with microsatellite markers TNFa, D6S273, D6S291 in DNA samples prepared from the serial tissue sections.

Results: Low Ki-67 nuclear expression was found in 45% of CIN1-2 and 25% of CIN3 lesions. Namely, less then 30% of cells in lower two thirds of the epithelium thickness were Ki-67 positive. The rest of CIN lesions revealed Ki-67 nuclear expression in 60-90% of cells in all layers of cervical epithelium. Expression of Ki-67 in microcarcinomas varied from 30% to 90% of cells while invasive SCC had 50-70% of stained nuclei. Survivin expression increased with the severity of CIN lesion, reflecting the reduction of apoptosis. Thus survivin expression was detected in 25% of CIN1, 50% of CIN2 and 67% of CIN3. Cytoplasmic expression of survivin was found in 70% of CC. Only few CIN cases (22%) were negative for Ki-67 and survivin that correlated with the retention of microsatellite heterozygosity at 6p21.3. We suppose these CIN lesions may regress with apoptosis.

Conclusions: The obtained results suggested that the combination of immunostaining for Ki-67 and survivin might be helpful in early diagnostic of cervical lesions and evaluation of further CIN progression.

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Informative comprehension of detection of chimeric genes PAX3/7-FKHR in prognostically unfavourable forms of rhabdomiosarcomas in children

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Background. Alveolar rhabdomyosarcoma (ARMS) is an aggressive soft tissue malignancy of children. Most ARMS patients express PAX3-FKHR or PAX7-FKHR gene fusions resulting from t(2;13) or t(1;13) translocations, respectively. The availability of them in tumor correlates with sensitivity to cytostatics and efficacy of the treatment. These markers can be detected also in bone marrow (B?), that is a feature of micrometastases or minimal residual disease.The objective of the work is to determine informative comprehension of detection of expression of chimeric gens PAX3-FKHR and PAX7-FKHR in cells of tumor and BM in prognostically unfavourable forms of rhabdomyosarcomas (RMS) in children. Materials and methods. Tumor tissue and BM, obtained during the treatment (from 3 aspirates per one examination), from 26 childhood RMS patients, aged 3-11. The treatment of patients was carried out in accordance with protocols: Protocol EpSSG RMS 2005 for non-metastatic embryonal and alveolar RMS; CWS-96 for metastatic (IV stage) and recurrent RMS. Choice of protocol was based on determination of PAX3-FKHR and PAX7-FKHR fusion status. Fusion status was determined using the real-time RT-PCR method. Results. Chimeric genes in cells of tumor have been detected in 11 patients that evidence on belonging of these tumors to RMS of alveolar type (aRMS), which are of unfavourable prognosis and require different from embryonal RMS protocols of polychemotherapy (PCT). At a moment of making out a diagnosis in 8 of 11 patients with aRMS chimeric transcripts PAX3-FKHR and PAX7-FKHR in BM were detected that evidence on IV stage of disease. In 1 patient they were detected in all 3 points of BM. During a year from diagnosis and beginning of the treatment 5 of 8 patients of this group died. After course of PCT in two patients chimeric genes in BM were not detected, that evidence on the efficacy of the treatment. Conclusions. High sensitivity of real-time RT-PCR assays are capable of identifying PAX3-FKHR and PAX7-FKHR fusion status both in tumor and submicroscopic metastatic disease in sites such as the BM. Our study has demonstrated the clinical utility of fusion gene detection in differential diagnosis, prognosis, and minimal disease monitoring, as well as allows determine the rate of achieved remission.

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Prognosis and recurrence pattern of patients with cervical carcinoma and pelvic lymph node metastasis

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Objective to investigate the prognostic risk factor(s) and pattern of disease relapse of patients with cervical carcinoma and pelvic node metastasis. Methods 124 cases of FIGOIB1~IIA cervical carcinoma with pelvic node metastasis treated from January 1991 to December 2001 were selected for this study. Prognosis and recurrence were retrospectively analyzed using the clinico-pathological data. Results The over all 5 year survival and disease-free survival (DFS) was 63.3% and 61.4% respectively. Overall recurrence rate was 39.5% (49/124). Intra-pelvic relapse (25/41, 61.0%) was significantly more frequent than extra-pelvic relapse (13/41, 1.7%, P=0.008).Multivariate analysis identified involvement of common iliac node as independent prognostic factor (P=0.035). According to this factor, node-positive patients could be divided into low risk group (without common iliac node involvement, 104 cases) and high risk group (with common iliac node involvement, 20 cases). The DFS were 69.4% and 24.5% respectively, and the difference was significant (P=0.003). Intra-pelvic relapse was observed in 22.1% of low risk and 25.0% of high risk group respectively, the difference was not significant (P>0.05), however extra-pelvic relapse was seen in 7.7% of low risk and 40.0% of high risk group, and the difference was significant (P<0.001). Conclusions Common iliac node involvement is the significant factor that influences the prognosis of patients with cervical carcinoma and pelvic node metastasis. According to this factor, survival and recurrence pattern differs significantly. These findings provide important reference for individualized modification and investigation of treatment mode.

