

time-to-onset of bone spread. LEPR Lys656Arg may be involved in tumour differentiation, thus influencing Gleason grade.

98 Association between primary brain tumours and specific IgE levels, measured in participants of the EPIC cohort study

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Background: Epidemiological studies investigating the association between allergic or atopic diseases, including asthma, hay fever or eczema (atopic eczema), and primary brain tumours (glioma, meningioma, schwannoma) showed nearly all an inverse association between glioma, with the less consistent results for meningioma. Only few cohort studies exist presenting with conflicting results. At the moment, no conclusive biological mechanism is known.

Our study investigates in frame of a large international cohort study based on specific IgE-levels the association between atopic condition and primary brain tumours.

Material and Methods: A nested case-control study has been conducted in frame of the EPIC (European Prospective Investigation into Cancer and Nutrition) brain tumour cohort. The serum samples were collected from the participants of this large international, multi-centric prospective cohort study, in general several years before the diagnosis of the brain tumour. 216 glioma, 137 meningioma and 39 schwannoma cases were available for testing. In total 595 controls sera were randomly selected from the cohort and matched to the cases according study centre, gender, data of birth, age, date and time of blood selection, and length of follow-up. ORs and their 95% CI were calculated using conditional logistic regression analyses. Adjustment has been done for educational level, smoking status, alcohol consumption, physical activity and hormone replacement therapy (for women only).

Results and Conclusion: In general the results of this cohort study confirm those of the case control studies. Using sera for testing specific IgE levels instead of questionnaire data from case-control participants avoid information and recall bias due to memory gaps and to missing symptoms although an atopic condition exists. Limitations and strength of the study design should be discussed, especially the issue of possible biological hypotheses.

99 Cyclooxygenase-2 (COX2) expression in transitional cell carcinoma of the bladder does not confer independent prognostic properties

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Background: Cyclooxygenase-2 (COX2) is responsible for maintaining an acute inflammatory state in the body and its aberrant overexpression can trigger chronic inflammation and cancer. The link between inflammation and bladder cancer has provided the impetus for many studies to evaluate the prognostic significance of COX2 in this tissue with no clear consensus on independent prognostic potential having been made. Using, one of the largest cohorts of Transitional Cell Carcinomas (TCC), we attempted to further elucidate the independent prognostic potential of COX2 expression in bladder cancer.

Methods: Tissue microarrays containing 557 non-muscle invasive (NMIT) and 216 muscle invasive (MIT) bladder tumours collected as part of the Spanish Bladder Cancer study, were analyzed by immunohistochemistry using computerized quantitative image analysis technology. COX2 expression was assessed as a product of staining intensity and area, providing a continuous protein expression gradient. Univariate and multivariate Cox-proportional hazards statistics were then applied to determine whether COX2 expression was an independent prognostic marker for recurrence and progression in NMITs, and progression and disease-specific survival in MITs.

Results: COX2 protein expression was associated with tumour stage ($p < 0.0001$) and grade ($p < 0.0001$) in NMITs. Maintaining COX2 expression as a continuous variable in univariate analysis yielded an association with increased recurrence in NMITs (hazard ratio [HR] 1.019 [95% CI 1.000–1.038], $p = 0.048$), while a dichotomous expression score was associated with a decrease in progression of NMITs (HR 0.448 [0.252–0.795], $p = 0.01$). These associations did not maintain significance in the multivariate analysis.

Conclusions: To our knowledge, this study makes use of the largest cohort of TCCs to be analyzed for COX2 expression, and recapitulates the association of COX2 expression with other established cancer markers. However, there is lack of evidence to support that COX2 expression can be used as an accurate, independent prognostic marker in bladder cancer.

100 Cancer incidence in the Republic of Belarus: from 1970 to 2030

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Background: Cancer incidence rates grow dramatically in the world. 25.0 million new cancer cases are expected to be registered in 2030. This cancer burden is supposed in Belarus too. Thus we will need to prepare the medical service for new conditions and design new approaches in cancer prevention.

Methods: The data of obligatory cancer registration were studied for the past 39 years. Age Standardized Incidence Rates (ASR_{World} per 100,000) in males and females (urban and rural) were calculated. Absolute numbers of cancer incidence were analyzed and predicted up to 2030 in compliance with age-specific rates trends and demographic situation prognosis.

