of mature lymphocytes admixed with plasma cells and histiocytes. The whole picture was reminiscent of that of lymphoepithelioma of the nasopharynx. Immunohistochemistry showed positivity for cytokeratin in the neoplastic cells.The lymphoid infiltrate was positive for LCA, with a predominant T-cell and scattered B-cell positivity. No PSA positivity was detected. Among the 15 cases that have been reported since 1991, 7 showed pure LELC histologic pattern , 5 tumours were reported to show a predominant (>50%) and 3 cases only focal (≤50%) LELC pattern. The pure LELC cases had a favourable outcome with chemotherapy (methotrexate, vinblastine, adriamycin and cisplatin) alone.

**Conclusions:** Neoplasms with a histologic character similar to nasopharyngeal lymphoepithelioma may occur in the urinary bladder. It should be differentiated from malignant lymphoma and from severe chronic cystitis so when a dense lymphoid infiltrate is present, pathologists should search carefully for neoplastic epithelial cells.

## P-720

**Expression of cyclin E protein in prostate cancer:correlation with proliferative activity.**

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Cyclin E is a G1 cyclin which has been proposed to be one of the key regulators of the important G1/S transition. Recently, it has been observed that cyclin E is overexpressed in several cancers such as colorectal, uterine, breast and gastric carcinomas.The aim of our study has been to examine the expression of cyclin E in prostate carcinomas and to correlate this expression with Ki-67, a cell proliferation marker that labels the G1/S/G2/M phase.

Eighty-six prostate carcinomas(including 28 grade I , 42 grade II and 16 grade III ) were immunohistochemically analyzed using formalin-fixed, paraffin embedded tissue sections and monoclonal anti-cyclin E and anti-Ki-67 antibodies.

Cyclin E and Ki-67 nuclear immunostaining was observed in 22 (25,5%) and 29 (33,7%) carcinomas respectively. A significant percentage 75,8% (22/29) of the Ki-67 positive tumors exhibited simultaneous labelling of cyclin E ($p<0.05$).

No correlation between cyclin E or between Ki-67 expression and tumor grade, was noted.

These results suggest that the expression of cyclin E may be involved in carcinogenesis and may be of importance in the development of prostatic carcinoma in correlation with high proliferation.

# P-721

**IMMUNOHISTOCHEMICAL VALUE OF KI-67 AND P53 EXPRESSION AS PREDICTIVE FACTORS OF THE OUTCOME OF RENAL CELL CARCINOMA. A RETROSPECTIVE STUDY OF 73 CASES.**

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**Aims:** Although many factors as nuclear grade or tumor staging have been considered to be the most important predictors of patient outcome in renal cell carcinoma (RCC), RCC continues to behave impredictably. To improve the prognostic evaluation and determine patients at high risk, another prognostic factors are needed.
**Methods and results:** Specimens of 73 RCCs of different nuclear grade (20 Furhman I/II, 32 Fuhrman III and 21 Fuhrman IV) and different stage (10 pT1, 23 pT2, 36 pT3 and 4 pT4) were immunostained with monoclonl antibodies against Ki-67 and p53. Tumor size ($p<0.001$), nuclear grade ($p<0.01$), tumor stage ($p<0.01$), Ki-67 index ($p<0.001$) and p53 immunostaining ($p<0.03$) were significantly correlated with poor prognosis. Moreover Ki-67 index ≥ 20% was a powerful predictor of survival in all patients ($p = 0.00001$) and in patients with non metastatic at time of surgery ($p = 0.0056$). In patients with a high tumor stage, Ki-67 index seems to be the most important predicator of prognosis with the better predictive values.
**Conclusions:** Ki-67 immunostaining discriminates cases of poorly prognosis especially in patients with a high tumor stage, who could be included in a therapeutic protocol just before the surgery.