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Clinical and pathological features of primary lymphoma of bone

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Introduction: Primary lymphoma of bone (PLB) is a rare disease, first described by Oberling in 1928. Even today the diagnosis of PLB can be difficult due to the relatively non-specific clinical signs and ambiguous radiographic features. Here we have reviewed the patients presenting with PLB, their clinical features, especially the relation between clinical stages and patients' outcome.

Patients: In this study, PLB was defined as follows. 1) Malignant lymphoma presenting a single or multiple bone lesion(s) with or without invasion to surrounding soft tissue. 2) There is no evidence of visceral or nodal involvement at the time of first diagnosis after the several radiographic screening, including whole body CT scanning, gadolinium scintigraphy.

According to the definition, we have experienced 17 cases of PLB from 1991 to 2005 in National Cancer Institute, Japan. All patients were diagnosed as PLB with needle and/or open biopsies. There were eight males and eight females varied 8 to 73 years old (Median 41 years old) at the time of diagnosis. The median follow-up period was 7.2 years (8 month to 11 years). The affected bone lesions were as follows. Femur and ilium: 5cases, thoracic vertebrae, sacrum and tibia: 3 cases, lumber vertebrae and rib: 2 cases, skull and humerus: 1 case. There were no specific radiographic images on PLB, except for the occasional extensive abnormal bone marrow signal on MRI whereas plain X-ray images were negative. Histopathologically, there were 11 cases of diffuse large B cell lymphoma, 3 cases of anaplastic large cell lymphoma (K1-lymphoma) and lymphoblastic lymphoma (precursor B cell type) and low grade B cell lymphoma (unclassified) accounted for the rest of two cases. Clinical stages (Ann Arbor) at the diagnoses, Stage IE: 9cases, stage IV: 7cases.

Result and Conclusion: All cases were treated with the combination of systemic chemotherapy(including anti-CD20 antibody; rituximab)with or without local radiotherapy. Surgical treatments were performed in 6 cases; laminectomy and instrumentation of thoracic vertebrae 2 cases, osteosynthesis of pathological fracture 3 cases, total hip arthroplasty 1 case. Overall survival rate was 81% (13/16). (Mean survival time; 46 months) Overall survival rate of stage IV was 71% (5/7), relatively good outcome compared to that of the historical control of stage IV patients including nodal or visceral involvement. These clinical outcomes might suggest the possibility of specific biological features of lymphoma cells only with skeletal involvement.

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Assessment of vascularity in gastric malignant tumors

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Introduction: Assessment of angiogenesis is possible nowadays by various methods: imaging methods, molecular biology and pathological exams. A high value of vascularity index is correlated with an advanced disease, thus the angiogenesis assessment could offer important preoperative parameters.

Method: In our study we proposed to assess the vascularity index in gastric adenocarcinoma using imaging method comparing to pathological markers (microvessel density, CD34, VEGF).

We included 8 patients with gastric cancer assessed by endoscopic ultrasound with color Doppler, power Doppler and pulse Doppler possibilities. We computed the vascularity index using a custom-made application based on the free ImageJ open-source software. The ROI

(region of interest) and the gain were constant during the entire exam. Other vascular parameters were assessed also: velocity (V), resistivity and pulsatility indexes (RI, PI) derived from flow velocity. The diagnosis of cancer was confirmed by pathological exam in all patients. We compared also the vascularity index with the tumoral stage.

Results: We found a significant correlation between the power Doppler vascularity index and the microvessel density. The resistivity index and pulsatility index were variable.

Conclusions: Vascularity index is an accurate parameter for assessment of tumoral vascularity. The vascularity index is correlated to the tumoral stage also. The results of our study are limited due to the small number of patients, extended studies being necessary in the future.

618 Poster

Investigation of the relationship between dna-dependent protein kinase and lymphatic metastasis in colorectal cancer

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Aim: To investigate DNA-dependent protein kinase (DNA-PK) expression, and its relationship with lymphatic metastasis in colorectal cancer.

Methods: Tumor tissues from 60 patients, divided into two groups according to lymphatic metastasis, were immunohistochemically stained to detect the DNA-PK expression including Ku70, Ku80 and PKcs proteins.

Results: Positivity of both Ku70 and Ku80 in colorectal cancer was negatively correlated with lymphatic metastasis with an r value of -0.57 and -0.38, respectively. Similar correlation was found between Ku expression, especially Ku70, and long-term survival. PKcs, however, displayed no significant correlation. Statistical analysis failed to detect any correlation between DNA-PK expression, and clinical characteristics, such as age, sex, tumor location, tumor thickness and distant metastasis ($p>0.05$).

Conclusions: DNA-PK expression, especially Ku70 expression, is negatively correlated with lymphatic metastasis, and the survival of patients with colorectal cancer. Ku70 expression may be a potential indicator for the preoperative evaluation, and prognosis in colorectal cancer.

