19th European Congress of Pathology

Ljubljana, Slovenia, September 6-11, 2003

Abstracts

Abstract Review Board

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Contents

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Prologue

Seven hundred and twenty one abstracts from 62 countries have been reviewed and accepted for 16 oral presentation and 40 poster presentation sessions at the 19th European Congress of Pathology in Ljubljana, Slovenia, September 6-11, 2003. This important contribution, mostly by younger pathologists and scientists, provides an excellent complementary programme to the 104 congress sessions and to the plenary keynote lectures prepared with invited speakers, internationally recognised experts in more than 20 different areas of our medical discipline.

The Abstract Review Board, chaired by R. Golouh and J. Lamovec, accepted most of the abstracts in the form submitted, with minor mostly linguistic changes being made in some cases. The preferences of authors for oral or poster type of presentation have been respected whenever possible. The high quality of most of the abstracts expresses the vitality, richness and prominence of our branch's contribution to biomedical science and the medical care of patients. The final selection of the best six of 23 pre-selected abstracts, for oral presentation at the plenary award session, was made by the Selection Committee, A. Llombart-Bosch, M. Sobrinho-Simões, and G. Bussolati, based on their scientific quality and originality of contribution. Furthermore, from 20 preselected best poster abstracts, the three best will be honoured by Congress Awards that will be presented at the same plenary session of the closing day of the Congress. Additionally, the best free paper contribution in the field of liver and digestive tract pathology will be selected by the Selection Committee complemented by D.G. Tiniakos and will be given the special, George Tiniakos Award.

It has become obvious that, for both scientific and professional reasons, our discipline requires organ and technique oriented sub-specialisation. However, congresses covering all areas of current pathology by means of invited lectures and free paper presentations, play an important role in establishing an appropriate balance between the need for sub-specialisation and the harmful effects of a complete splitting of our discipline.

Another important aim of the European Society of Pathology from its inception has been to keep together pathologists from all European countries, irrespective of their geographical and political borders, to promote professional and scientific exchange and to contribute to harmonisation among countries in relation to our discipline. There is no doubt that the collapse of the Iron Curtain has been a crucial turning point. In that sense, the significantly increasing number and quality of submitted free paper abstracts, with a concomitant increase in attendance at congresses, of pathologists from countries of the former Warsaw Pact, contributes to exchange and harmonisation within our medical branch and, to some extent, may represent a step towards the forthcoming European unity.

Finally, the Congress organisers would like to express our sincere gratitude to the Congress main sponsor, Ventana Medical Systems, for its financial support of this publication, and to the publishing house Springer-Verlag and the editor, G. Klöppel, for making it possible for all abstracts to be published in the September 2003 issue of the European Society of Pathology official, highly respected journal, Virchows Archiv.

Gianni Bussolati
President
European Society
of Pathology

Dušan Ferluga
President
19th European Congress
of Pathology

O-001

Congenital tumours at foetal and neonatal autopsies during 17-years period

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Introduction Nowadays, congenital tumours can be successfully diagnosed antenatally by means of ultrasound. Some of them may be an ultrasonic marker of foetal chromosomopathy. Depending upon their morphology and volume, some can be treated surgically immediately after birth, but in many cases the outcome is poor. The aim of this study was to assess the frequency and types of congenital tumours at autopsy of aborted foetuses, stillbirths and neonates that died within 28 days after birth.
Material and methods Autopsy protocols from the period 1986 to 2002. were analysed retrospectively, and the tumour tissue samples were retrieved from the archive (when available) and re-examined.
Results In the 17-years period 5139 autopsy of foetuses, stillbirths and neonates were performed. Congenital tumours were diagnosed in 65 cases (1.26%). Most frequent, in 45 cases (69.2%) were those considered to be hamartomas: cavernous lymphangiomas or cystic hygromas (43) and haemangiomas (2). Teratomas were next in frequency, with 13 cases (20%); 8 cases (61.5%)were sacrococcygeal; 3 cases (23.1%) originated from the skull base and 2 cases (15.4%) from the anterior mediastinum (one of them contained a relatively well formed foetus). Neuroblastoma and (lipo)fibromas were diagnosed in 2 cases (3.3%) each. Mesoblastic nephroma, rhabdomyoma of the heart and leukaemia were diagnosed in 1 case (1.6%), each. Congenital malformations were diagnosed in 23 cases (37.7%): in 24 cases (39.3%) generalized foetal hydrops was present.
Conclusions Congenital tumours are rare. The most frequent are hamartomas, which was confirmed also in this study. Oncogenesis and teratogenesis seem to be closely connected during embryonal development as can be presumed by frequent concurrent appearance of congenital tumours and malformations.

O-002

Potential prognostic value of the expression of P-glycoprotein in chosen solid tumours of childhood

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Solid tumours in children are diagnostically and prognostically a very difficult group for both: clinicians and pathologists. Still we have to use complicated procedures to predict the clinical course of neoplasmatic disease. Current studies indicate that P-gp is one of the most important factors of multidrug resistance in some of malignant neoplasms. P-gp is a mdr1 gene product localized on the long arm of 7th chromosome. 95 formalin-fixed and paraffin-embedded tissue sections from cases diagnosed in the Laboratory of Pathology Institute of Pediatry, Medical University of Lodz (48 cases of nephroblastoma and 37 cases of neuroblastoma, 5 cases of rhabdomyosarcoma and 5 cases of EWS/pPNET) were selected for our study. In our research we used mouse anti-human P-glycoprotein (P-gp) (CHEMICON International, Inc.). We found the positive correlation with the expression of P-gp protein and poor clinical course in children with nephroblastomas, but we did not observe such a coincidence in children with neuroblastomas and PNETs.

O-003

C-kit protein expression as a poor prognostic factor in human neuroblastoma

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Introduction Neuroblastic tumours (NTs) are childhood embryonal neoplasms of neuroectodermal cells of neural crest origin. The NTs are quite heterogeneous. In a subset of NTs, regression and maturation of neoplastic cells are widely documented. Other NTs are highly malignant and poorly responsive to therapeutic schemes. Aims: The recent development of specific small molecules that block the activity of tyrosine kinases suggested to investigate the prognostic relevance of the c kit oncogene expression in NTs.
Materials and methods One hundred and sixty eight NTs were investigated for expression of c kit protein and its ligand stem cell factor (SCF) using immunohistochemistry and Northern blot analysis. Mutations in exon 11 of the c kit gene were studied by PCR.
Results c kit protein expression was detected in 21 out of 168 NTs (13%). Immunostaining was observed in neuroblasts either diffuse in the cytoplasm and at cell membrane. Twenty out of 21 c kit positive cases were poorly differentiated neuroblastomas with high mitosis/karyorrhexis index. Nineteen out of 21 (90%) presented at III-IV advanced clinical stage, 17 out of 21 (81%) showed MYCN amplification and 1p36 deletions. Co-expression of c kit protein and SCF was documented by double immunostaining. These results were also confirmed by Northern blot analysis. Nine c kit positive cases were investigated for the presence of activating mutations in exon 11 of the c kit gene. In none deletion mutations were revealed.
Conclusions Our results show that c kit protein expression identifies a subset of NTs with unfavourable clinical and molecular parameters.

O-004

Congenital hepatic fibrosis with liver failure in an infant

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Introduction Congenital hepatic fibrosis (CHF) is rare developmental disorder, which is usually diagnosed in older children presenting hepatomegaly, normal liver functions and portal hypertension. It is defined as a ductal plate malformation. CHF is usually

associated with polycystic disease of the kidneys. It may be either sporadic or hereditary. We are reporting a very rare case of CHF in an infant who died of liver failure.
Case report A 2-month-old girl was admitted to the hospital because of fever, cough, jaundice, irritability and poor feeding. The physical examination revealed a jaundiced infant with hepatomegaly. Laboratory tests revealed an elevated WBC, low haemoglobin and normal platelets, prolonged PT and PTT. Bilirubin, ALP, GGT and LDH were all elevated and albumins were very low. Ultrasound showed an enlarged and diffusely echogenic liver, size and echo of both kidneys were normal. Ascites was present. Despite treatment the child's condition deteriorated and she died on the ninth day of admission. Clinical diagnosis was: Hepatitis? Cirrhosis? with secondary coagulopathy. Postmortem examination: hepatosplenomeagaly, ascites, hydropericardium, jaundice. Poorly aerated lungs with focal haemorrhages, PDA. In histology: ductal plate malformation-namely CHF, hemorrhagic necrosis of the liver and haemorrhages in different organs.
Conclusions 1. The early presentation of CHF at 2 months of age with fatal liver failure is unusual. Mortality affects older children and is related to variceal bleeding, cholangitis and/or kidney disease. 2. Our case was sporadic and associated with PDA, but not with Caroli's disease or kidney pathology.

O-005

Neurologic disorders in children patients with scoliosis

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Aims The purpose of our work was to study neurologic disorders in children at a scoliosis.
Materials and methods It is surveyed in 25 patients with a scoliosis 1-2 degrees in the age of 10-14 years. To all children was carried out general clinical research, including the general analysis of blood, urine, electrocardiogramm (ECCG), roentgenography of organs of chest and a backbone, and also instrumental research of parameters of haemodynamics by a method of rheoencephalography (REG).
Results Among surveyed children in 13 is diagnosed left-hand scoliosis (52%), right-hand scoliosis and disturbance of bearing in 12 children (48%). In all patients with a scoliosis of children is diagnosed vegetative vascular dystonia. Neurologic disorder in all patients were characterized: complaints to headaches, nausea, vomiting, the common weakness, fatigue, memory impairment disorder of the dream, raised perspiration, paresthesia, emotional instability, fears, cardialgia. In the neurologic status prevailed: easy asymmetry of nasolabial fold, weakness of convergence is more often from both sides, distal heperrhirosis, red proof dermographism, hernias. On electroencephalogram in 56% surveyed children are revealed moderate diffuse changes and decrease of a threshold of proximal readiness. On ultrasonic doplerography in 30% patients is revealed asymmetry of blood flow on supratrochlear arteries, acceleration of blood flow on common carotid artery, internal carotid and iliac arteries. On REG in surveyed children is revealed dystonia type REG-in 24% patients, hypotonic type-in 12%, hypertonic type – 24%, hypertonic REG due to increase of a tonus of arterial and venule – in 28% children, that is whom or specific deviations REG is not found in these patients.
Conclusions Thus, long displacement of position of a vertebral column at a scoliosis in children results in infringement of blood circulation in spinal cord and cerebrum already at early stages of a scoliosis that is revealed with the help of instrumental researches. With a deepening of gravity of current of a scoliosis are formed various functional disorders of the central and vegetative system.

O-006

Integrated optical density and nuclear volume in Down syndrome

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Introduction Down syndrome (trisomy 21) is the most common numeric aberration of autosomal chromosomes in man; the incidence in newborn is about 1 in 700. Intrauterine growth retardation and endocrine disorders are a consistent finding in infants with Down's syndrome. The aim of this study was to estimate nuclear size and integrated optical density of parenchymal cells of various organs in patient with Down's syndrome and control group.
Material and methods During the years 1988 to 2000 fourteen cases of Down's syndrome were found (8 male and 6 female). Ten babies without congenital anomalies, died with respiratory distress syndrome, were analysed as a control group. Five nuclear variables were estimated: area, equivalent diameter, volume of equivalent sphere, roundness, and integrated optical density (IOD).
Results The mean nuclear volume and IOD of thyroid follicular cells were significantly lower in patient with Down's syndrome (65.46±15.31 mm^3, and 234.58±32.85) than in control group (43.82±8.95 mm^3, and 173.81±32.85, respectively) ($p<0.01$). The mean hepatocyte nuclear volume and IOD were significantly higher in control group (165.54±55.42 mm^3, and 220.84±51.75) than in trisomy 21 (110.39±32.97 mm^3, and 176.58±28.53, respectively) ($p<0.05$).
Conclusion The present results suggest altered gene expression in excessive genetic material, especially in thyroid follicular cells.

O-007

Comparison of Giemsa staining and immunohistochemical staining for the detection of Helicobacter pylori infection in gastric biopsies of urea breath test negative and positive paediatric patients

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Introduction Helicobacter pylori (HP) is a common cause of gastritis in both children and adults, and its incidence increases every year. Aim of the study was to evaluate the immunohistochemical and Giemsa stain of paediatric gastric biopsy specimens for the detection of HP infection from Urea Breath Test (UBT) negative and positive patients.
Materials and methods Eighty-two patients with positive UBT and thirty-eight patients with negative UBT were included to the study. Gastric biopsy specimens were evaluated histopathologically and graded according to the Sydney system. Giemsa staining immunohistochemical staining for HP were performed for the identification of HP.
Results With Giemsa staining, 7/38 (18%) UBT negative biopsies were HP positive. Biopsies from 79/82 (90%) UBT positive patients were HP positive with Giemsa staining. Immunohistochemical staining ratios of these cases were compared statistically.

Conclusion The results of this study suggested that, UBT may cause false positive HP infection diagnosis because of HP-like organisms. We conclude that Giemsa staining and immunohistochemical staining on gastric biopsy materials must be performed for the detection of HP infection.

O-008

Neuronal intestinal malformations in children - A review of 288 biopsy cases

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Introduction Neuronal intestinal malformations (NIM) encompass classical aganglionosis or Hirschsprung's disease (HD) and allied disorders (intestinal neuronal dysplasia - IND, hypoganglionosis, immaturity of ganglion cells, not classifiable dysganglionosis). According to histological and histochemical analyses of rectal biopsy, these entities may well be differentiated from HD. However, the diagnosis of IND remains controversial with a wide variation in the frequency in different series. The aim of this study was to determine the frequency of NIM in our series of children biopsied because of primary constipation.
Material and methods The morphological differentiation of NIM in rectal (suction or full thickness) biopsy or resected surgical specimens from 452 patients was performed using histological, specific histochemical and in some cases immunohistochemical methods.
Results Of the 452-biopsy cases, 288 or 63.72% had a form of NIM. Male/female ratio was 3:1, and age in ranged from 3 days to 18 years. Hirschsprung's disease was the most frequent, presented in 135 cases (46.88%). Twenty-nine of them were associated with IND proximal to the aganglionic zone. Isolated intestinal neuronal dysplasia type B (IND B) has been diagnosed in 66 cases (22.92%); not classifiable dysganglionosis in 42 (14.58%); hypoganglionosis in 30 (10.42%) and immaturity of ganglion cells in 17 cases (5.90%). In 164 cases (36.28%), the histology was reported to be normal.
Conclusion Rectal biopsy of children with primary constipation shows the spectrum of abnormalities. Some of them had to be specified. According to recent obligatory diagnostic criteria for NID it is easier to recognize this entity.