# P-722

**PROGNOSTIC VALUE OF THYMOSIN β15 IN MODERATELY DIFFERENTIATED PROSTATIC ADENOCARCINOMA**

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**Aims:** Identifying patients with Gleason Grade 3 stage M0 prostate adenocarcinoma who are at high risk for developing distant metastasis poses a great clinical challenge. Thymosin β15 (Tβ15 ) is an actin binding protein expressed at higher levels in prostate cell lines with high metastatic potential and in high grade prostatic adenocarcinoma. Gleason Grade 3 tumors express a variable amount of Tβ15. This study was performed to determine whether Tβ15 level at initial prostate biopsy of Gleason Grade 3 prostate tumor correlates with clinical outcome.
**Methods:** Thirty-two patients with Gleason Grade 3 tumors were randomly selected. All patients underwent transrectal biopsy and were treated with radiation without hormone therapy five years prior to this study. Of these patients, 11 were disease free (NED), 11 showed biochemical failure with elevated PSA (+PSA), and 10 showed distant metastasis with positive bone scans (+BS). The initial biopsy tissues were stained immunohistochemically for Tβ15. The staining and interpretation (weak, moderate or strong) were performed in a double blind manner.
**Results:** One of the 11 NED patients, 4 of the 11 +PSA patients and 8 of the 10 +BS patients showed strong Tβ15 staining. Overall, 13% (1/8) with weak staining developed clinically evident distant metastasis (+BS) compared to 61% (8/13) of patients with strong staining ($p=0.01$ by exact trend test).
**Conclusion:** Clinical outcome (5 year follow up) of patients with Gleason Grade 3, M0 prostate cancer correlated with Tβ15 staining of initial prostate biopsy specimens. Strong Tβ15 staining identifies a cohort of high risk patients with moderately differentiated tumors who may benefit from systemic as well as local therapy.

# P-723

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHANGES OF BENIGN PROSTATIC HYPERPLASIA AND PROSTATIC INTRAEPITHELIAL NEOPLASIA AFTER THE CHERNOBYL ACCIDENT IN UKRAINE

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**Aims:** The present study serves to evaluate the proliferative changes using morphological and immunohistochemical analysis of p53, bax, bcl-2 and apoptosis in prostatic tissue with BPH from 168 patients who underwent surgery in the Institute of Urology & Nephrology in Kyiv, Ukraine.
**Methods:** Group I - 76 cases from patients living in the radiocontaminated areas of Ukraine, group II - 47 cases from patients inhabiting Kyiv-City and the control group III - 45 cases from patients who underwent surgery before the Chernobyl accident were analyzed. Immunohistochemistry was performed using the avidin-biotin-peroxidase complex method. The ApopDETEK Cell Death Assay System was used to identify apoptosis.
**Results:** Both groups I and II showed the significant increasing of the incidence PIN as compared with the control group III (35.5%, 29.8% and 15.5% respectively). The expression of the p53 protein and the apoptosis significantly increased in group I as compared with the control group III (p53 index in areas of PIN in group I - 23.60 ± 0.14%, in group III - 12.40 ± 0.09%; apoptotic index in areas of PIN in group I - 9.72 ± 0.56%, in group III - 0.44 ± 0.06%). p53 and apoptotic indices in group II were closer to the indices in the group I, than to the indices in the group III.
**Conclusions:** This pilot study showed that after the Chernobyl accident the incidence of PIN in the BPH of patients living in the radiocontaminated areas of Ukraine was increased. The amount of the apoptotic cells in the prostate of such patients was increased too with the p53 expression in nuclei of PIN. These changes could be the result of the influence of the long term low doses of the ionizing radiation.