Results: In 1970 13,983 new cancer case were established in Belarus. This number has grown to 40,744 to 2008. This increase was caused partially by population ageing and by growth of age-specific rates due to cancerogenic factors affection. Constant growth of ASR was noted for colon cancer and melanoma of skin in both males and females and for breast, corpus uteri and renal female cancers. Incidence rates for skin cancers in the both sexes, prostatic and renal cancer in males slowly increasing from the 1970s started growing rapidly in the middle of the 1990s. But considerable decrease was shown in ASR of males and females stomach cancer as in lip cancer in males. ASR for female and male recto-sigmoidal cancer and male cancers of oesophagus, larynx, lung and bladder had been increasing till the middle of the 1990s to be fixed at a certain level then. Thyroid cancer incidence jumped immediately after Chernobyl disaster from 0.45 in 1970 and 0.77 in 1986 to 3.1 in 2003 (males) and from 0.81 in 1970 and 1.71 in 1986 to 14.7 in 2003 (females). Since 2003 morbidity has been flatten out in males and started decreasing in females. Thyroid cancer incidence rates have returned to before-Chernobyl level in children age-groups but they continue increased in elder cohorts. It is expected that the population of Belarus will decrease by one million persons (10% from the 2008 level) but the proportion of 55 years old people will increase in 25% from 2008 to 2030. Most important cancers incidence rates grow rapidly exactly in people who are 55 years and elder. Both ageing of population and cancerogenic factors impact allow us to predict the 60,000 new registered cancer cases in 2030.

Conclusions: To ease the result of our expectancies we need to start realizing cancer screening programs, especially reinforcing prevention activity and expanding health care and medical education systems in part of oncology.

101 Tumours of salivary glands from diagnosis to management

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Background: The three major salivary glands plus the hundreds of small minor salivary gland locates within the submucosa of the oral cavity and oropharynx are capable of giving rise to a wide range of neoplasms. The vast majority of salivary neoplasms are epithelial in origin. The ratio of benign to malignant salivary gland tumours is gland dependent. Epithelial salivary gland tumours are relatively uncommon and constitute a wide spectrum of variable morphologic and biologic entities. Among these cytological and morphological properties of salivary gland tumours, one of the most important criteria for measuring its biological behaviour and aggressiveness is cell proliferation. The cell proliferation/death balance is most important in the development of salivary gland tumours.

Material and Methods: Forty nine formalin fixed paraffin embedded tissue blocks of epithelial salivary gland tumours were used in this study. Haematoxylin and Eosin stain was used for reassessment of the histopathologic diagnosis. The cell proliferation activity was examined by proliferating cell nuclear antigen (PCNA) and Ki 67 immunohistochemistry and proapoptotic cell death Bax and Bcl-2 mRNA genes was analysed by in situ hybridization techniques.

Results: The parotid glands expressed a high frequency of affected site and about 25% demonstrated malignant behaviors while the minor salivary glands the frequency rate were account for 25% and have ability to demonstrated malignant behavior to 50%. Immunohistochemical analysis show high expression of PCNA and Ki 67 was noted in 8 of 12 pleomorphic adenoma cases (66.67%), 15 of 19 adenoid cystic carcinoma cases (78.95%), 6 of 7 mucoepidermoid carcinoma cases (85.71%), and 3 of 5 adenocarcinoma cases (60%). Significant difference was found between labeling index of benign and malignant salivary gland tumours, while no significant relationship was noted in labeling index between adenoid cystic carcinoma and mucoepidermoid carcinoma neither between mucoepidermoid carcinoma and adenocarcinoma.

In situ hybridization detection show low expression of Bax and was noted in pleomorphic adenoma cases (25%), in adenoid cystic carcinoma cases (52.63 %), however, mucoepidermoid carcinoma showed high expression of these markers than other salivary gland tumours, whereas adenocarcinoma show equal number of cases expressed both PCNA protein and Bax mRNA. No significant relationship was demonstrated between the immunostaining PCNA,

Ki 67 or Bax, Bcl-2 and the morphological growth pattern or patient clinical profile.