O-009

Placental villi maldevelopment and intrauterine hypoxia

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Introduction Formation of terminal villi with syncytiocapillar membranes is necessary for optimal maternal-foetal exchange and especially for oxygen diffusion. Immature placentas with little number of syncytiocapillar membranes may worse foetal nutrition and gas exchange. The aim of this study was to estimate the role of villous tree immaturity in the development of intrauterine hypoxia.
Materials and methods 337 placentas from singleton pregnancies were investigated. According to ultrasound and doppler velocimetry data, Apgar score, placentas were divided into groups with chronic hypoxia (132), IUGR (70), acute intrauterine hypoxia (64), from normal pregnancies (71). Estimation of the degree of maturity according to gestational age was made on routine histological slides. Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) concentration in placental tissue homogenates were investigated by ELISA method in 40 placentas.
Results The frequency of placental immaturity was 2-fold higher in placentas with hypoxia in compare with control group. Preterm maturation of placental tree accompany with severe foetal hypoxia, a very high mortality and morbidity. Concentration of VEGF in placental tissue was elevated in placentas with terminal villi deficiency, and concentration of PlGF was elevated in placentas with synchronous immaturity.
Conclusion Due to importance of the placental maldevelopment it is necessary to devise methods of its early diagnostics and investigate mechanisms of villous tree development. Determining of VEGF and PlGF may be useful for this purpose.

O-010

SAGE profile and MLH1/MSH2 expression in colorectal adenocarcinomas: correlation with the tumor phenotype

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Background The role of DNA mismatch proteins on tumor cell kinetic in sporadic colo-rectal adenocarcinomas (CRC) by topographic compartments remains unknown.
Design We selected 90 consecutive sporadic CRC (73 low-grade, 17 high-grade; 48 stage I, 18 stage II, and 24 stage III). Necrosis, inflammatory response, and growth pattern were evaluated. Mitotic figure counting, Ki-67 index, G2+M phase fraction, in situ end labeling (ISEL) of DNA fragments (scored by compartments, above muscularis propria vs muscularis propria) were studied regarding topographic mlh-1/msh-2 expression (positivity threshold 15%) and considered statistically significant if $P<0.05$. Oligo(dT)-primed double strand cDNA was produced from total RNA and libraries were produced from 3 normal colon specimens and 18 CRC. Sequential analysis of gene expression (SAGE) data was correlated with mlh-1 and msh-2 expression.
Results mlh-1/msh-2-negative CRC showed non-polypoid growth, necrosis, and inflammatory response, but no correlation with differentiation and stage. Superficial mlh1/msh2 expression showed direct correlation with mitotic figure counting, Ki-67 index, and G2+M phase fraction and inverse with ISEL index. Deep mlh1/msh2 expression showed direct correlation with Ki-67 and inverse with mitotic figure counting and G2+M phase fraction. MLH1/MSH2 SAGE revealed significant positive correlation with RAS homolog gene, and PI3P phosphatase, and negative correlation with Trefoil factor 3, Caspase recruitment domain (member 9), and Meprin A.
Conclusions mlh1/msh2-positive sporadic CRC show down-regulated apoptosis (Caspase 9), inflammation (Trefoil factor 3), and stromal degradation (Meprin A), and up-regulated proliferation (RAS homolog and MAPK activation by PI3P phosphatase). This gene profile explains both tumor phenotype and cell kinetic (↑proliferation, ↓apoptosis).

O-011

Molecular analysis of hereditary hemochromatosis

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Hereditary hemochromatosis (HH) is an autosomal recessive disorder, common in individuals of Northern European descent (prevalence close to 1:400). HH is characterized by increased iron absorption and iron deposits in various organs. HH patients may develop liver and pancreas cirrhosis, primary liver cancer, diabetes mellitus, and cardiomyopathy. In the majority of cases, HH is caused by mutations of the HFE-gene on chromosome 6p. HFE encodes an MHC class-I gene, a putative negative regulator of iron uptake. Three HFE mutation are common, C282Y, H63D and S65C. Patients with a homozygous C282Y genotype most often endure a severe clinical course, whereas homozygotes for H63D may develop mild forms of the disease. Heterozygotes are usually symptom-free, but show elevated serum ferritin levels. It has been speculated that secondary iron overload (heavy alcohol, chronic hepatitis, ?-thalassemia) may be a critical co-factor in those heterozygotes that develop symptoms of HH. HH not associated with HFE mutations is much less common. Within this heterogeneous group of non-HFE HH, three subtypes have been attributed to genetic alterations. Juvenile hemochromatosis (HFE2) is a rare but severe disorder mapped to chromosome 1q. HFE3 has recently been mapped to the transferring receptor 2 gene (TfR2) on chromosome 7q22. HFE4 follows an autosomal dominant trait and is associated with mutations of the ferroportin 1 gene (SLC11A3) on chromosome 2. Except for juvenile hemochromatosis, HFE, HFE3 and HFE4 are amenable to mutation analysis. The advent of modern high throughput technologies such as denaturing high performance liquid chromatography (DHPLC) has sparked clinical mutation screening programs, which also include screening of patients suspected to be affected by HH. In addition, DHPLC-based mutation screening can also be performed using archival formalin-fixed, paraffin embedded tissues (FFPE). We present here results from HFE-analyses of FFPE from autopsy cases using DHPLC and direct sequencing.

O-012

p63 expression pattern in squamous cell carcinoma of the oral cavity

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Introduction p63/p73L/p51/p40/KET is highly expressed in basal cells of the epithelium. P63 generates two main splice variants the TAp63/p51 and DeltaNp63/p73L. TAp63/p51 is responsible for squamous cell differentiation; DeltaNp63/p73L is a self-renewal agent. Recently Senoo et al. (Br J Canc 2001, 84: 1235-1241) described a new isoform called DeltaNp73L that correspond to the DeltaNp63/p73L lacking exon 4. This novel splice variant has been found only in cell lines of neoplastic squamous cells and in SCC. The aim of the study was to define the expression pattern of TAp63/p51, DeltaNp63/p73L and DeltaNp73L in 33 cases of squamous cell carcinoma (SCC) of the oral cavity compared with non-neoplastic keratinocytes.
Material and methods Immunohistochemistry was performed in order to analyse p63 tissue distribution in all the 33 samples. P63 expression was studied, by RT-PCR and nested-PCR. Controls were obtained by non-neoplastic gingival mucosa and skin.
Results In invasive SCC a strong nuclear positivity for TP63 antibody in all the cases studied was found. The DeltaNp73L isoform was more frequent in metastasising tumours (18/20, 65%) compared with tumours without metastases (5/13, 38.5%). Analysing the TAp63 expression a band about 250bp shorter than the TA isoform was seen. The sequence of this new mRNA form was identical to TAp63/p51 lacking exon 4, thus we named this variant DeltaNp51 for homology with DeltaNp73L. DeltaNp51 was more frequent in metastasising tumours (9/20, 45%) rather than non metastasising (4/13, 30.7%).
Conclusions DeltaNp73L seems to be associated with higher metastatic potential of SCC. More experiments are needed to clarify DeltaNp51 function.

O-013

The synthetic ligand of peroxisome proliferator-activated receptor gamma ciglitazone affects viability, proliferation and apoptosis in human glioblastoma cell lines

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Introduction Peroxisome proliferator-activated receptors (PPARs) are transcription factors, members of nuclear receptors superfamily, playing an important role in carcinogenesis, inflammation, atherosclerosis, lipid metabolism and diabetes. There is evidence that activation of PPARs by specific ligands inhibits the growth of different malignancies. The aim of our study was to determine whether ciglitazone, the synthetic ligand of PPAR gamma, affects proliferation, differentiation, apoptosis and expression of some cell cycle related proteins in glial tumor cell lines.
Material and methods The study was performed on U-87 MG, A172, T98G, and U-118 MG human glioblastoma cell lines. The lines U-87 MG, A172 and U-118 with inactive form of PTEN were transiently transfected with cDNA of wild type PTEN. MTT, flow cytometry, TUNEL assay and immunoblotting were used for detection of changes of the cell viability, proliferation, differentiation and apoptosis.
Results We have found that ciglitazone inhibited cell growth in studied glial tumor cell lines. The effect is accompanied by decreased expression of pRb protein, cyclin D1 and increased expression of c-myc and p27 after 12/24 hours of treatment by IC50 of ciglitazone. The flow cytometry confirmed the arrest in the G1 phase of cell cycle. We did not find any significant changes in expression of Bcl-2 family proteins and flow cytometry did not revealed any significant sub-G1 fraction. However, we have confirmed a degradation of PARP protein, caspase 3 and TUNEL staining showed an increase of the number of cells with DNA breaks. The level of GFAP, the marker of astrocytic differentiation, remained without change in U-87 MG and A172 cell lines. The effect of ciglitazone on cell lines transfected with functional PTEN is also discussed.
Conclusions We conclude that PPAR ligands may be potentially used in anticancer treatment. Supported by grants NC 6726-3/2001 and MSM 151100001.

O-014

Evaluation of real-time quantitative RT-PCR as diagnostic tool for the detection of carcinoma cells in routinely paraffin-embedded sentinel lymph nodes from various cancer patients

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Introduction A prerequisite for the use of quantitative RT-PCR (qRT-PCR) in routine pathological evaluation of sentinel lymph nodes (SLNs) is its application to paraffin-embedded tissue. Its reliability has to be proved by direct correlation with (immuno)-histochemistry. The aims of the study were development of reliable protocols for the expression analysis of tumor markers in paraffin sections of SLNs, determination of their sensitivity and specificity for detection of micrometastases of diverse cancer types, and correlation between qRT-PCR results and micromorphometrically obtained data.
Methods RNA was extracted from 8 µm sections of routinely paraffin-embedded SLNs taken from prostate cancer, breast cancer, and melanoma patients. For several tumor markers qRT-PCR was performed and results were correlated with the (immuno)histochemical and micromorphometric data of adjacent tissue sections. When available, clinical follow up was included in the analysis.
Results Amplifyable RNA could be isolated from all analyzed specimens. Tyrosinase qRT-PCR was performed with 306 SLNs from melanoma patients with good correlation with S-classification. For detection of prostate cancer micrometastases qRT-PCR for PSA and PSM was performed on 108 SLNs. The obtained values for PSA expression correlated well with the micromorphometric data. From breast cancer patients 50 SLNs were analyzed for expression of mammaglobin and maspin. The detection rate of (micro)metastases increased significantly when both tumor markers were used.
Conclusions Quantitative RT-PCR on paraffin sections enables a further improvement of sensitivity for the detection of micrometastases in SLNs without impediment of the histomorphological diagnosis. The determination of expression thresholds for certain tumor markers can be of prognostic value.

O-015

Human Papillomavirus (HPV) and cervical carcinoma: Physical state of HPV DNA and HPV oncogenic expression

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Introduction HPV is a necessary cause of cervical carcinoma. Persistent high-risk HPV infection, integration, and E6/E7 oncogenic expression are required for development and maintenance of a malignant phenotype. The aims of this study were to compare DNA and RNA based methods for detection of HPV, to study the physical state of HPV DNA and the incidence of E6/E7 oncogenic expression in squamous cell carcinoma of the cervix.
Material and methods DNA and RNA were extracted from 204 squamous cell carcinomas. The HPV DNA prevalence was investigated using the L1 Gp5+/6+ consensus primers and E6/E7 type specific primers for HPV 16, 18, 31, 33, and 45. PreTect HPV-Proofer (NorChip AS, Norway) was used to study the presence of HPV E6/E7 mRNA, detecting HPV 16, 18, 31, 33, and 45. The physical state of HPV DNA was tested by in situ hybridization (ISH) for HPV type 16, 18, 31, and 33 using a cloned HPV DNA probe labeled with biotin. The ISH was carried out with an alkaline phosphatase anti-alkaline phosphatase detection system.
Results The HPV prevalence was 97%, as detected by consensus PCR, type-specific PCR, and PreTect HPV-Proofer. HPV 16 was the predominant type (65%), followed by HPV 18 (11%), 45 (10%), 33 (6%) and 31 (5%). ISH analyses revealed that 166 cases tested positive. All cases had punctate signals indicating integrated virus DNA, whereas 48% contained both punctate and diffuse signals indicating both integrated and episomal virus DNA.
Conclusions This study documents the presence of HPV E6/E7 transcripts from a limited number of high-risk HPV types in cervical carcinomas. HPV seems to be integrated in the human genome in the majority of cervical carcinomas.

O-016

A comprehensive human disease bank for functional genomics

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Introduction Diseased tissues contain all the information on the genetic and epigenetic changes that caused the disease and determine its outcome (prognosis). Recent developments in genome and proteome research offer the opportunity to unravel this information and provide new insight into the molecular pathogenesis of diseases. Aims: We are establishing a unique human tissue resource for genome research based on a collection actually comprising 2.5 Mio paraffin-embedded and 28 000 frozen tissue samples stored at the temperature of liquid nitrogen.
Materials and methods This collection is being expanded by national and international interdisciplinary cooperations. Blood samples, patient history, laboratory parameters, treatment and outcome information are included in addition to tissues. Special emphasis is placed on quality control and compliance with ethical and legal criteria. All protocols follow established standard operating procedures in accordance with guidelines of the FDA and recommendations of European countries. Informed consent is obtained from patients and appropriate security measures guarantee patient confidentiality.
Results To make this resource available to a wider scientific community, we generate disease-specific gene libraries and produce tissue micro arrays. Well defined expandable cell lines and nude mice xenografts are established for characterization of gene function.
Conclusion Proper access to collections of diseased human tissues is currently a major limiting factor for genome research in medicine. There is an urgent need of tissue banks that meet highest quality and legal criteria.