# P-724

**MORTALITY DUE TO SYSTEMIC SECONDARY (AA) AMYLOIDOSIS IN RHEUMATOID ARTHRITIS**

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**Objective**: *Systemic secondary (AA) amyloidosis* was studied in a randomized autopsy population of 234 in-patients with rheumatoid arthritis (RA). The aim of this study was to determine the prevalence of systemic secondary (AA) amyloidosis in RA, the clinically missed diagnosis of (AA) amyloidosis, the correlation between (AA) amyloidosis and coexistent complications: *vasculitis (SV), sepsis (SI),* active *tuberculosis (Tb),* and *malignant tumours (mTu)*, the mortality due to (AA) amyloidosis , and the contribution of (AA) amyloidosis to mortality due to coexistent complications: *SV, SI, Tb, mTu* in RA.
**Methods**: The tissue specimens were fixed in 8% formaldehyde solution and embedded in paraffin. Serial sections were cut, and stained with HE and Congo red. Amyloid deposits were characterized histochemically according to Romhányi. The correlation between (AA) amyloidosis and coexistent complications were determined by $\chi^2$-test.
**Results**: Systemic secondary *(AA) amyloidosis* was observed in 48 (20.5%) of 234 cases. Twenty (41.7 rel%) of 48 complications with AA amyloidosis led to death by uraemia, and only 15 (31.3 rel%) of these 20 was clinically recognised. *SV* was accompanied by (AA) amyloidosis in 11 ($\chi^2$=0.0408), *SI* in 4 ($\chi^2$=0.6414), *Tb* in 2 ($\chi^2$=0.0037), and *mTu* in 6 ($\chi^2$=0.3302) of 48 cases. There was no significant correlation between (AA) amyloidosis and any of the coexistent complications as mentioned.
**Discussion**: *(AA) amyloidosis is a major complication in RA.* Amyloidosis may be regarded, based on the low value of correlation coefficients, as an exclusive complication of RA in the presented autopsy population. Massive amyloid deposition in the kidneys cause a renal insufficiency and uremia in nearly one half of the RA patients who had (AA) amyloidosis. Systemic secondary (AA) amyloidosis associated with SV may play an additive role in causing myocardial necrosis, multifocal myocardiocytolysis, and heart failure. Cardiovascular amyloidosis may lead to circulatory failure. Vascular, and interstitial amyloid deposition in the lungs may contribute to respiratory insufficiency. Gastrointestinal amyloidosis may cause malabsorption, ulcer, bleeding, perforation and peritonitis. Amyloid deposition in the anterior pituitary, or adrenal cortex may impair endocrine functions, ect. Clinically recognised amyloidosis - diagnosed at the time of renal failure - represented in our autopsy population only about one third of de facto existing amyloidosis („tip of the iceberg"). The clinical diagnosis of amyloidosis was based on decreased renal function. Clinical signs of renal involvement (nephrotic syndrome) corresponded histologically an advanced stage of systemic amyloidosis. Missed diagnosis, or late recognition of amyloidosis limits the therapeutical possibilities and means a poor prognosis.

## P-725

**FAMILIAL ALZHEIMER'S DISEASE WITH AN USUALLY EARLY ONSET AND SEVERITY OF DISEASE**

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**Aims:** Estimation of histopathological features of familial Alzheimer's disease (FAD) with a very early onset (24-33 yr.) and short duration of the disease (3-4 yrs.) on the basis of the neuropathological examination of two autopsied cases (two sisters in Polish family) was performed.

**Methods:** Formalin fixed paraffin embeded tissue sections were stained with conventional (HE, PAS, Congo red, modified Bielschowsky silver method), and immunocytochemical (anti-A4 amyloid protein mAb, 1:20; anti-Tau-2 mAb, 1:50, Novocastra) methods, using a standard PAP technique.

**Results:** The leading histopathological features were: especially numerous senile plaques in the cerebral cortex, abundant congophylic angiopathy and relativelly scarse neurofibryllary degeneration. DNA analysis in presented two cases and other living family members revealed novel mutation (Pro117Leu) of presenilin-1 (PS-1) gene localized on 14[th] chromosome.

**Conclusions**: The severity of the clinical picture and high intensity of histo-pathological features indicate the great importance of PS-1 in pathogenesis of Alzheimer's disease.

## P-726

**Improved objectivity of grade in $T_{a,1}$ urinary bladder tumors by morphometric proliferation and differentiation associated features and Ki-67 immunoquantitation**

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Background: The grade of $T_{a,1}$ urinary bladder tumors has therapeutic impact, but intra- and interobserver reproducibility is poor. Previous studies have shown that morphometric analysis of mean nuclear area of value for objective grading.

Objective: To analyse with single- and multivariate analysis whether mitotic activity index (MAI), nuclear area features and Ki-67 immunoquantitation, have additional discriminative value to each other in different grades of $T_{a,1}$ bladder tumors.