Conclusion: The histogenesis and morphogenesis of these neoplasms demonstrated wide spectrum of histologic features depends on their biologic behavior. Salivary gland neoplasms are clinically diverse group of neoplasms and the frequency of these neoplasm outcomes is probably not recognized when analyzing short term survival results in patients to whom the disease has a long natural history.

102 Large melanoma of skin: level of invasion or tumour volume what is superior?

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Background: The main prognostic factors for malignant melanoma are Breslow and Clark criteria, ulceration, growth patterns and localization of tumour [Balch, 2001; Cascinelli et al., 2009]. But, on the other hand, the exophytic nodular part of large melanoma often is ignored. Purpose: to access prognostic significance of nodular melanoma tumour burden in comparison with its Clark level of invasion.

Material and Methods: 107 patients of 28–70 years of age (M – 35, F – 72) with melanoma T4N0M0 were enrolled in the retrospective study. The tumour volume of exophytic nodular part of melanoma was calculated in vivo by formulas for spherical and hemispherical patterns after measurement of tumour's thickness and base. All patients underwent surgery – wide local excision of melanoma. Survival analysis estimated by using the Kaplan-Meier method and the log-rank test. Spearman and Kendall's correlation coefficients (τ) were used for comparison of prognostic signification between Clark's levels and tumour mass.

Results: The 5-year overall survival rates in 15 patients with nodular melanoma 5–10 mm of thickness (or tumour volume about 0.5 cm³) were 86.6%; in 40 patients with nodular melanoma 11–20 mm (tumour volume 0.5–4.0 cm³) – 52.5%; in 33 patients with nodular melanoma 21–30 mm (tumour volume 4.1–14.0 cm³) – 36.4%; and nobody of 19 patients with tumour more than 31 mm thickness (>14.0 cm³) lived out 5 years. Equation, which shows dependence of survival rate of patients on tumour volume, was made on the base of regressive analysis of received data. It estimates that in case of large melanoma tumour volume is significantly superior ($\tau = 0.67$) in prognosis of patients' overall survival than Clark's levels of invasion ($\tau = 0.10$).

Conclusion: The assessment of tumour volume in the nodular exophytic part of melanoma could be the additional diagnostic and prognostic criterion, which will determine the strategies of patient's treatment.

103 KRAS mutational spectra in routine diagnostic analysis of metastatic colorectal cancer

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Background: KRAS tumour mutational status is now widely recognized as a predictive biomarker in patients with colorectal cancer (CRC). Large clinical trials have shown that only CRCs with wild-type KRAS respond to epidermal growth factor receptor (EGFR) targeted antibody treatment. Seven different DNA base pair substitutions within codons 12 and 13, each leading to an amino acid substitution in the protein, are the most frequently observed genetic events within KRAS gene in CRC. Therefore, analysis of these hotspot clustered mutations is compulsory before treatment and for large routine diagnostic tests reliable frequency and types of KRAS mutations have yet to be established.

Material and Methods: Formalin-fixed paraffin-embedded tissue blocks were collected and DNA was extracted from tissue sections from 259 cases of metastatic CRC. Mutation analysis of KRAS codons 12 and 13 was performed by allele-specific real-time polymerase chain reaction.

Results: KRAS point mutations in codons 12 and 13 were present in 96 cases (37.07%) of 259 analyzed CRCs. All seven tested mutation types were observed; among them the frequencies of mutations in codon 12 were GAT (p.G12D, 11.58%), GTT (p.G12V, 10.42%), GCT (p.G12A, 4.25%), TGT (p.G12C, 3.47%), CGT (p.G12R, 1.16%), AGT (p.G12S, 1.16%) and the only one in codon 13 was GAC (p.G13D, 5.02%). Furthermore, the rate of transversions (52.08%) was found to be higher than the transitions' rate (47.92%). All the transitions were of the G>A type (the most prevalent alteration in our study) affecting the first base of codon 12 or predominantly the second base of both codons. Two types of transversions (G>T, G>C) occurred only in codon 12, mainly at the second base.