O-017
Withdrawn

O-018

Male microchimerism in fetal, infantile and female liver: role of pregnancy in hepatocyte microchimerism

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Introduction Feto-maternal microchimerism has been described for many years. Besides an hypothetical role in the pathogenesis of autoimmune diseases, these fetal cells should provide stem cells which can participate to repair of damaged tissues. Their capacity to differentiate into hepatocytes is largely unknown. Our aim was to evaluate the frequency of male microchimerism in normal or diseased female livers, the nature of chimeric cells and the influence of a current pregnancy.
Material and methods 46 female liver specimens obtained from 29 women (chronic hepatitis C: 10; fulminant hepatitis: 4; acute liver disease during male-bearing pregnancy: 6; normal controls: 9) were studied for the detection of Y chromosome by combining fluorescent in situ hybridization (FISH) and 2 distinct PCR assays. Real-time PCR for quantification of Y chromosome was normalized on X chromosome. 11 fetal and 6 infant female livers were used as putative negative controls, 5 male livers (4 adult and 1 fetal) were used as positive controls.
Results Y chromosome was detected by at least one technique in 19/29 adult women but also in 6/6 children and in 7/11 fetuses. The two PCR assays were highly concordant and more sensitive than FISH. Mean Y/X ratio was 0.01 ±0.014 and was correlated to FISH
Results Hepatocyte microchimerism was observed only in 4/6 women with acute liver disease during pregnancy and in 4/9 fetuses while non-hepatocyte microchimerism was observed in all the groups (adult, children, fetuses) for normal and diseased livers.
Conclusion Male cells are frequently present within female livers from the fetal to the adult age. In case of children or fetuses, this demonstrates the transplacental transmission of fetal (male) cells preexisting in the mother, phenomenon we propose to call feto-fetal microchimerism. Their differentiation into hepatocytes was only observed in female fetuses and pregnant women.

O-019

The role of proximal tubular cells in macrophage migration and crescent formation

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Introduction Recent studies confirmed that proximal tubular cells played an important role in local macrophage proliferation. Locally produced macrophage inflammatory protein may be involved in development of cellular crescents.
Material and methods We analyzed 573 glomeruli of renal biopsy specimens of 40 patients with crescentic glomerulonephritis. Glomeruli with both segmental and circumferential crescents and cross section of urinary pole were studied. Macrophages were confirmed with CD 68 monoclonal antibodies.
Results 158/573 glomeruli presented both crescents and urinary pole at the same time. Segmental crescents affected urinary pole in 96.5% glomeruli and circumferential in 100%. Positive CD 68 cells were noted: 1) in cellular crescents, 2) surrounding proximal tubules close to urinary poles and 3) scanty positive proximal tubular cells.
Conclusion There are two possibilities: 1) crescents tend to migrate towards urinary pole, or 2) macrophages, originated from de-differentiated proximal tubular cells migrate to the nearest position.

O-020

NCAM expression on renal interstitial cells: a marker for renal progenitors?

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Introduction Neural cell adhesion molecule (NCAM) is highly expressed on cells of the murine metanephrogenic mesenchyme at early stages of kidney development. During further development, NCAM can still be detected on epithelial cells of comma and S-shaped bodies as well as on interstitial mesenchymal cells. During maturation of the fetal kidney NCAM gradually disappears from all epithelial structures and is confined to small areas of interstitial medullary cells. In the adult murine kidney NCAM expression is virtually absent.
Materials and methods In the present study NCAM expression was analyzed by immunohistochemistry and Western blots in adult human kidneys, including healthy kidneys and kidneys with different glomerular diseases associated with incipient interstitial fibrosis (IF). NCAM positive cells were characterized by triple immunofluorescence staining using antibodies against human NCAM combined with a panel of antibodies against neurofilaments, a smooth muscle actin (aSMA), HLA class II, CD133 and CD34, and DAPI for nuclei staining. Digital pictures from every fluorescence channel were taken and superimposed for the specific staining using the Analysis DOKUTM software from Soft Imaging Systems.
Results In adult human kidneys, NCAM expression was restricted to rare interstitial cells with a dendritic morphology, long cytoplasmatic extensions and elongated nuclei, which were localized predominantly in the outer medulla. In comparison to healthy kidneys, the number of NCAM-positive interstitial cells increased in the initial phases of IF in different forms of glomerulonephritis. Western blot analysis of renal tissues with incipient IF showed the exclusive expression of the 140 kDa isoform of NCAM; other isoforms were not detectable. Staining with antibodies against neurofilaments clearly differentiated nerve cells from NCAM-positive interstitial cells, which are neurofilament negative. They are also aSMA negative, though the number of the aSMA+ cells increased at the early stages of IF. However, some of the NCAM-positive cells clearly express the stem cell markers CD133 and CD34.
Conclusion These data indicate that a subpopulation of NCAM-positive interstitial cells may be renal progenitor cells, which can participate in the initial phases of IF.

O-021

Development of intrinsic innervation in human fetal kidney

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Introduction We aimed to define, for the first time, the ontogeny of intrarenal innervation and to assess the distribution and nature of nerves in human fetal kidney.
Material and methods We studied routinely-processed tissue sections from 17 human fetuses (20-24 gestational weeks-gw n=6, 25-40 gw n=11) and 3 adults using immunohistochemistry and antibodies to pan-neural markers S100, neuron specific enolase (NSE), neurofilaments (NF), PGP9.5 and the adrenergic marker tyrosine hydroxylase (TH).
Results NSE-, NF-, S100-, PGP9.5-positive nerves, associated with arterial and venous vasculature, were identified in renal medulla and cortex at 20gw onwards and their density appeared to increase with gestation reaching adult levels at 28 gw. Nerve fibres extended from the cortico-medullary region to the outer cortex, reaching the renal capsule in the 3rd trimester. In renal medulla, S100-, PGP9.5- and TH-positive nerves were detected around tubular cells as early as 20 gw. However, their density gradually decreased during the 3rd trimester. Most of the intrarenal nerves were TH-positive. In detail, NSE-, NF-, S100-, PGP9.5- and TH-immunoreactive fibres were observed in close apposition to renal artery and its branches, occasionally reaching the afferent and efferent arteriole (3rd trimester). Nerve fibres were detected in close apposition to the juxtaglomerular apparatus in 2nd and 3rd trimester.
Conclusions Human kidney appears richly innervated during the 2nd and 3rd trimester. There is progressive increase in the density of parenchymal nerve fibres towards term from the cortico-medullary region to the cortex. Most intrarenal nerves are adrenergic and have a predominant perivascular distribution, implying that renal innervation plays an important functional role in intrauterine life.

O-022

Analysis of chimerism in human renal transplant biopsies by fluorescent in-situ hybridisation (FISH) and laser-microdissection based RT-PCR (LM - RT-PCR)

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Introduction Cell migration from the host to the transplanted organ results in chimerism (1), and seems to be associated with chronic transplant rejection (2,3). However, early detection of recipient cells might provide an important clue for anti-rejection therapy.
Materials and methods 15 cases from sex-mismatched renal transplants (male kidney into female receiver) with 3 consecutive biopsies (one, six, and twelve months) were analysed by FISH and immunohistology (c-kit and smooth muscle actin). In 10 additional cases of renal transplants each at day 0 and twelve months after transplantation, glomeruli were microdissected and a RT-PCR for c-kit and ß-actin was performed. Umbilical vein endothelial cells served as positive control.
Results Glomeruli: Transplant glomerulopathy shows increasing numbers of y-positive cells with progression of the sclerosing lesion. By LM - RT-PCR, increased c-kit transcription is observed compared to controls, while immunohistology is negative. Arteries: Recognition of vascular lesions as chronic transplant vasculopathy was easily possibly by FISH, but not by light microscopy. Tubuli/interstitium: Infiltrates of acute interstitial rejection change sizes from focal/large to disseminated/small over time. Chimeric cells are seen in tubulitis but also in tubular epithelium with ischemic damage.
Conclusion Transplant glomerulopathy is a lesion of chronic rejection associated with the appearance of c-kit positive, recipient cells. Chimeric cells can be demonstrated in transplant vasculopathy and in damaged tubular epithelium, possibly homing to ischemic lesions as part of a repair process.
1. Lancet 1992; 339:1579; 2. Liver Transp 2000; 6:686; 3. Transfusion 2001; 41:419

O-023

Unique congenital glomerulopathy in a child with suspected Ehlers-Danlos syndrome

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Introduction The aim of our demonstration is to call attention to a unique pediatric case with as yet unknown ultrastructural changes in the glomerular basement membrane (GBM).
Case report The full-term female patient – 3100 g/49 cm – has been developing well with the exception of early (intrauterine?) unilateral intraocular hemorrhage, which necessitated the enucleation of the bulbus at the age of 1 month. Nine months later, varicose enlargement of the left saphena vein was observed, and microscopic hematuria was detected. Later selective proteinuria and mild hypertension was found. At the age of 1 year an open renal biopsy was performed. Light microscopy showed thick-walled and dilated capillaries of glomerular tufts. Collagen IV staining demonstrated corrugated positivity in the GBM, also seen in COL IV alpha 1 chain. Chains alpha 3 and 5 appeared normal. Immunofluorescence gave only strong global IgM positivity. Electron microscopy showed peculiar mostly circular splitting of the GBM lamina densa with inclusions of small amorphous densities as well as of some remnants of cellular organelles. Analogous changes were observed in adjacent arterioles. At present, the 6 year-old child with signs of somatic underdevelopment is being treated for mild hyperazotemia, anemia and renal hypertension. Her body shows some asymmetry, the skin is thin, pink, but no areas of hyperextensibility are seen as well as any hypermobility of joints. NMR investigation of the brain disclosed a slowly growing mass in the left thalamus.
Conclusion The classification of the case among connective tissue disorders is difficult, type IV of Ehlers-Danlos syndrome seems to be the closest.

O-024

Chronic renal thrombotic microangiopathy (TMA) following radiation: study of 5 cases

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Introduction Renal TMA may develop in various etiological conditions including infections, drug toxicity, immune disorders and radiation.
Case reports We described 5 cases of chronic TMA associated with radiation injury. Patients 1, 2 and 3 underwent bone marrow transplantation (BMT) together with intensive chemotherapy and total body irradiation of 10 to 12 Gy without renal shielding. Two patients had received cyclosporin (CsA) and 1 aziathioprine for graft versus host reaction prevention. Three to 5 years later these 3 patients developed moderate proteinuria associated with slowly progressive renal failure in one, and hypertension in two. Biological signs of hemolytic anemia were no detected in all. Renal biopsy was performed. Patient 4 had been treated by chemotherapy and abdominal irradiation (20 Gy then surimposition of 20 Gy on the left lombo-aortic region) for splenic lymphoma. At this time carcinoma in the left kidney was discovered. The renal function was normal and no proteinuria was found; left kidney was removed 1 year later and pathologic study was performed. Three months after surgery the renal function remained normal and no proteinuria was noted. Patient 5 had a renal biopsy for discrete chronic renal failure and hypertension without proteinuria. She has been successfully treated, 26 years ago, by abdominal irradiation for sub-diaphragmatic lymphoma. In all patients the renal lesions were similar and associated glomerular chronic TMA with subendothelial expansion, mesangiolysis, aneurysmal dilatations and diffuse severe arteriolar lesions with hyalin obstruction. The glomerular lesions were focal in patient 5 and diffuse in the others. No immune deposits were found by immunofluorescence.
Conclusion This study shows that chronic severe lesions of TMA develop following renal radiation associated or not with BMT. These lesions may be clinically and biologically silent over long period of time and are probably underestimated. Although the role of irradiation seems pivotal, chemotherapy and cyclosporine may add deleterious effects in BMT patients.

O-025

Expression of cyclin-dependent kinase inhibitors (CDKI) in human mesangioproliferative glomerulonephritis: their role in mesangial cell proliferation (morphometric study)

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Introduction There is evidence for roles of the Cip/Kip family of CDKI (p21, p27) in mediating growth arrest in glomerular cells. Mature podocytes, highly specialized cells with a complex cellular structure (interdigitating foot processes) leave cell cycle and may undergo nuclear division, but not cytokinesis, because this would involve the breakdown of the actin cytoskeleton. It has been recognized that mesangial cells proliferate and accumulate matrix in response to certain forms of injury. The aim of the current study was to elucidate the role of CDKI expression in human glomerular diseases.
Material and methods The analysis was performed on 4 groups of renal biopsies (every consisting of 10 cases): minimal change disease (MCD), membranous glomerulonephritis (MGN), mesangioproliferative proliferative glomerulonephritis: IgA (IgAN) and IgM nephropathy (IgMN). We examined the expression of p27, p21, Ki 67 and PCNA by immunohistochemistry. Adult nephrectomy specimens (N=5) without evidence of glomerular disease served as controls. Morphometric analysis was additionally done in MCD, IgAN and IgMN cases. Color sampling was performed to extract "brown" nuclei stained for p27 and all nuclei of glomerular cells. Then the number of segmented "brown" nuclei was calculated per area of glomerular profiles and related to the number of all nuclei.
Results Immunostaining for p27 was abundant in normal podocytes and some mesangial cells. There was no evidence of p21 and proliferation markers Ki 67 and PCNA in normal glomeruli. In MCD and MGN expression did not change. In contrast there was a marked decrease in staining intensity for p27 in mesangial cells in patients with IgAN and IgMN. The ratio of stained for p27 nuclei was 56.1% and 39.1% for MCD and IgAN, IgMN group, respectively (p=0.03). Interestingly also podocytes focally - in sclerotic portions of glomeruli - revealed a marked decrease of p27. This loss of immunostaining occurred predominantly in mesangial cells and podocytes expressing Ki 67 and PCNA. This was accompanied by the de novo nuclear immunostaining for p21.
Conclusion The decrease in p27 level is required to induce podocyte and mesangial cell proliferation. Also the increase of p21 was observed. Thus changes in cell cycle regulatory molecules play central role in determining the renal response to injury and may underlie the development of progressive glomerulosclerosis. Understanding mechanisms regulating cell proliferation may target future therapies on these molecules.