Material and methods: 150 consecutive $T_{a,1}$ urinary bladder tumors have been analysed. Duplicate blind assessment of grade was performed by three independent observations in standard HE-paraffin sections, which were also used for morphometric analysis. The QPRODIT 6.1 system (Leica, Cambridge, UK) was used for morphometric analysis and immunoquantitation of Ki-67. Single and multivariate analysis was applied to discriminate the different grades.

Results: In the learning set (n=17 grade 1, 30 grade 2, 28 grade 3) the best discriminating features were MAI and Ki-67, and to a lesser degree the mean of the area of the ten largest nuclei. With multivariate analysis, all three features were included. The best discriminating features between grade 1 and 2 were MNA-10 and MAI, and between grade 2 and 3 the MAI and Ki-67. With these features, 93.6% of the original grade 1 and 2 cases were correctly classified (3 of the grade 2 cases were classified as grade 1). Of the grade 2 and 3 cases, 96.6% was correctly classified (2 grade 3 cases were classified as grade 2). In the test set, the classification results were similar (89,4% of the grade 1 versus 2 and 94.8% of the grade 2 versus 3 were correctly classified).

Conclusions:

1. Combination of MAI, Ki-67 and MNA-10 gives much better discrimination between grade 1, 2 and 3 $T_{a,1}$ urinary bladder tumors than MNA-10 alone.
2. The similarity of the classification results of the test set and the learning set encourage us to apply the classification model in a prospective analysis.

Literature: Blomjous et al, 1989, Anal. Quant. Cytol. Histol. 11:426-437

## P-727

CORRELATIONS OF CLINICAL AND HISTOLOGICAL PARAMETERS IN CHOROIDAL MALIGNANT MELANOMA

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**Aim:** To emphasize the importance of correlating some clinical and morphological parameters in assessing the outcome of the patient with malignant melanoma of the uveal tract (MMUT).

**Methods:** Twenty-nine patients with choroidal melanoma were examined and fragments collected by enucleation were processed by routine techniques. 17 cases were male and 12 female. 26 tumors were located in the posterior pole and 3 at the equator.

**Results:** The modified Callender's histological classification was used considering the type and size of cells and nuclei, mitotic activity, pattern of tumoral vessels and invasion into the sclera. The histological parameters were correlated with the clinical ones of location, size, shape, and pigmentation of the tumor, and presence of metastases. The extraocular extension was of little prognostic value, except when associated with the epithelioid type cells and largest dimension of the tumor.

**Conclusions:** Our study supports the need of a complex clinical-morphological analysis, the selection of some factors with predictive value (cell type, vascular pattern, number of mitoses) being useful both for establishing the treatment and assessing patient outcome.

## P-728

HOW BIASED IS THE STATIC TELEPATHOLOGY? THE IMAGE PYRAMID SYSTEM AS AN UNBIASED, INEXPENSIVE AND BROADLY ACCESSIBLE METHOD

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**Aims:** Although computerized information technology became widely accepted in global communications, using the Internet apparently does not play a significant part in the daily routine diagnostic pathology. Therefore, an easy, inexpensive method of telepathology (TP) utilizing the Internet should be developed. The highest is the explicit/implicit ratio (EIR) of transferred graphic information the least biased is the consultation.

**Methods:** Several thousands of images of histological sections as well as cytological smears have been digitized, stored and delivered to remote locations. EIR has been analyzed to estimate a degree of bias in various static telepathology systems. Five different ways of text and graphic file transmissions were tested and compared. These include electronic mail transfer with graphic attachments, static World Wide Web (WWW) pages completed with e-mail transfer, and the so called image pyramid (IP) system on the Internet.

**Results:** We found that the EIR of transferred still image captured from an analog microscopic slide strongly depends on the objective lens magnification. At the lowest magnification the EIR is approximately 1:1 on small biopsies, but it is about 1:100 on large materials. Unbiased static information can be transferred by methods including using a large series of individual images on the WWW. This is slow and proved to be an unpractical method. One of the other methods which may also lead to unbiased static TP consultations is increasing the resolution of the camera. However, this results in large images with high pixel numbers and increased EIR. Neither electronic mail transfer nor WWW-page storage is able to handle giant files easily in the routine diagnostic practice. The best and broadly accessible method for unbiased static TP proved to be the IP-system which includes a hierarchical order of still images organized in the "z" plane.