Conclusions: According to the previous studies G12V and G12R mutations may predispose to more aggressive biological behavior in advanced CRC. Though, G12D and G12S mutants have less aggressive transforming phenotypes. In addition, G>A nucleotide substitutions score the second place among the point mutations in human cancers in the Human Gene Mutational Database for all human somatic missense mutations and in our study they represent the most common alterations. Since the performed analysis detected

all tested KRAS mutation types, which may have different impacts on the outcome of CRC patients, we want to emphasize as a conclusion the need for defining individual mutational spectra for developing therapeutic strategies targeting KRAS mutations in clinical practice.

104 HPV infection in epithelial ovarian cells of women at high risk of developing ovarian cancer

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Background: The causal role of high-risk (HR) HPV has been well established for cervical and other anogenital cancers but our understanding of the role of HPV in ovarian cancer etiology is controversial. Attempts to identify molecular risk factors for ovarian cancer in women at high risk of developing ovarian cancer which include family history of ovarian or/and breast cancer as well as personal history of breast cancer lead to the discovery that their ovarian epithelial cells are characterized by cross-linking-agent-induced chromosomal instability analogous to Fanconi Anemia phenotype and therefore can be susceptible to environmental risk factors including HPV infection.

The objective of the study is to investigate the frequency of HR HPV in women at high risk of developing ovarian cancer.

Materials and Methods: 20 ovarian samples were obtained from women undergoing risk reducing oophorectomy because of significantly elevated risk of developing ovarian cancer. The patients considered to be at high risk for ovarian cancer are defined as women with two or more first degree relatives with ovarian and/or breast cancer, or a personal history of breast cancer and a first-degree relative with breast and/or ovarian cancer. HPV testing was performed using polymerase chain reaction (PCR) with type-specific primers for E6 fragment of high-risk HPV type 16, 18 and 33. Real-time quantitative PCR was performed with ABI 7500 Fast Real-time PCR system and SYBR-Green master mix to calculate HPV copy number. Immunohistochemistry by using polyclonal anti-HPV 16 E6/18 E6 antibody (clone SC-460, Santa Cruz Biotechnology) to detect the E6 expression in the ovarian sections was performed.

Results: In the tissues derived from women at high risk of developing ovarian cancer, 30% of the samples were positive for HPV-16, 35% positive for HPV-18 and 10% positive for HPV-33. About 20% of the studied samples had a mixed infection. HPV copy number varied from 0.04 to 851 copies/reaction in HPV-16 positive samples and from 0.27 to 2793.51 copies/reaction in HPV-18 positive samples. By immunohistochemistry we determined that E6 HPV16/18 protein is expressed in follicular and surface ovarian epithelium. It is necessary to note that some vessel endothelium of the ovaries was focal positive for E6 protein.

Conclusion: Our study suggests that HR-HPVs ascending from the cervix to the ovaries might have a role in susceptibility of ovarian surface epithelium to neoplastic changes in women at high risk of developing ovarian cancer. Our broader goal is to define tools and methods to better understand the environmental risk factors that account for ovarian cancer risk and to translate these mechanisms into strategies for early ovarian cancer prevention.

105 Role of IL-1RN VNTR polymorphism in host immune susceptibility to viral associated neoplasias

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Background: A common 86bp VNTR polymorphism within intron 2 of the IL-1 receptor antagonist (IL-1RN) gene seems to be associated with the balance between host immunity and viral infections. Several authors have referred that the IL-1RN A2 allele is responsible for shorter immune responses to viral infections and therefore might be associated with increased risk of viral associated cancers development.

Methods: We have developed a cross-sectional study to analyse the role of this 86bp VNTR polymorphism on the development of viral associated neoplasias: cervical and nasopharyngeal carcinoma. Genotyping of IL-1RN VNTR polymorphism was performed by PCR in DNA extracted from peripheral blood samples from both healthy individuals (n = 446), individuals with cervical lesions (n = 346) and nasopharyngeal carcinoma (n = 122) from the Northern Region of Portugal.

Results: Our study revealed that the IL-1RN A2 allele was significantly increased in both patients with cervical and nasopharyngeal carcinoma when compared with healthy individuals. Statistical analysis revealed that it is associated with increased risk for both invasive cervical cancer (p = 0.027; OR = 1.84) and undifferentiated type of nasopharyngeal carcinoma (p < 0.001; OR = 3.73).