O-026

Thin-basement-membrane nephropathy (TBMNP): global and segmental types

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Aim Characterization of the clinico-pathologic spectrum of TBMNP.
Methods Retrospective determination of the harmonic mean GBM thickness (nm + SD) in a consecutive series of 33 adult patients whose GBM had been classified as "thin" during the original evaluation, who had no IgA nephritis or familial haematuria, and for whom at least 2 glomerular profiles were available for measurements. Controls: 5 living kidney donors and 5 patients with minimal change NP.
Results Three cohorts were distinguished. The first cohort, involving TBMNP consisted of 22 patients with microhaematuria (proteinuria in 15 patients), no glomerular immune complexes, histologically normal glomeruli, and an attenuated GBM. The attenuation was global in 14 patients, and segmental in 8. The second cohort, involving coexisting TBMNP and an established glomerular disease (4 focal sclerosis, 2 focal proliferative GN, 1 minimal change, 1 membranous, and 1 hypertensive arteriolosclerosis)

comprised 9 microhaematuric patients. The thinning was global in 5 patients, and segmental in 4. The third cohort contained 2 non-haematuric patients who had a globally thinned GBM and focal proliferative lupus GN or minimal change NP, respectively. In summary, 19 cases of global TBMNP (234 + 19 nm; controls: 334 + 43 nm; p<.0000), and 12 cases of segmental TBMNP (299 + 21 nm; p=0.02) were verified.
Conclusion TBMNP manifests with either a globally or a segmentally thinned GBM. TBMNP is not infrequently associated with other glomerular diseases. The clinical and morphologic data should be considered simultaneously when the diagnosis of TBMNP is made. Supported by OTKA T-038271(Budapest, Hungary) grant to I.B.

O-027

Simultaneous quantitative gene expression analysis in human renal biopsies in patients with lupus nephritis (LGN)

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Introduction Inflammatory flares, enhanced proliferation and delayed apoptosis are important pathomechanisms in LGN. These are associated with increased expression of genes previously studied by semi-quantitative immunohistologic detection of an individual gene product. The aim of this study was to perform a simultaneous quantitative comparison of expression of genes tentatively relevant to pathogenesis of LGN.
Material and methods Renal tissue samples, fresh frozen (18) and formalin-fixed (8), obtained by percutaneous biopsy of 18 patients with LGN (WHO class II, III and IV) were used. Formalin-fixed zero transplant biopsies served as controls. From 5 µm thick sections, 25 glomeruli were laser-microdissected, RNA was extracted and transcribed. A real-time, quantitative PCR analysis was performed using VCAM-1, fractalkine as markers of inflammation, IL-6, PDGF-ßR as markers of proliferation, Bax, Fas ligand and TNF-R1 as regulators of apoptosis and ß-actin as housekeeping gene.
Results Frozen biopsies allowed detection of all genes; formalin-fixed only of some.
There was no correlation between levels of gene expression and age, sex, proteinuria or activity/chronicity indexes. WHO class II cases showed increased levels of VCAM-1, PDGF-ßR and Bax but not fractalkine as compared to controls. WHO class III and IV cases showed higher expression of fractalkine, IL-6 and PDGF- ßR than WHO class II cases. In WHO class III cases, IL-6, and fractalkine levels were clearly, while Bax levels were slightly higher than in WHO class IV cases.
Conclusions 1. Real-time quantitative PCR is useful for a simultaneous analysis of gene expression in frozen samples of human renal biopsies. 2. In this limited study, class II lesions, as expected, showed lower expression of genes in comparison with classes III and IV. However, class III lesions seemed to exhibit higher expression levels of genes involved in proliferation, inflammatory cell infiltration and apoptosis regulation compared to class IV, possibly relating to a different pathogenesis of those two classes.

O-028

Haemorrhagic glomerulonephritis (GN) and massive pulmonary haemorrhage in Goodpasture syndrome - unique case report

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Aim We present the autopsy study of an unique case of Goodpasture syndrome with a rapidly progressive fatal course.
Case report A 38-year-old previously healthy woman was admitted to hospital with a brief history of fever, chest pain and haemoptysis. She was anemic, dyspnoic with a blood oxygen saturation of 47% and WBC count of 17.7 x 103/µL. Chest x-ray showed pulmonary alveolar densities. Despite negative haemocultures for bacteria, broad spectrum antibiotics were introduced. Anti-GBM antibodies were positive by indirect immunofluorescence, while ELISA for α3(IV) chain was negative and she additionally received prednisone. She expired 3 days after admission due to rapidly progressive respiratory and renal failure. The autopsy study revealed massive intraalveolar pulmonary haemorrhage, leukocytoclastic alveolar capillaritis and linear immunofluorescence of IgG and C3. Kidney autopsy samples showed a significant glomerular influx of neutrophils (10-20 per glomerulus), diffuse effusion of blood in the Bowman's space and tubular lumen accompanied by linear IgG and C3 deposits along the glomerular and tubular basement membrane.
Discussion and conclusion Our patient fulfills diagnostic criteria for Goodpasture syndrome. Pulmonary haemorrhage associated with alveolar capillaritis and linear deposits of IgG is a characteristic finding which can be fatal when massive. However, renal involvement in Goodpasture syndrome is characterized by anti-GBM diffuse necrotising and extracapillary crescentic GN. To the best of our knowledge, this is the first reported case of Goodpasture syndrome with GN, which we have termed haemorrhagic due to similarities with lung pathology. It might be hypothesised that this unique renal involvement is related to the finding of atypical anti-GBM antibodies in our case, obviously not directed against the Goodpasture epitope on α3 chain of type IV collagen, but probably against another antigen.

O-029

Progressive diffuse proliferative and crescentic recurrent IgA glomerulonephritis (GN) in transplanted kidney

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Introduction Recurrent IgA GN in renal transplant has been reported in 40-50% of patients, and it is even less frequently progressive than in the native kidney, probably due to immunosuppressive therapy. We present two patients with a severe form of recurrent progressive IgA GN.

Case reports A 53-year-old man with chronic mesangiopro-liferative GN developed terminal renal failure 7 years after the onset of the disease. The patient almost certainly had IgA GN according to the clinical and laboratory findings, histologic picture and confirmed IgA GN in his son, although immunofluorescence was not done on his native kidney biopsy. His first kidney allograft was lost due to surgical complications. The second allograft functioned well for 10 years, when decreased renal function, hypertension, nephrotic syndrome and erythrocyturia were observed. In addition, IgA ANCA was positive 1:1280. Renal biopsy revealed an IgA GN, severe diffuse proliferative with 25% crescents.
A 39-year-old man with advanced diffuse proliferative and crescentic (15%) sclerosing (38%) IgA GN received a cadaveric renal allograft. Six years later, decreased renal function, hypertension, proteinuria and erythrocyturia were found. Renal transplant biopsy disclosed severe diffuse IgA GN with 70% crescents.
Conclusions Only 4 cases of recurrent rapidly progressive crescentic IgA GN in transplant have been reported so far. In our first patient, severe IgA GN was probably causally related to capillary wall immune deposits accompanying mesangial deposits, and the occurrence of ANCA after transplantation. A peculiar immune process resulting in a severe progressive and crescentic IgA GN in the native and transplanted kidney could be hypothesised in our second patient. Arterial hypertension, established as the most frequent risk factor of non-immune progression of IgA GN, did not play a significant role in our patients.

O-030

Collapsing variant of FSGS associated with interferon-α treatment for hepatitis B infection

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Introduction Use of recombinant interferon-α (IFN-α) has been associated with protean kidney manifestations, including episode of acute renal failure (ARF) secondary to ATN, nephrotic syndrome due to either FSGS or minimal change disease and chronic interstitial nephritis.
Patient and methods 45 year old African American male with long-standing hypertension, on diuretic and ACEI and normal kidney function, who initiated treatment for hepatitis B with IFN-α developed proteinuria and lower extremity edema. Laboratory testing revealed creatinine (Cr) 5.1 mg/dL (449 μmol/L), albumin 1.7 mg/dL and abnormal urine sediment. Proteinuria was estimated at 10 gm/d. All serologies were either normal or negative. US guided kidney biopsy was performed about three months after the initiation of IFN-α treatment.
Results Thirteen glomeruli were available for review, two were globally sclerotic. All glomeruli showed various degree of mesangial sclerosis, diffuse mild mesangial hypercellularity and variable degree of glomerular collapse. Four glomeruli had segmental scars. Interstitium showed diffuse moderate interstitial fibrosis and tubular atrophy with mild to moderate lymphoplasmocytic infiltrate. IF studies were nonspecific. EM revelaed hyperplastic visceral epithelial cells and diffuse effacement of the foot processes. Kidney function did not improve after the discontinuation of IFN-α treatment, however four months of prednisone was effective with partial remission of proteinuria to 1.5 gm/d and decrease in Cr to 3.0 mg/dL (264 μmol/L). Due to elevated liver enzymes prednisone was discontinued. Kidney function and proteinuria remained stable over the next 6 months.
Conclusion IFN-α should be included on growing list of medications associated with collapsing FSGS. It seems that discontinuation of IFN-α treatment and steroids may prevent rapid progression to advanced kidney disease. Further studies are needed to delineate the extent of this association and the potential mechanism of injury.

O-031

Use of TTF-1 in diagnosis of metastatic pulmonary adenocarcinoma in FNAB samples

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Introduction Diagnostic use of TTF-1 as an organ specific marker for thyroid and lung origin of carcinomas was documented mostly in tissue sections, while the information on TTF-1 in cytology samples is still insufficient. The aim of the study was to analyze the expression of TTF-1 by immunocytochemical detection in cytology samples.
Materials and methods We obtained material by fine needle aspiration biopsies (FNAB) of primary and metastatic neoplasms (104 cases). First, methodological approach with TTF-1 was tested in thyroid tissue (16 goiters, 4 adenomas, 13 carcinomas). Afterwards, we analyzed 3 primary and 18 metastatic lung adenocarcinomas, 5 primary and 13 metastatic extrapulmonary adenocarcinomas, 3 primary lung tumors and 11 metastatic lung carcinomas of other types and 10 metastatic carcinomas of different types. Additionally, 3 metastatic malignant melanomas were also included in the study. Finally, we tested 5 metastatic tumors of unknown origin.
Results All primary and 12/18 metastatic lung adenocarcinomas stained positive with TTF-1. Two of 5 metastatic tumors of unknown origin stained positive, however primary tumor was not found. Except for thyroid samples, normal lung, 3 metastatic microcellular and 1 macrocellular carcinoma of lung origin, none of other primary or metastatic tumors stained positive for TTF-1.
Conclusion In FNAB specimens, TTF-1 can confirm thyroid origin of carcinoma and more importantly, determine the lung origin of metastatic or primary adenocarcinoma. Therefore, TTF-1 could be used for early identification of primary site (pulmonary vs. extrapulmonary) in FNAB of metastatic adenocarcinomas skipping further expensive investigations.

O-032

Immunoprofile of adenocarcinomas in effusions: a retrospective study of 102 cases

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Introduction Adenocarcinoma is the most common metastatic neoplasia among malignant serous effusions. An effusion is often the initial presentation of a malignancy. The aim of the study was to assess the validity of immunocytochemical reactivity patterns in determining the origin of metastatic adenocarcinomas in serous effusions.
Material and methods We reviewed 102 non-consecutive specimens of serous effusions, containing adenocarcinomas, obtained from our department files. Of these, 100 had prior or posterior histological confirmation of the primary tumour. A battery of

monoclonal antibodies was applied (Ck7, Ck20, TTF-1, CA19.9, CA125, CA15.3 and HMFG-1) to Papanicolaou stained smears.
Results Positivity ratios for Ck7 in lung, breast, stomach, ovary, pancreas, colon and endometrial adenocarcinomas were 18/19, 19/20, 19/21, 20/20, 6/6, 0/6 and 6/6 respectively. The same ratios for Ck20 were 3/19, 4/20, 11/21, 3/20, 2/6, 6/6 and 1/6 respectively. We obtained positive immunoreactivity for TTF-1 in 15 of the 19 lung adenocarcinomas and in none of the other tumours. The positivity ratio for CA19.9 in stomach, pancreas and gallbladder adenocarcinomas were 18/21, 4/6, and 1/1 respectively. CA125 was positive in 18/20 of ovarian carcinomas. Of the 20 breast carcinomas, 13 were positive for HMFG-1 and 11 for CA15.3.
Conclusion The Ck immunoprofile was the single most important determinant in the assignment of the primary tumour. In some cases the Ck pattern was confirmatory in combination with additional immunostains (TTF-1, CA125, CA19.9, CA15.3 and HMFG-1). TTF-1 proved to be sensitive and highly specific in metastatic lung adenocarcinomas.

O-033

Interlaboratory quality control (QC) of PAP smear cytology as a part of the multi-centre trial comparing six optional screening tools in Latin America (The LAMS Study*)

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Aims As part of the external quality control (QC) programme incorporated in controlling all the diagnostic tests in the LAMS study, the reproducibility of the cytological diagnosis is being controlled using an inter-laboratory QC approach.
Study design Four sets of slides (20 in each), containing in different proportions 1) inadequate smears, 2) negative for SIL, 3) ASC, ASC-US, AGC, LSIL, 4) HSIL or CIN2, 5) HSIL or CIN3, and 6) invasive cancer, have been compiled, with consensus diagnoses by three cytopathologists as the gold standard. The reproducibility (inter-laboratory- and laboratory/consensus diagnosis) will be tested using both non-weighted and weighted kappa (ICC, intra-class correlation coefficient), as well as calculating three indexes measuring variability: A) between CIN1 and CIN2; B) between CIN2 and CIN3, and C) CIN1 versus CIN2, CIN3, invasive cancer.
Results The overall agreement with the laboratory- and the consensus diagnosis in two slide sets is substantial using non-weighted k=0.712 (95%CI 0.548-0.876) and almost perfect with weighted kappa ICC=0.963 (95%CI 0.929-0.980). The non-weighted k=0.873 (95%CI 0.705-1.00) for set 1 and k=0.608 (95%CI 0.350-0.866) for set 2. Using weighted kappa, ICC is almost perfect: ICC=0.989 (95%CI 0.977-0.996) and 0.940 (95%CI 0.836-0.977) for set 1 and 2, respectively. Further details will be presented in the conference.
Expected outcome measures This study design provides valuable data on the quality standards of individual laboratories and helps pinpointing the possible problem areas in their performance, where continuous quality improvement (CQI) could be helpful. *(LAMS: Latin American Screening Study, funded by EC, INCO-DEV Contract # ICA4-CT-2001-10013).