**Conclusion:** Potential survey of the whole remote microscopic slide is enabled by using diverse dynamic telepathology systems. But, perhaps, due to their high costs their usage is very limited. The limitations of static systems including low EIR can be overcome by the use of the IP system. Static TP applications can also be used for other purposes involving education and/or archiving.

## P-729

PROGNOSTIC IMPACT OF TUMOR CELLS IN WOUND BLOOD SHED DURING CANCER SURGERY

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**Aims:** The impact of disseminated cancer cells in bone marrow, in the circulation , or in the peritoneum on outcome and tumor recurrence is under intensive investigation. We recently have demonstrated and characterized tumor cells in the blood shed during various cancer surgery procedures as a sample from the surgical field. 54 month later we now have evaluated the local tumor recurrence, metastasis and survival time of these patients dependent on the tumor cell number in the wound blood.

**Methods:** In 83 patients with R0-resection of their primary tumor shed blood was collected and cancer cells detected by analysis of cytokeratins, nucleolar-organizer-regions and morphology after density gradient centrifugation. For follow-up of the patients the data bank of the Tumor Center Regensburg and a questionaire for the patient's doctor was used. Kaplan-Meier curves and Cox regression were derived for survival analysis. Spaerman correlation was analyzed to compare the results with the median survival times from a local cancer register.

**Results:** Patients that died within 54 month had significantly higher tumor cell numbers in their intraoperative wound blood. Survival curves were steeper for patients with cell numbers exceeding 4000 in the whole shed blood volume. Even after stratification for matching T, N, or G status this tendency remained. In univariate and multifactorial analysis Cox regression revealed the tumor cell number in wound blood as a separate prognostic factor (regr.coeff.0.79, $p<0.003$). In addition, patients with higher cell numbers had a shorter tumor-free survival time than expected from their staging and grading, and vice versa.

**Conclusions:** The number of tumor cells in the blood shed during cancer surgery is an independent predictor of survival time, recurrence rate and metastasis rate with statistical significance, and might become a useful parameter in clinical routine. Further investigations on these cells in wound blood are needed to define their role in cancer disease.

## P-730

**IMPLANTATION OF CRYOPRESERVED FETAL SUBSTANTIA NIGRA: COMPARISON OF GRAFT SURVIVAL AND HOST-GRAFT REACTION**

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**Aims:** Studies concerning fetal neural transplantation in Parkinson's disease provide evidence that the clinical results depends on the transplantation technique, including the preparation and preservation of tissue. Cryopreservation is one of method of preservation used in clinical transplantation, and two models of fetal tissue are used alternatively: solid tissue block and cell-suspension. The aim of this study was to carry out of comparative analysis of survival and maturation of intracerebral allogenic grafts of two models of cryopreserved fetal substantia nigra, solid tissue block and cell-suspension and the host tissue reaction against both graft models.

**Methods:** A fetal cryopreserved rat substantia nigra was grafted into striatum of 153 healthy adult rats, apllying two different forms: solid block and cell-suspension. The fetal tissue was preserved in liquid nitrogen for 30-80 days. The controls were subjected to sham transplantation. Results were evaluated by means of histological and immunocytochemical methods (HE, TH, GFAP, Ferritin, W3/3).

**Results:** It was found that fetal cells of cryopreserved rat mesencephalon transplanted into adult rat striatum in two models, survive similarly. The host cellular reaction against the graft was nonspecific and similar to that found in the control groups. Over a posttransplantation period of 21 days no graft rejection was observed in any of experimental groups.

**Conclusions:** There is no difference between two forms of implanted tissue as far as survival and maturation of grafts and cellular reaction against graft is concerned.