619 Poster

Methodology matters - prognostic significance of HER2 protein expression versus HER2 gene amplification in metastatic breast cancer

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Background: After so many years of research, clinical value of HER2 (Human epidermal growth factor receptor 2) is unclear. Clinical use of HER2 status for prognosis of breast cancer patients remains controversial, mostly because of different testing methods used in different studies and consequently variability of results. There is a lack of studies regarding prognostic value of CISH especially in metastatic breast cancer (MBC) when risk evaluation is based on different parameters than for primary breast cancer. Aim of this study was to compare prognostic relevance of HER2 status in MBC tested by two different methods i.e. immuno-histochemistry (IHC) and chromogenic in situ hybridization (CISH).

Material and methods: In the same group of 107 MBC patients HER2 protein overexpression was determined by IHC and HER2 gene ampliication was determined by CISH in a primary tumor tissue.

Results: There was significant correlation ($p<<0.001$) between HER2 protein overexpression determined by IHC and HER2 gene amplification determined by CISH, beside the existence of discrepant results. However, there was a difference in prognostic value of compared methods during the course of metastatic disease. In a whole group of MBC patients there was no significant difference when patients are stratified by CISH or IHC results. There was significant difference in metastatic breast cancer survival between HER2 nonamplified and HER2 amplified cases in subgroups of patients determined by available clinicopathological parameters i.e. in a subgroup of patients older than 50 years, postmenopausal subgroup and node-positive subgroup. When patients are stratified by IHC results there was no significant difference in survival in these or any subgroups of patients.

Conclusion: These results indicate a discrepancy in the ability of two methods to predict patient's survival. CISH grading system in contrast to IHC grading, offers a real cut-off value for determination of different breast cancer prognostic subgroups. CISH seems to be more accurate and more informative than IHC in prediction of clinical outcome in metastatic breast cancer. Beside that, these results show that HER2, although as biomarker with limited significance in different subgroups of patients, has relevant prognostic value in metastatic breast cancer. In that context, our results confirm that methodology matters and those gene-based testing methods should be really accepted as a gold standard for assessment of HER2 status.

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Utility of p53 gene expression for early diagnosis in oral leukoplakias

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Background: The last advances in cellular biology demonstrated the precise mechanisms regulating the cell cycle and show that abnormalities in cell proliferation are a very common manifestation in some cancers and precancerous lesions. Nevertheless tumor suppressor genes, like p53, and other proteins associated to the cell cycle also mediate in this sequence. Oral leukoplakia is a precancerous stage that constitutes a canzerisable lesion due to the genetic alterations that mediate in the evolution of lesion. Since p53 disturbs lead to a loss in cell cycle control, routine molecular study of p53 gene expression would contribute to an improved diagnosis and treatment of oral premalignant lesions. Objective: Study the utility of p53 gene expression as diagnosis factor in oral leukoplakias by means of Quantitative Real Time PCR (qPCR). Materials and Methods: Expression levels of p53 gene, in 24 unique freeze samples from 24 patients with leukoplakia, were measured. From each patient, 2 samples were obtained: opposed lateral oral mucosa and leukoplakia mucosa. As control, a pool of healthy human oral mucosa from healthy donors (n=4) was used. qPCR experiments were performed on a LightCycler 480 Instrument (Roche) using LightCycler 480 SYBR Green I Master (Roche). A constitutively expressed gene, HPRT, was used as internal control. Results: The expression levels of p53 were higher in opposed lateral oral mucosa and leukoplakia both from patient, with regard to the pool of healthy human oral mucosa from healthy donors. When comparing opposed lateral oral mucosa and leukoplakia from the same patient, 54.2% of samples showed higher p53 expression in opposed lateral oral mucosa than in leukoplakia. This could be explained by the "field cancerization" hypothesis in oral cavity, since when the field alteration occurs it is followed by progression of the lesions or the appearance of recurrences or second lesions. Conclusions: qPCR assays confirmed that p53 is up-regulated in premalignant oral epithelial lesions, indicating that p53 may contribute to carcinogenesis. On the other hand, although in other kind of tumors p53 over-expression is a late event, in oral cavity it can be observed in more initial phases of the precancerous lesion. Therefore p53 could represent an attractive diagnosis factor in oral leukoplakias. Support: S. Díaz Prado is beneficiary of an Isidro Parga Pondal contract from Xunta de Galicia (Spain).

621 Poster

Gene expression analysis in pheochromocytoma - searching for new pathways involved in the hereditary susceptibility and the malignant outcome

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Introduction: Phaeochromocytomas and paragangliomas are rare neuro-endocrine tumors that arise from adrenal and extra-adrenal chromaffin tissue respectively, usually causing secondary hypertension by oversecretion of catecholamines. Nowadays, it is widely accepted that 25-30% of patients carry a germline mutation in one of six susceptibility genes: SDHB, SDHC, SDHD, RET, VHL and NF1. Nevertheless, there are still some familial cases not associated with any predisposing gene. On the other hand, malignancy occurs in 15-40% of cases (depending on location and genetic alteration), and is defined as the presence of metastases, with no other clinical features useful for an early diagnosis.

Aim: On this basis, new strategies are needed in order to find new susceptibility genes responsible for the remaining familial cases. In addition, new molecular tools that help us to characterize those patients at risk of malignancy are needed in order to intensify the follow up.