O-034

Similar MAC signals in buccal mucosa of breast and lung cancer patients

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Background Malignancy associated changes (MAC) were found in normal cells of patients with different types of cancer. It has been suggested that they could be used as a test for detection of early cancers. Any diffrences in MAC expression among different cancers remain to be determined. The aim of the study was to compare MAC signals detected in buccal mucosa of breast and lung cancer patients as measured by image cytometry.
Materials and methods 106 patients with lung cancer and 89 control subjects, and 100 breast cancer patients and 93 control subjects were included in the study. Scrapings of buccal mucosa were suspended in transport medium. Monolayer filter preparations were stained by Feulgen-Thionin method. Image cytometric analysis was performed by Cyto-Savant. Classifier, using nuclear texture features (NTF), was trained on lung cancer patients and corresponding controls and tested on breast cancer patients and their controls and vice versa.
Results When trained on lung cancer samples, the classifier with optimal combination of three NTF representing MAC recognized correctly 74% of breast cancers, while the correct recognition of lung cancer was 87% with the same classifier. When the classifier was trained on breast cancer samples, 78% of lung cancers were correctly classified, while 81% of breast cancers were correctly recognized with the same classifier.
Conclusions Our results indicate that MAC signals in buccal mucosa of breast and lung cancer patients are very similar. Therefore we now postulate that different cancers (at least of the epithelial origin) induce similar MAC changes, which would allow more universal test for detection of early cancers.

O-035

Rhabdomyosarcoma development in mice lacking p53 and Fos: tumor suppression by the Fos proto-oncogene

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Introduction Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma of childhood. Animal models of RMS are rare and the application of available models is limited due to low tumor incidence and long latency periods. The Fos protein, a major component of the AP-1 transcription factor, acts as an oncogene, mediates transforming signals, and controls invasive growth and angiogenesis during tumor progession.
Methods To investigate a potential genetic interaction between the p53 and Fos pathways, p53/Fos double knock-out mice were generated.
Results In contrast to p53 and Fos single mutant mice, these mice specifically develop RMS of the facial and orbital regions with more than 90% penetrance at 6 months of age. In cell lines established

from these tumors, re-expression of Fos is associated with enhanced apoptosis and reduced Pax-7 expression, whereas cell proliferation and the expression of other AP-1 components are not altered.
Conclusions Our results suggest that Fos acts as a tumor suppressor during RMS tumorigenesis and that p53/Fos mutant mice may be a suitable animal model for human RMS.

O-036

Long-term effect of neonatal monosodium glutamate (MSG) treatment on hypothalamus-pituitary-adrenal-thymus axis of rats

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Introduction The study aimed at determining effects of MSG, introduced in the perinatal period, on hypothalamus-pituitary-adrenal-thymus (HPAT) axis of Wistar rats.
Material and methods In days 2, 4, 6, 8, 10 the newborn rats received s.c. injections of MSG (4mg/g body weight). The rats were killed at 10 months of age. The paraffin sections of investigated organs were stained with HE, PAS and Van Gieson methods.
Results Macroscopically, the treated rats showed: stunted skeletal development, Cushing's obesity of "buffalo" type, both pituitary and adrenal hypertrophy. Microscopically the hypothalamic arcuate nuclei were completely destroyed along with neuronal constituents in the median eminence. Gliosis, the most important histopathologic indicator of CNS injury, was found instead of these tissues. Astrocytes were hypertrophic and hyperplastic, with small and dark nuclei, located in a dense net of glial fibrils. Multifocal small loci of microglial nodules, associated with neuronophagia, were also observed. Basophile cells of pituitary gland were hyperplasic, with foamy aspect and infiltrating neurohypophysis. Cortical cells of the adrenal gland, symmetrically, were also hyperplasic. The adrenal fasciculate zone was disturbed by increased number of cells and obviously dilated sinusoids. The thymus atrophied and lymphocytes proliferation was apparently decreased.
Conclusion This study supports the idea that the disturbance of hypothalamus-pituitary-adrenal (HPA) axis is accompanied with cell-mediated immunodeficiency.

O-037

Development and routine evaluation of the digital histology laboratory

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Background Comparable to the digital radiology, recent significant developments in the microscopic slide scanning, data storage technology can contribute to the development of digital histology laboratory techniques. The aim of the study is to evaluate this technology in a routine setting.
Methods In the daily routine in a university pathology department 100 to 300 slides were prepared from 50 to 150 cases in a day. From the usual paraffin blocks 5 µm sections were prepared and processed. The bar-coded slides were digitized and stored on a digital slide server. Commercial short and long term storage may be used. The evaluation of the slides was done in local and remote access. For specific tissue types (colon, gastric) automated tissue component segmentation and analysis modules were developed. For the 3D reconstruction of serial sections a dedicated program was developed.
Results The daily prepared slides (100-200 pcs) could be scanned in the night. The virtual microscopy analysis yielded concordant results with the optical microscopy analysis. Teleconsultation based on digital slides and virtual microscopy was applicable in selected cases. The automated algorithms could classify gastric biopsy specimen cases with high accuracy (95%). 3D reconstruction yielded a more detailed insight and diagnosis. Reports including the low resolution digital slide image, high resolution selected field of views, alphabetical report were sent in e-mail to the referring specialists in higher safety and speed as compared to the traditional way.
Conclusions The digital histology laboratory technology improves the specialisation in pathology allowing remote access to selected tissue types, the reporting process' safety and efficiency.

O-038

Experiences in the production of digital slides by an automated high-resolution scanner system after automated slide preparation

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Background Automated slide preparation systems are available for histological routine use. We recently developed and installed the Hi-Scope slide scanner system in a routine environment. The aim of the present work was the evaluation of Hi-Scope for everyday applications.
Methods Hi-Scope and a digital storage environment were installed in the biopsy specimen laboratory. 5 µm sections from gastrointestinal and surgical pathology material were prepared as usual. The slides were deparaffined, stained and coverslipped using automated systems (Medite Inc., Germany). The barcode labeled slides were placed into the Slide-Scope cassette and scanned by the Hi-Scope system (3DHISTECH Ltd, Hungary) automatically. A day 100 to 200 slides were scanned. Hi-Scope is an autoloader, autoscanner, and auto focusing slide scanner. Slides were stored on dedicated slide server computers equipped with fast short term and slow long-term storage components.
Results In the experiment 4000 slides were scanned altogether and 6 cassettes were reused continuously. From the 80 cassette loading cycle the cassette stuck once. From the three type of slides used the machine could not load 3% of the cheap, low-quality no name slides, 0.5% of the Superfrost slide with square edges and corners and 0.1% percent of the Starfrost slide with grounded edges end corners. A slide load/unload cycle is 10 seconds. Slides were identified using 6 decimal digit barcodes. The resolution was 0.16µm / pixel. The scanning speed of the system was 2.5 images / second including auto focusing. With this resolution 1 mm^2 was scanned in 20 seconds and resulted 20 Mbyte digital data using 1:5 JPEG compression.
Conclusions Hi-Scope can be used in the routine for slide digitization and archiving.

O-039

Cytogenetical findings in a case of non-ossifying fibroma

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Introduction Non-ossifying fibroma (NOF) is a benign osseous lesion with favourite localisations in the metaphyses of the tibia and distal femur. NOF is mostly asymptomatic and is therefore usually incidentally identified on radiographs. Most lesions stabilize at puberty and shows signs of healing by peripheral sclerosis or solid opacification. Only a few cases have been analysed cytogenetically.
Case report Clinical history: A 21-year old female presented with a lesion in the methaphysis of the tibia, which produces an eccentric cortical-based lesion with distinct sclerotic margins and ground glass aspect on radiographic imaging. A fracture line is observed. Surgical curettage is performed. Histopathology: This lesion is composed of short fascicles of benign appearing oval to spindle-shaped cells. The fascicles are arranged in a storiform pattern. In this stroma few multinucleated giant cells and some xanthomatous cells are present. In several stromal cells a substantial amount of hemosiderin pigment is clearly visible. Small numbers of mononuclear inflammatory cells are noticed. No atypical mitoses are found. Cytogenetics: G-banding analysis revealed following karyotype: 46, XX, der(2)t(2;?)(q22;?),-22, +mar[4]/46, XX[15].
Discussion and conclusion NOF is a benign osseous lesion confined to the metaphyses of the long tubular bones of skeletally immature patients. Most lesions stabilize at puberty. Malignant transformation is extremely rare and some individual cases of associated fibrosarcoma and osteosarcoma are described. These molecular findings together with earlier reported cytogenetical data are remarkable as NOF is generally considered to have frequently a spontaneous resolution indicating a self-limiting process which is most likely related to incomplete ossification. Further studies are needed to clarify the contribution of these clonal changes in these lesions.

O-040

Ret/PTC and p53 expression in normal, benign and malignant thyroid lesions

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Introduction This study aims to investigate the expression of ret/PTC and p53 in the local normal, benign and malignant thyroid lesions in order to shed light on the pathogenesis of papillary carcinoma and explain the high prevalence of this condition among the nodular hyperplasia (multinodular goiter) cases in the local population.
Materials and methods Archival blocks from 50 follicular adenomas, 66 nodular hyperplasia and 53 papillary carcinoma cases were retrieved from HUSM pathology department files. They were studied by immunohistochemistry for the presence of ret/PTC and p53 mutant protein. Normal tissues from 74 cases served as controls.
Results 5.4% normal thyroid tissue, 18% follicular adenomas, 22.7% nodular hyperplasia cases and 71.7% papillary carcinomas expressed ret/PTC mutation. Ret/PTC expression in papillary carcinoma was not associated with coexistence of nodular hyperplasia lesion. P53 is expressed by 17% of papillary carcinoma. No association was found between p53 expression of nodular hyperplasia with or without coexisting papillary carcinoma. p53, not ret/PTC was an excellent predictor of tumour lymph node metastasis and capsular invasion. It is also a significant prognosticator of survival outcome.
Conclusion Ret/PTC mutation is highly prevalent in local papillary carcinoma, indicating a significant role in the pathogenesis of this tumour; with no apparent role in tumour behaviour and survival outcome. P53 on the other hand appear to be a significant factor in the latter events. The two genes appear to act in two different pathways; the former being the initiator, and the later perpetuator of papillary carcinoma.

O-041

Vascular invasion in pleomorphic adenoma of salivary gland: a possible consequence of FNA biopsy

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Introduction Benign intravascular invasion in association with pleomorphic adenoma (PA) of salivary glands is uncommon. In none of the reported cases has it been associated with subsequent development of metastases. It may cause a diagnostic and therapeutic dilemma in salivary glands, which appears otherwise benign. No explanation of the etiopathogenesis has been offered in previously reported cases. Aim: We suggest that pre-operative procedures undertaken such as fine needle aspiration (FNA) of the salivary gland may cause iatrogenic seeding of tumour cells.
Materials and methods Two cases of PA of the parotid gland showed vascular invasion. Both presented with a palpable nodule in the parotid gland. One patient had two FNAs the other one prior to surgery. Both underwent superficial parotidectomy.
Results Histologically both tumours were PAS positive with little chondromyxoid component but both were rich in myoepithelial cells. Clusters of myoepithelial and epithelial cells were seen within muscular and thin walled blood vessels, both outside the capsule and within the tumour. In spite of genuine vascular invasion there was no cytological evidence of atypia or malignancy. Our speculation is that the previous FNAs may have caused spillage of PA cells within vessels giving an impression of a potentially malignant tumour.
Conclusion We recorded two cases of PA with associated vascular invasion. We speculate that preceding operative procedures such as FNA may be relevant to the pathogenesis.

O-042

Extra-cellular signal-regulated ERK-1/ERK-2 pathway activation in human salivary gland mucoepidermoid carcinoma: association to aggressive tumor behaviour and tumor cell proliferation

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Information on oncogenetic events in salivary gland mucoepidermoid carcinoma is so far limited. Activation of extracellular signal-regulated kinases ERK-1 and ERK-2 is strongly correlated to cancer. Using an antibody specific for phosphorylated (active) ERK-1/ERK-2, we examined human salivary gland mucoepidermoid carcinoma samples by immunohistochemistry. The comparison in paired tumor and normal tissue samples showed that phosphorylated ERK-1/ERK-2 expression was higher in tumor cells as compared to surrounding normal salivary parenchyma. Using a threshold below which > 95% of normal samples expressed phosphorylated ERK-1/ERK-2, we showed that ~39 % of mucoepidermoid carcinomas expressed high levels of phosphorylated ERK-1/ERK-2. Those tumours where ERK-1/ERK-2 pathway was activated had a more aggressive tumor behaviour as compared to the group where this pathway was inactive. The association of ERK-1/ERK-2 phosphorylation to a worse prognosis was independent of histological grade. ERK-1/ERK-2 phosphorylation was associated with an increased tumor cell proliferative index. There was no relationship between ERK-1/ERK-2 phosphorylation and HER-2/neu expression. In conclusion, ERK-1/ERK-2 pathway is active in salivary gland mucoepidermoid carcinoma and this activation is associated to a more aggressive tumor behaviour and a higher proliferative activity. These data suggest that deregulation of ERK-1/ERK-2 pathway contributes to mucoepidermoid carcinoma phenotype and, possibly, represent a target for new anticancer drugs.

O-043

Sinonasal acinic cell carcinoma: what do we know about this rare entity?

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Introduction Acinic cell carcinoma(ACC) is a low-grade salivary gland carcinoma with a predilection for the parotid gland. ACC originating in the sinonasal tract has rarely been reported. Four cases of ACC involving the sinonasal tract were identified in the Pathology files of M.D. Anderson Cancer Center from 1984 to 2002. The clinicopathologic information was reviewed.
Results There were 2 males and 2 females, aged 45 to 65 years, mean 58. The patients presented with unilateral nasal obstruction. All tumours were confined to the nasal cavity. Histologically, they were composed of round to ovoid cells with clear and/or granular basophilic cytoplasm and round, hyperchromatic, small, eccentrically located nuclei. The growth pattern was lobular, solid, and follicular. Histochemically, PAS diastase resistant cytoplasmic granules were demonstrated in all cases. Immunohistochemical stains were non-specific. Surgical resection was the treatment of choice. All patients are alive and well from 4 to 17 years.
Conclusion Sinonasal acinic cell carcinoma is a distinct low-grade carcinoma, which can be distinguished from any other neoplasm by light microscopy and histochemical stains. Pathologists should be aware of the occurrence of this salivary gland carcinoma in the sinonasal tract.