## P-731

CARCINOID OF THE PANCREAS WITH HEPATIC, ADRENAL AND LYMPH NODES METASTASES

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Carcinoid of the pancreas is extremely rare; literature research was done using Medline from 1966 to 1996 and includes 34 published cases. Clinical presentation in the reported case was atypical carcinoid syndrome, including pain, diarrhea and weight loss. Elevated urinary 5-hydroxyindolacetic acid and seric amylase were the main laboratory findings. Intraoperatively, the pancreas was partially replaced by a tumor 5/5/4 cm, the liver displayed multiple tumoral nodules between 1 and 3 cm diameter, the right adrenal gland appeared compressed by a tumor 4/3/3 cm and pericaval lymph nodes were enlarged, with the largest diameter about 1,5 cm.; the bowel was free of tumor.

Microscopically, the neoplasm belongs to the classic insular type of neuroendocrine tumors, occasionally presenting acini and rosettes. Immunohistochemical studies revealed reactivity for serotonin, chromogranin, synaptophysin, neuron-specific enolase, TBO1 (CD 57) and negative staining for gastrin, vasoactive intestinal polypeptide, pancreatic polypeptide, somatostatin; insulin and glucagon were positive only in normal islets. These results converge to the diagnosis of carcinoid tumor and assure the differential diagnosis with other neuroendocrine tumors, as insulinoma, glucagonoma, gastrinoma, vipoma, somatostatinoma, PP cell tumor. The absence of palpable intestinal tumor and the voluminous metastases infirm a secondary origin of the pancreatic tumor.

The clinical presentation and the presence of distant metastases in the reported case are those common to the pancreatic carcinoid (Maurer, 1996), unlike the behavior of the more frequent intestinal counterpart.

## P-732

**PRIMARY MULTIPLE TUMOURS STUDIED AT AUTOPSY MATERIAL**

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**Aim: to study primary multiple tumours at autopsy material for the period of 20 years (1978-1997).**

**Methods: Morphologic study was performed at the level of light microscopy using histologic and histochemical methods. Clinico-morfologic characteristic of the material was given.**

**Results: For the period of 20 years we revealed 51 cases of polyneoplasia that constituted 2,5% to the number of performed autopsies (1993).**

**There were 29 male and 22 female patients.**

**Various combinations of tumours, the variety of clinical symptoms, complications and causes of death were determined. Depending on the time of occurrence polyneoplasia was characterised as synchronous or metachronous. Autopsy allowed to reveal tumours not recognized during lifetime but playing a certain role in thanatogenesis, leading to definite clinical symptoms and sometimes being the main disease and the cause of death.**

**Conclusion: Autopsies allow to solve some problems connected with morpho- and histogenesis of primary multiple tumours.**

# P-733

## THE EXPRESSION OF VHL-PROTEIN IN HUMAN INVASIVE DUCTAL CARCINOMA OF THE BREAST

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**Introduction**: Angiogenesis is a very complex process, which is controlled by many pro- and antiangiogenic factors. One of the recently described antiangiogenic factors is the VHL-protein, a protein which does not work in presence of Von-Hippel-Lindau disease and which is thought to be an antagonist of vascular endothelial growth factor.
**Aims**: We investigated the expression of VHL-protein in human invasive ductal carcinoma of the breast and examined the relationship between VHL-expression, intratumoral micro-vessel density (MVD), histological grading and local tumour spread (TNM-system).
**Methods**: Representative paraffin blocks of formalin-fixed tumour tissue from 46 patients with invasive ductal carcinoma of the breast were selected for immunohistochemistry. Micro-vessels were stained by anti-CD31 (Clone: JC70/A, Dako) and 10 vascular hot-spots were counted following the method of Weidner. VHL expression in tumour tissue was detected using a polyclonal anti-VHL antibody from rabbit (Phar Mingen).
**Results**: We found a negative correlation between VHL-expression and MVD ($r = -.751$, $p = .01$). There was a significant negative correlation between VHL-expression and histological grading ($p = .041$). A higher expression of VHL-protein was more often related with a lower tumour stage – but this was not statistically significant ($p = .058$).
**Conclusions**: The results of our study could suggest an important role of VHL-expression in the regulation of angiogenesis in human invasive ductal carcinoma of the breast. The VHL-expression seems to be associated with prognosis in breast cancer.