O-044

Aetiological correlates in Nigerian laryngeal cancers

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Introduction A recent review of cancer data from the cancer registry of the University College Hospital Ibadan, showed an increase in the relative ratio frequency of carcinoma of larynx (CaL) in the last decade. An increase of appearance was found especially among adult males. The aims of the study were to characterise the epidemiology of laryngeal carcinoma and to compared with nasopharyngeal carcinomas, both representing upper airway lesions with some common environmental aetiological associations. The objective was to assess, if possible, the role of purported aetiological correlates with CaL.
Materials and methods Data from the cancer registry of University College Hospital, Ibadan was reviewed for cases with carcinoma of larynx and nasopharyngeal carcinoma over a 15-year period, 1986-2000. Information of the age, sex and histological type of tumours were recorded and analysed. Clinical information regarding social habits was sought from the histology request cards. Data was subjected to statistical analysis.
Results A total of 17,132 cases of cancer occurred in that period (7,283 in males and 9,849 in females). There were 256 laryngeal cancers (1.5%) with mean ages of 53.8 years for males and 58.8 years for females and male: female ratio of 6: 1, while there were 332 cases of nasopharyngeal carcinoma (1.9%) with mean ages of 42.8 and 37.5 and a male: female ratio of 2.7: 1. Amongst male cancers carcinoma of larynx constituted 3% while NPC constituted 3.3% of cases, compared with 0.38% and 0.9% respectively in females. Quinquenial analysis showed no increase in the number of cases of CaL while cases of NPC increased consistently during this period.
Conclusions Prevalence of the male population in Nigerian CaL suggest that additional hormone receptor studies might help to elucidate aetiopathogenesis of laryngeal cancer.

O-045

Immunohistochemical characterisation of adenocarcinoma of the nose and paranasal sinuses in woodworkers

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Introduction Adenocarcinoma of the nose and paranasal sinuses is rare but has been shown to occur with increased frequency in workers exposed to wood dust. Histologically the tumours resemble adenocarcinoma of the GI tract and show the same spectrum of

histological types and differentiation. Therefore tumours at this site may present a diagnostic problem in distinguishing between primary and metastatic disease. Immunostaining has shown colonic adenocarcinomas to express cytokeratin CK20 and carcinoembryonic antigen (CEA) but not cytokeratin CK7. Lung and squamous carcinomas of the head and neck are usually CK7 positive and CK20 negative. This prompted assessment of the immunohistochemical expression of CK7, CK20 and CEA in a retrospective series of primary nasal adenocarcinomas. The aims of the study were to establish the pattern of expression of CK7 CK20 and CEA in primary sinonasal adenocarcinoma and to compare it with that seen in colonic adenocarcinoma.
Materials and methods 22 cases of primary sinonasal adenocarcinoma were available from archival files. These were reviewed and classified into 4 histological subtypes including papillary, tubular, mucinous and signet ring. Representative paraffin sections were immunostained for CK7, CK20 and CEA.
Results Normal respiratory epithelium included in the sections was positive for CK7 and negative for CK20 and CEA. 13/22 (59%)of tumours expressed CK7. All of the tumours expressed CK20 and all but one of the tumours expressed CEA, with 3 showing only focal positivity. There were 7 tumours designated mucinous or signet ring type only one of which expressed CK7.
Conclusion Primary sinonasal adenocarcinomas express CK20 and CEA. There is variable expression of CK7, which may help to distinguish between primary sinonasal adenocarcinoma, which are positive, and metastatic colonic adenocarcinoma of papillary and tubular subtype, which are negative. Poorly differentiated mucinous tumours are indistinguishable by immunohistochemistry alone.

O-046

Provisional extracellular matrix – indicator of oral squamous cell carcinoma (OSCC) progression and target for therapy

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Introduction Progression of OSCC embraces carcinoma cell invasion into the stroma but also includes invasion of blood vessels and stromal cells into the tumour. Both processes share a provisional matrix. The aims of the study were review of provisional matrix synthesis, distribution and co-localisation, demonstration of suitability of ED-B fibronectin for OSCC targeting and experimental tumour therapy.
Material and methods OSCC shock frozen tissue, cell lines; confocal microscopy; ISH or immunohistochemistry for ED-B fibronectin (ED-B Fn), high molecular weight tenascin (Tn-CL), and gamma2-chain of laminin-5 (Ln-gamma2), molecular engineered recombinant ED-B Fn antibody, experimental tumour therapy with fusion protein, recombinant ED-B Fn antibody - tissue factor, in mice.
Results OSCC progression is associated with ED-B Fn, Tn-CL and Ln-gamma2 synthesis and arrangement in strand-like matrix structures at the deep invasive OSCC edges. The strand-like organisation of ED-B Fn by stromal fibroblasts is the pre-condition for the formation of the multiprotein complexes. Additionally, ED-B Fn and Tn-CL are synthesised and deposited by/in newly formed blood vessels. At deep invasive OSCC edges Ln-gamma2 may be translocalised to newly formed blood vessels. The provisional ED-B Fn matrix is highly restricted in normal tissue. Fusion proteins, recombinant ED-B Fn antibody - tissue factor, are able to target mice tumours and to selectively induce thrombosis in tumour blood vessels.
Conclusions OSCC and angioneogenesis may use ED-B+Fn-Tn-CL matrix for invasion. The translocalisation of the invasion factor Ln-gamma2 to blood vessels of invasive front may contribute to angioinvasion. ED-D Fn is presented as ideal antigen for angioneogenesis-related therapy strategies.

O-047

On the application of the Ljubljana classification in the diagnosis of epithelial hyperplasic lesions of the oral mucosa. Pilot study

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The currently used systems of classification of the hyperplastic lesions of the oral mucosa are not strictly relevant to the picture and clinical data. In these systems a three step classification describing the lesions as 1st, 2nd and 3rd degree of epithelial changes is proposed. In our research we applied the specimens from 40 patients obtained from the Clinic of Periodontology Medical University of Lodz. Leukoplacic lesions with no or weak respond to the standard treatment were observed in most patients. Suspicion for carcinoma was another reason for biopsy. What we found microscopically was very useful for the description of the biopsed material, which was histologically classified according to the Ljubljana classification. Such terms as “simple hyperplasia”, as well as “abnormal hyperplasia” were strictly relevant to the histopathological picture and were by definition not related to malignant transformation. For some materials term “atypical hyperplasia-risky epithelium” was better accepted by clinicians than, for example, 3rd degree of dysplasia. In conclusion we found the Ljubljana classification to be a very useful tool for the diagnosis of epithelial hyperplastic lesions of oral mucosa, which is better than currently used other systems.

O-048

Gene expression profiling of squamous cell carcinomas of the head and neck - Fak-1 a potential predictive marker for loco-regional metastasis

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Introduction Despite numerous studies about possible molecular mechanisms leading to squamous cell carcinoma of the head and neck (HNSCC), specific markers indicating tumour stage or prognosis have not been elucidated yet. The new technology of microarray analysis allows the simultaneous examination of a broad spectrum of genes to assess a carcinoma-specific gene profile with the aim to reveal possible prognostic markers.
Methods We examined fresh frozen material of 25 patients with squamous cell carcinoma of the oropharynx (15 patients) and lar-

ynx (10 patients). A selective tumour cell sampling was achieved by laser capture microdissection. The expression profiles obtained by the cDNA PiquorTM Arrays (Immuno-Onco, Biozym) were compared with the pooled array data of normal mucosa taken at the resection margins. Controls were performed by immunohistochemical analyses, in-situ hybridisation on tissue microarrays and Western blot.

Results The analysis revealed 52 differentially expressed genes, 37 up- and 15 downregulated. As in previous studies, factors of proliferation, growth, apoptosis and differentiation were differentially expressed. Not reported yet is the differential regulation of several members of the caspases as well as the SOCS family. Upregulation of FAK-1 (focal adhesion kinase) was significantly correlated with the presence of lymph node metastasis.

Conclusion FAK-1 was elucidated as a potential predictive marker for lymph node metastases in HNSCC. Furthermore, the deregulation of signalling pathways controlled by SOCS and caspases, to date not associated with HNSCC, have been identified.

O-049

Correlation between cytogenetical and histopathological findings in 61 pleomorphic adenomas of the parotid gland

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Introduction Pleomorphic adenoma is the most common tumour of the parotid gland. Histomorphologic features and peculiarly tumour capsule play a major role in recurrences of parotid pleomorphic adenomas. Cytogenetically, pleomorphic adenomas are characterized by recurrent chromosome rearrangements. The aim of this study was to correlate cytogenetics and histopathology features in pleomorphic adenomas in order to define cytogenetical characteristics of tumours with high risk of recurrence.

Material and methods Histological and cytogenetical studies were performed in 61 patients with pleomorphic adenomas of the parotid gland treated in our institution during the period may 1992 – November 2002. Structural and numerical chromosomal abnormalities were: rearrangement of chromosome 12 (breakpoint at 12q13-15), rearrangement of chromosome 8 (breakpoint at 8q12), translocation between chromosome 3 and 8: t(3;8)(q21;q12), hyperdiploidy.

Results The studied population was distributed into 3 histological subtypes according to Seifert classification. Hypocellular (stroma rich) pleomorphic adenoma was reported in 61%, hypercellular pleomorphic adenoma was reported in 26% and “classic” in 13%. Thirty patients (49%) had normal karyotype while 31 (51%) had clonal chromosomal abnormalities. Chromosome 12 rearrangement tumours and hyperdiploid ones tend to be hypocellular. Tumours showing chromosome 8 abnormality tend to be hypercellular.

Conclusions As recurrences most likely appear in hypocellular tumours, patients with hyperdiploidy and chromosome 12 abnormalities could have a greater risk of recurrence because of their histomorphological characteristics. Because of the lack of patients, not sufficient statistical significance was demonstrated.

O-050

Histomorphological study of pleomorphic adenoma: Preliminary study for long term follow-up of recurrence

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Introduction Pleomorphic adenoma is the most common tumour of the parotid gland. The main issue of pleomorphic adenoma surgery is postoperative recurrence. Histomorphologic features and peculiarly tumour capsule play a major role in recurrences of parotid pleomorphic adenomas. The aim of this retrospective study was to define histomorphological characteristics of pleomorphic adenoma for better understanding of recurrence processes. This work corresponds to the preliminary prospective study of a long term follow-up for detection of recurrence.

Material and methods Histological study was performed after reviewing of slides originating from 100 patients with pleomorphic adenomas of the parotid gland. These randomly selected patients underwent parotid surgery in our institution during the period may 1992 – November 2002.

Results The studied population was distributed into 3 histological subtypes according to Seifert classification for better analysis. Hypocellular (stroma rich) pleomorphic adenoma was reported in 56%, hypercellular pleomorphic adenoma was reported in 29% and “classic” in 15%. Thinness of capsule was significantly related to hypocellularity. Pseudopodias and satellite nodules were reported in 72% of this series.

Conclusions Due to capsular characteristics, surgical exerecise should avoid dissection in the vicinity of the tumour to minimize the risk of surgical induced recurrence. According to these findings, enucleation surgery on a pleomorphic adenoma should not be performed anymore. These tumours should now be followed up in order to detect and define risk factors of recurrence.

O-051

Trend of nasopharyngeal cancer at the Ibadan Cancer Registry (1981-2000)

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Introduction Only two comprehnsive reports on nasopharyngeal carcinoma (NPC) are available from this large centre covering the periods from 1961 to1966, and from 1966 to 1980.

Objective This study describes the trend and pattern of nasopharyngeal cancer at the Ibadan Cancer Registry and shows that there have been some significant changes.

Method This is a retrospective review of all histologically confirmed cases of nasopharyngeal cancer accumulated in the Ibadan Cancer Registry from 1981-2000.

Results 223 cases were recorded; 156(70%) males and 67(30%) females with a male: female ratio of 2.3:1. It constitutes 2% of all cancers, 1% of female and 3.5% of male cancers. There is a steady

increase in the incidence of NPC over the last here decades. Overall, the mean age was 41.1 years (age range 10 to 81 years). The females had a mean age of 36.1years (age range 11-80 years) while the mean age for the males was 43.2 years (range 10-81 years). The peak age group of incidence for the females was 20-29 while in the males it was the 50-59 age group with an almost an equal incidence in the preceding three decades. The ratio of Regaud to Schmincke type cancer reversed with increasing age amongst the females with the Schmincke type being more common in the first decade but this was not reproduced in the males.

Conclusions There is a steady rise in the incidence of nasopharyngeal cancer over the last three decades. The two main histological types showed differential variation between the sexes suggesting a biological effect in manifestation of this disease. There is a need for health education and increased physician awareness at all levels of health care to stem this trend and especially to detect a role of Epstein Barr virus infection in aetiopathogenesis of the nasopharyngeal cancer.

O-052

Lactate dehydrogenase (LDH) in peripheral blood lymphocytes (PBL) of patients with solid head and neck tumours

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Lactate dehydrogenase(LDH)is intracellular enzyme, which take part in process of anaerobic glycolyse. It is located in all cells, but in higher amount in the stromal cells. LDH activity increased in the malignant cells, as well as in the peripheral blood lymphocytes (PBL) of patients suffered from head and neck tumours. The authors present results of their investigations in activity of intracellular LDH in PBL, spontaneous LDH releasing from PBL and correlation between spontaneous releasing of LDH and increasing of intracellular level of LDH in patients with solid head and neck tumours. Spontaneous releasing of LDH in all the patients with solid head and neck tumours were 2 to 4 times higher then in the health controlling group. There are correlation between clinical stage of the tumours and spontaneous releasing of LDH. The authors concluded that measuring of level of spontaneous released LDH from PBL, can prove the presence of head and neck tumours in the very early stage.

O-053

Protein expression profiles of cell cycle and apoptosis factors in head and neck cancer using tissue microarrays

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Introduction Squamous cell carcinoma (SCC) constitutes more than 90% of the malignancies from the head and neck. Thereafter, the survival of patients in stage III-IV is between 33%-11% at 5 years. As most stromal types, head and neck squamous cell carcinomas (HNSCC) harbour deregulation in the expression levels of many proteins that participate in critical biological pathways leading to cancer development. The aim of the study was to evaluate the correlation between several molecules implicated in cell cycle and apoptosis in an attempt to define their importance as prognostic indicators in HNSCC.

Material and methods 110 HNSCC specimens from stage III-IV patients were collected prior to chemotherapy. We constructed a tissue-microarray with paraffin-embedded tumours to analyse, by inmunohistochemistry, the expression levels of 18 proteins implicated in cell cycle arrest and apoptosis (P53, cyclin-D1, cyclin-A, RB, HDM2, topoisomerase, chk2, cdc2, Survivin, BCL-2, CDK-2, CDK-6, cyclin D3, cyclin E, cyclin B1, P21, P27 and P16).

Results Alterations in the expression levels of proteins that participate in the G1/S checkpoint were frequent. RB and P16 proteins were absent in 15% and 15% of the tumours, respectively, whereas high expression of cyclin D1, CDK-4 and CDK-2 was observed in 42%, 8% and 13% of the cases, respectively. On the other hand, des-regulation of markers implicated in the apoptosis were also evident as shown by over-expression of BCL-2 protein in 23% of the tumours. This last observation seems to be related to bad prognosis.

Conclusions Definitive conclusions from our results concerning expression levels of each marker isolately, according to tumour location and in relation to survival are still being evaluated.

O-054

p53 and MIB1 expression in basaloid squamous cell carcinoma of the larynx inversely correlates with p27 expression

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Introduction Cell cycle regulators play a crucial role in cancer and their functional alteration may play an important role in the clinical progression of basaloid squamous cell carcinoma (BSCC) of the larynx. BSCC is a biologically aggressive tumour with high proliferative activity and a propensity to destructive local growth and early regional and distant metastases.

Methods Herein we report nine cases of BSCC of the larynx, selected from January 1987 to January 1999 by a review of all laryngo-hypopharyngeal cancers collected from our files. Clinical follow-up was obtained for all patients. The potential prognostic value of DNA ploidy status and immunohistochemical expression of Ki-67, p53, and p27 kip1 were analysed.

Results Histologically, all tumours were composed of squamous cell carcinoma with an intimately associated basaloid component, showing an irregular lobular pattern of growth. p53 expression was demonstrated in 8 cases, while Ki-67 (MIB-1) resulted positive in 7 of them. The positivity range scored from moderate (++ = <60%) to intense (+++ = >60%). p27 was expressed in 5 cases, two of them showing a moderate staining (++ = >60%), the others showing a weak staining (+ = <30%). It was either absent or weakly expressed in 7 cases, 5 of which with both p53 and Ki67 overexpression accompanied by a very poor outcome. Of the three alive

patients, two showed an inverse correlation between p53 and Ki-67 expression and p27 expression.
Conclusion An inverse correlation between p27 and Ki-67 and p27 and p53 was demonstrated in 7 cases. Image analysis of DNAc showed aneuploidy in all cases. The present study shows that over-expression of p53, and Ki-67, together with a down-regulation of p27 kip1 correlates with biological aggressiveness and consequent shortened survival in BSCC of the larynx. The DNA status doesn't correlate with any of the analysed parameters since all tumours in our series displayed an aneuploid pattern.

O-055

Human telomerase catalytic subunit (hTERT) gene re-expression is an early event in oral carcinogenesis

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Introduction The exact role and timing of telomerase re-activation, a key enzyme implicated in cellular immortalisation and transformation in the multistep process of oral carcinogenesis is still unknown. The aims of the study were to determine whether a.) quantitative differences exist in the levels of telomerase catalytic subunit (hTERT) mRNA expression among different grades of oral epithelial abnormalities classified according to the Ljubljana classification; b.) telomerase re-activation is an important, most probably early event in oral carcinogenesis; and c.) to analyse whether the relative quantity of hTERT mRNA can be used as a molecular biomarker in the early detection of precancerous lesions.
Material and methods The relative quantity of hTERT mRNA was analysed in 45 frozen oral epithelia representing different morphological stages of oral carcinogenesis and in 37 oral squamous cell carcinomas (OC) by using a commercially available LightCycler Telo TAGGG hTERT Quantification kit (Roche Diagnostics, Branchburg, NY).
Results hTERT mRNA was not detected in normal and reactive hyperplastic oral epithelia, but was present in 43% of atypical hyperplasias (precancerous lesion), 60% of intraepithelial carcinomas and 68% of OCs. Statistical analysis revealed two groups of oral epithelial changes with significant differences in the levels of hTERT mRNA expression: 1. normal and reactive hyperplastic oral epithelium (simple and abnormal hyperplasia), and 2. atypical hyperplasia, intraepithelial and OCs.
Conclusions Telomerase re-activation is an early event in oral carcinogenesis, detectable already at the stage of precancerous oral epithelial changes. We believe that measurement of hTERT mRNA in oral tissue specimens may increase the sensitivity of standard microscopical analysis in early detection of those epithelial abnormalities associated with increased risk of progression towards OC.

O-056

Survivin is a negative prognostic marker in laryngeal squamous cell carcinoma and is associated with p53 accumulation

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Introduction Survivin, a novel inhibitor of apoptosis, is expressed in cancer cells but not in normal tissues. The cancer-specific expression of survivin, coupled with its importance in inhibiting cell death and regulating cell division, makes it a useful diagnostic marker of cancer. Since both over-expression of survivin and loss of wild-type p53 are associated with cancer development, we investigated a possible connection between these two events in laryngeal squamous cell carcinoma (LSCC). The aim of our study was also to evaluate the prognostic value of survivin expression and its relation to apoptotic activity.
Methods The expression of survivin, p53 and apoptotic index were evaluated by immunohistochemistry on biopsy specimens of 68 patients with LSCC. An antibody against cleaved caspase 3 was used to detect apoptotic cells.
Results Overall, 98.5% of LSCC were positive for survivin. The proportion of survivin positive tumour cells varied from 8.2% to 100%, its localisation was nuclear and/or cytoplasmatic. The number of survivin positive cells was significantly higher in the p53 positive group. A positive correlation between survivin expression and the apoptotic index was found. In multivariate analysis, high level of survivin expression was an independent adverse prognostic factor in LSCC.
Conclusions Survivin is expressed in a varying proportion of tumour cells in virtually all cases of LSCC. A high level of its expression predicts poor survival. Loos of wild-type p53 could be responsible for survivin upregulation. The importance of survivin in inhibiting apoptosis remains unclear, as its expression is related to a higher apoptotic activity. Understanding the role of survivin in LSCC development and progression could give rise to improved therapeutic strategies.

O-057

Morphology, p53, Bcl2 and Bax spectrum of 125 surgically removed parathyroid lesions

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Altogether 125 patients were operated on at the Department of Transplantation and Surgery of the Semmelweis University in the past four years, because of clinical symptoms of hyperparathyroidism. Chronically impaired renal function was found in 60 cases. The removed parathyroid glands showed histologically hyperplasia in 62, adenoma in 60 and carcinoma in 3 cases. The oxyphil/chief cell ratio and occasional mitotic and apoptotic figures were determined. The oxyphil component predominated both the hyperplastic and tumorous lesions. Apoptosis and mitosis were rarely seen in hyperplasias (0-2%) more frequently in adenomas (2-5%) whereas in carcinomas 3-5% of the tumor cells showed signs of mitosis and 3-5% were apoptotic. Immunohistochemical detection of p53, bcl2 and bax revealed diversity between the various lesions. Cytoplasmic p53 positivity could be observed in the majority of adenomas and in some of the oxyphil cells of the hyperplasias. The carcinomas and about one sixth of the adenomas showed nuclear p53 positivity. Bcl2 was detected in the cytoplasm of the tumor cells of adenomas and in lower extent in the oxyphil cells of hyperplasias. Co-localistion of bcl2 and bax was found randomly in all types of lesions. No significant differences in the p53, bcl2 and bax

spectrum were found regarding the primary and secondary (i.e. renal failure) parathyroid alterations. The very low incidence of carcinomas, the low mitotic and apoptotic ratio in adenomas and hyperplasias point to the potent antiproliferative defense mechanism in the parathyroid cell-population. This may be also reflected in the cytoplasmic co-localisation of various gene products which regulate cell death and cell proliferation.

O-058

Factors influencing the presence of sentinel lymph node metastasis in patients with breast carcinoma

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Introduction Primary tumor phenotypic characteristics influencing development of the SLN metastasis are not known. Loss of SYK tyrosine kinase expression and over-expression of CXCR4 chemokine receptor were recently described as important characteristics in establishment of mammary carcinoma metastasis in the lymph nodes in experimental models.
Material and methods 173 mastectomies with sentinel lymph node dissections from 172 female patients were included in the study. Radiolabeled sulfur colloid technique with or without blue dye was used to identify the SLN(s). Entirely submitted SLNs were formalin fixed, and stained with H&E for evaluation. Expression of SYK and CXCR4 was evaluated with standard immunohistochemical (IHC) procedures.
Results 45 patients had positive SLN as examined with H&E alone (26%). Cytokeratin IHC identified two more cases, yielding an overall total of 47 positive patient (27.2%). Both of these cases were invasive lobular carcinomas. 100 SLN biopsies were followed by completion axillary dissection. Predictive value of negative test was 0.98. No in-situ or pT1a carcinomas were associated with SLN metastasis. pT1b was associated with 17% SLN positivity, pT1c with 20%, pT2 with 53% and pT3 with 67% SLN positivity. No difference in SYK expression between SLN positive and negative cases was observed (1.9±0.8 vs. 2.0±0.72), and no difference in CXCR4 expression between these two groups was observed (1.13±0.68 vs. 1.18±0.56)
Conclusions False negative SLN was rare (2%), while overall predictive value was high, which supports the value of this treatment in low stage breast carcinomas. The frequency of SLN positivity is directly proportional to the size of the primary tumor. No evidence to support the role of SYK and CXCR4 expression in establishment of lymph node metastasis was found in this group of patients.

O-059

Prognostic importance of occult metastases in early breast cancer

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Introduction At present the significance of occult axillary lymph node metastasis (OM) in early breast cancer patients is not known. The aim of the study was to evaluate the prognostic role of OM in stage I breast cancer.
Materials and methods We studied 96 patients with T1 breast carcinoma who underwent axillary dissection and originally had negative lymph nodes by routine microscopic examination. 48 patients who developed distant metastases within 15 years after surgery (M group) were compared to 48 age-matched patients who were disease free for 15 years (NM group). We examined 1539 lymph nodes by three deeper H&E levels and one section stained with immunohistochemistry for keratin.
Results We detected OM in 21/96 patients. All 21 had micro-metastases (<2.0 mm). OM was identified in 16 patients (34%) in the M group and in 5 (11%) in the NM group (P=0.007). Micrometastases larger than 0.2 mm were 10x more common in the M group (10/48 vs. 1/48, P=0.004). Tumors in the M group were significantly more often of higher grade, had a higher mitotic index (MI) and showed lymphovascular invasion. The two groups did not differ in size, histologic type, estrogen receptor status or type of treatment. In multiple logistic regression, only high MI and presence of micrometastases larger than 0.2 mm showed an independent significant correlation with the occurrence of distant metastases.
Conclusion Axillary micrometastases are associated with occurrence of distant metastases and are important prognostic factor in T1 breast cancer patients. This was not significant for OM smaller than 0.2 mm.

O-060

Immunohistochemical study of the pathological response of neoadjuvant chemotherapy and radiotherapy in invasive breast carcinoma

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Introduction Current therapeutic protocols stratify patients to regimens of chemotherapy or associated chemo- and radiotherapy in locally advanced breast cancer. The aims of this study were to compare immunohistochemical breast evaluation after neoadjuvant chemotherapy and / or radiotherapy and to analyse post systemic and regional irradiation therapy prognostic markers, usefull for further treatment procedures.
Materials and methods 120 locally advanced breast cancer patients with preoperative chemotherapy, or combined chemo- and radiotherapy were semiquantitatively immunohistochemically assessed for the expression of ER, PR, pS2, c-erbB2 (DAKO – HercepTest), PCNA and CD34 (DAKO, LSAB2).
Discussion and conclusions Systemic therapy altered tumor cells retain immunohistochemical reactivity; ER, PR positive cases correlate with important decrease in tumor cellularity, difficulties in grading assessment, reduced rate of proliferation (20-25%). Single chemotherapy in negative ER, PR tumours correlates better with c-erbB2 expression ($p<0.02$). Complete histopathologic response for 12% cases (no residual tumor cells) was found after chemo and radiotherapy. The patients who express besides G3 tumor grading, an immunohistochemical pattern of negative ER, PR, 40-65% proliferating rate, could be included in a chemo and radiotherapy non responding group.

O-061

Reliability of HER-2/neu immunohistochemistry in Austria

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Introduction Practice and accuracy of immunohistochemistry in different laboratories is known to vary highly. Since the introduction of targeted therapy in breast cancer patients reliability of immunohistochemistry has become crucial. The aim of the study was to assess reliability of HER-2/neu immunohistochemistry in Austrian pathology laboratories and to provide data for benchmarking with respect to quality improvement.
Material and methods The trial consisted of six cases of invasive ductal breast carcinoma. Presence / absence of gene amplification was determined by FISH to be used as a gold standard. Laboratories were asked to stain and assess slides using their routine immunohisto-chemical staining protocol. Participation was voluntary.
Results Participation rate was 75% of all pathology departments in public hospitals. Overall sensitivity and specificity was 96 %. Positive and negative predictive value were 92% and 98%, respectively. False positive and false negative results occurred in either 4%. Analysis of the laboratory method revealed that all false negative cases (9%) were due to staining methods other than Hercep-Test. False positive results occurred in 3,8 % using Hercep-Test and 4,5% using other staining protocols. Thus sensitivity was lower in staining protocols other than Hercep-Test (90% versus 100%). Specificity was 96% for Hercep-Test and 95% for other protocols.
Conclusion Participation rate was high.
Results were highly reliable when using Hercep-Test. Using other staining protocols the main problem were false negative immunohistochemical results, neglecting a possible therapy option for these patients.

O-062

Immunohistochemically defined myoepithelial cells in the diagnosis of benign and malignant papillary breast lesions

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Introduction The presence or absence of myoepithelial (ME) cells has been considered as an important feature in the differential diagnosis of papillary breast lesions. The aim of this study was to evaluate the usefulness of monoclonal antibodies: alpha-actin (smooth muscle actin) and S-100 protein, which are known as good markers of ME cells in breast tissue.
Material and methods The distribution of ME cells in formalin-fixed paraffin-embedded tissue sections of 15 intraductal papillomas and 10 papillary carcinomas were immunohistochemicaly stained by ABC method.
Results Alpha-actin reacted strongly and consistently with the ME cells in all papillomas. In papillary carcinomas there were no alpha-actin reactive ME cells. Rare isolated ME cells were present in two cases, but they were inconspicuous and these reactions were also considered negative. The ME cells failed to express S-100 protein in four papillomas, while majority were weak positive. All the papillary carcinomas were S-100 protein negative.
Conclusions Consistency and uniformity of ME cells reaction is important because benign and malignant papillary lesions often coexist in the same tissue section. Although vascular smooth muscle in the papillary fronds and stromal myofibroblasts may mimic ME cells, the number of alpha-actin immunoreactive cells is much smaller and their distribution more irregular. S-100 protein has broad spectrum reactivity and it is of little value in the differential diagnosis of papillary breast lesions. Alpha-actin is more specific marker than S-100 protein for recognition of ME cells in breast tissue.

O-063

Prognostic significance of regional TIMP-2 mRNA expression in invasive breast carcinomas

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Introduction Despite wide agreement about TIMP-2 involvement in the progression of malignancy, there is uncertainty as to its precise role in the biology of cancer development. Aims: In order to elucidate the clinical significance of TIMP-2 in invasive breast cancer, TIMP-2 mRNA expression was investigated in 118 tissue samples in relation with clinicopathological parameters, overall and disease-free patients' survival, as well as c-erbB-2 and MMP-11 protein expression.
Materials and methods TIMP-2 mRNA transcripts were detected using non-radioisotopic mRNA in situ hybridization with a digoxigenin-labeled RNA probe. Immunohistochemistry (ABC-HRP) was performed for the detection of c-erbB-2 and MMP-11 proteins. Statistical analysis was univariate and multivariate.
Results TIMP-2 mRNA was detected in fibroblasts located within the tumor area in 57.6% of cases and at the tumor margin in 43% of cases. Intratumoral TIMP-2 mRNA expression demonstrated no significant correlations with the co-estimated parameters. On the other hand, TIMP-2 mRNA expression at the marginal portion associated significantly with the presence of lymph node metastases ($p=0.036$), c-erbB-2 overexpression ($p=0.022$) and MMP-11 (ST3) protein expression ($p=0.012$). In univariate analysis, TIMP-2 mRNA expression at the tumor margin, associated significantly with decreased recurrence-free survival ($p=0.019$). In multivariate analysis, TIMP-2 mRNA localization at the tumor margin, demonstrated a significant independent correlation with shortened overall patients' survival ($p=0.016$).
Conclusion TIMP-2 mRNA detection at the marginal portion of invasive breast carcinomas associates with lymph node metastases, c-erbB-2 overexpression and MMP-11 protein expression. Moreover, TIMP-2 mRNA detection at the marginal portion of tumors is associated with poor patients' outcome and may be a useful prognostic factor in invasive breast cancer.

O-064

Population screening for immunohistochemically evaluated HER2 status of breast carcinomas in Poland

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Introduction Aim of the study was so-called population screening for HER2 status of breast carcinomas in Poland and standardization of the immunohistochemical technique and reporting results**.**
Material and methods Study was done in 16 medical centers in Poland and during the period of 10 months 5088 breast cancers were diagnosed and evaluated. Histologic type and grade as well as the menopausal status were recorded. Number of patients evaluated in particular centers ranges from 85 to 1025. Immunohistochemical status of HER2 receptor was evaluated using Herceptest (DAKO) which is standardized kit for immunohistochemiacal evaluation. Four-tier classification proposed by Dako (0, +1,+2,+3) were used for describing strengths of the stain.
Results Among 5088 cases of breast carcinomas strongly positive results +3 was found in 1001 cases (19.7 %), +2 in 776 cases (15.3%), weakly positive in 1311 (25.8 %) and negative in 2000 cases (39.3%). There were no difference in the percentage of each group between premenopausal and postmenopausal women. Considering histopathologic type of tumor, ductal carcinoma (2993 patients) was HER2 positive in 21% (+3) and 16.7% (+2), while lobular carcinoma (541 patients) was HER2 positive in 7.6% (+3) and 13.5% (+2). Strong correlation was found between histologic grade of ductal carcinoma and HER2 expression, e.g. in G1 ductal carcinoma 3.3% were strongly positive (+3) while in G3 ductal carcinoma 28.1 % were strongly positive (+3).The striking feature in the results were differences in percentage of each group among the centers participating in the program. Percentage of HER2 +2 cases ranges from 4.1 to 27.1% and HER2+3 from 10.6 to 30.3%. Distribution of particular staining reaction were similar among the centers with largest number of cases
Conclusion Percentage HER2 positive breast cancer cases in our material corresponds with data from literature. To evaluate properly immunohistochemical stain certain experience is required.
*Representatives of 16 centers participating in the program. HER2 - population screening project was supported by Roche -Polska

O-065

Accuracy of the intraoperative sentinel lymph node examination by imprint cytology in breast cancer patients

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Introduction The utilization of lymphatic mapping for breast carcinoma has made intraoperative evaluation of sentinel lymph node (SLN) attractive, because axillary lymph node dissection can be performed during the initial surgery if SLN is positive. The aim of our study was to evaluate the accuracy of intraoperative SLN examination by imprint cytology (IC).
Material and methods 459 SLNs from 225 women were examined. For intraoperative cytological examination, touch imprints from bisected SLNs were prepared, air dried and stained with quick staining method (Hemacolor-Merck). SLNs were than formalin-fixed and embedded in paraffin. Histological sections were evaluated with 5 H&E stained levels and cytokeratin immunohistochemistry (CK-IHC).
Results Histological examination of the SLN revealed 84 (37,3%) cases with metastatic deposits: 41 (18.2%) macrometastases, 32 (14.2%) micrometastases and 11 (4.9%) cases with isolated tumor cells or clusters (ITC) detected by immunohistochemistry. Sensitivity and specificity of intraoperative IC was 41% and 99%, respectively. IC was significantly more sensitive for detection of macrometastasis: they were detected in 34 out of 41 cases (83%). IC generally failed to detect micrometastases or ITC: only one case of micrometatasis was cytologically suspicious for malignancy. Out of 141 histologically negative SLNs, one IC diagnosis was false positive.
Conclusions IC is a simple and quick method of intraoperative screening for the presence of SLN macrometastases in patients with breast cancer. If SLN micrometastases and ITC are used to determine the need for further lymphadenectomy, more sensitive intraoperative methods, such as rapid CK-IHC on touch preparations, will be needed to avoid second operation.

O-066

Comparison of fine-needle aspiration cytology and core biopsies of breast lesions

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Introduction Fine-needle aspiration cytology (FNA) is an established, highly accurate method for diagnosing breast lesions. However, in recent years there has been increased use of core biopsy (CB) as a diagnostic alternative. This study aimed at comparing the value of FNA and CB in palpable and non palpable breast lesions.
Materials and methods The material consisted of 4367 FNA samples performed in our institution between 1999 and 2001. Correspoding histology results from 1275 lesions including 463 CB were available for comparison. Quality assessment parameters were calculated using metodology detailed in the National Health Service Breast Screening Programme guidelines.
Results High specificity (100%) and sensitivity (92%) and low inadequacy rates were found for CB. FNA showed high sensitivity (87%). Low specificity (49%) for FNA due to inclusion of many cell-poor lesions was particularly seen in the submitted material sampled by physicians lacking experience with FNA procedures.
Conclusion CB may be an alternative method for preoperative diagnosis when experienced cytopathologists are not available. CB is superior to FNA in fibrotic and collagenous lesions such as lobular carcinoma and radial scar. FNA is most accurate when immediate assessment by cytopathologist is performed for evaluation of material adequacy so that additional aspirations can be performed if necessary.

O-067

Atypical ductal hyperplasia (ADH): practical relevance in diagnostics and therapy

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Introduction In contrast to ductal hyperplasias, ADH belongs to intraductal neoplasias. ADH can be distinguished from non-high grade DCIS (among other criteria) by its size. Therefore, a question that is asked quite frequently is whether such small findings can be removed with sufficient security by minimal invasive biopsy (MIB). We prospectively investigated: 1. relative frequency of ADH without associated intraductal or invasive carcinoma 2. relevance of such small findings in MIB specimens.
Materials and methods Between 1998 and 2002, 2825 breast-lesions could be clarified by means of MIB in the interdisciplinary team. Excisions were carried out in all invasive carcinomas and all DCIS as well as in all cases with minimal parts of intraductal non-high grade neoplasias (with the differential diagnosis ADH vs. DCIS). Additional ADHs were diagnosed in excision specimens as incidental findings associated with other targets (except for DCIS and invasive carcinomas). The MIB- and operation specimens have been examined prospectively by two team pathologists according to the criteria of Page and Tavassoli.
Results We diagnosed altogether 25 ADHs and 138 DCIS. The proportion of ADH in all operated intraductal neoplasias (ADH+DCIS) was 1.56% (25/163). Findings with the differential diagnosis of ADH vs. DCIS could be found in 25 cases of 2825 MIBs (0,88%).The following excision specimens showed in 16 of 25 cases (64%) non-high grade DCIS and in 9 of 25 cases ADH (36%). The other 16 ADH were coincidental findings in excision specimen of benign lesions.
Conclusions ADH is a very rare finding. In ADH-like findings in MIB specimens even specialised breast-pathologists cannot exclude a non-high grade DCIS with sufficient security when applying strictly the most common morphological definitions. The high rate of 64% of DCIS in the specimen of the following operation highlights the indication for clarifying by excision (according to the European Guidelines for Quality Assurance in Mammography Screening).

O-068

Multifocal stromal invasion in microinvasive squamous cell carcinoma of the cervix - how to measure and stage these lesions

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Objectives About 12% of microinvasive squamous cell carcinomas of the cervix have more than one invasive focus at time they first break through into the cervical stroma. It is unclear how the lateral extent of lesions with multiple invasive foci should be measured to distinguish FIGO stage 1A1 and 1A2 carcinomas from clinically occult stage IB disease.
Methods and results We believe that three presentations of multiple invasive foci should be distinguished. Type I shows plump and coherent infiltration of the cervical stroma at different sites in one ore more step-serial sections. They originate from the squamous epithelium outside the transformation zone or from metaplastic cervical gland epithelium. All foci are contiguous with the epithelium. In this case, the largest lateral spread of each individual invasive focus should be measured. These measurements are added up to determine the total of horizontal spread. The normal tissue separating the different early invasive foci is not measured. In type II the origin of invasion cannot be seen in all early invasive foci but the greatest lateral extension can be seen in one of the step-serial sections. Lateral extension is defined as the maximum distance from one most lateral point of the border between the invasion and the adjacent tissue to the other. The lateral extension of type II includes the normal tissue separating the early invasive foci. Type III shows the same pattern as type II, but the maximum horizontal spread cannot be measured in a single step-serial section. In such lesions the width needs to be measured across all the step-serial sections involved and the second dimension must be calculated by knowing the distances between the sections.
Conclusions These growth pattern of stromal invasion should be considered when measuring the width of early invasive squamous cell carcinomas with multiple invasive foci. Lesions with multiple invasive foci taken together measuring more than 7 mm in lateral extension should be classified as stage IB.

O-069

Expression of cyclooxygenase-2 (COX-2) in gynecologic tumors

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Introduction Cyclooxygenases regulate the production of prostaglandins and play a role in tumor development and progression. The aim of this study was to investigate the prognostic impact of expression of the cyclooxygenase isoforms, COX-1 and COX-2 in 221 primary breast carcinomas as well as in 86 primary ovarian carcinomas.
Materials and methods COX isoform expression was determined by immunohistochemistry.
Results Expression of COX-2 was detected in 42% of 86 ovarian carcinomas and was associated with reduced overall survival in univariate analysis ($p=0.04$) as well as multivariate analysis (0.004). There was no correlation with other tumor parameters such as grade, stage or type. In contrast, COX-1 was no prognostic parameter in ovarian carcinoma. Expression of COX-2 was detected in 36% of breast carcinomas and was significantly associated with several clinicopathological parameters, such as positive nodal status ($p<0.0005$), larger tumor size ($p<0.0005$), poor differentiation ($p<0.0005$), vascular invasion ($p=0.003$) and negative estrogen receptor status ($p=0.04$). In univariate survival analysis a significant association between elevated COX-2 expression and decreased disease-free survival ($p=0.0007$) as well as decreased overall survival ($p=0.02$) was observed. In multivariate analysis expression of COX-2 was of borderline significance for disease-free survival (RR(95%CI) 1.90 (1.00-3.59)).
Conclusions Our data suggest that increased expression of COX-2 may play a role in progression of primary breast carcinomas as well as ovarian carcinoma. It remains to be investigated if treatment with selective inhibitors of COX-2 may be an additional therapeutic option for patients with gynecological tumors.

O-070

Simple endometrial hyperplasia: Bcl-2, HLDF expression and clinical behavior

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The endometrial hyperplastic processes is characterized by major variety of clinical outcomes. The main feature of "spontaneous endometrial hyperplasia" is complete normalization after total curettage (Lindahl B., Willen R. 1994); there is also proof of recurring hyperplasia. The purpose of present research was to carry out clinical and morphological correlations between various types of endometrial hyperplasia and expression of pro- (HLDF) and antiapoptotic (BCL-2) proteins. Human leukemia cell differentiation factor (HLDF) with molecular mass 8.2 kd, having DNA/RNA hydrolyzing activity, was originally allocated from HL-60 cell line of promyelocyte leukemia medium, processed by retinoid acid or C2–ceramid (Kostanyan I.A. et al., 1994). Previously we have shown that HLDF is a useful tool for apoptosis visualization in prostate (Babichenko I.I. et al., 2001). We performed analysis of 139 patients with histologically documented simple endometrial hyperplasia without atypia: 74 women (53 %) had spontaneous recovery after total curettage, in 42 (30 %) patients endometrium recovered after gestagen treatment and in 23 (17 %) cases persistent recurring hyperplasic process was observed during 2-5 years. Immunohistochemical research showed that in samples of recurring hyperplasia HLDF was expressed in cytoplasm of glandular epithelium, while in sections with spontaneous or after-treatment recovery, HLDF was revealed as a thin line on the surface of glandular cells. BCL-2 protein was found only in the cytoplasm of glandular cells in all investigated groups. Thus expression of the HLDF factor in cytoplasm of glandular cells in endometrium allows revealing the disturbance of apoptotic processes in cells and predicting the subsequent recurrence of simple hyperplasia.

O-071

Surface epithelial changes in endometrial adenocarcinoma

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Introduction Epithelial changes on the surface of endometrial adenocarcinoma (EAC) show greater maturation and better differentiation than the underlying adenocarcinoma. These changes are relatively common and can be misdiagnosed as benign surface changes on curettings, including endometrial metaplasias (EM), papillary syncytial change (PSC), or microglandular hyperplasia (MGH). The aim of this study was to investigate surface epithelial changes in EAC.
Material and methods 122 cases with EAC (96 endometrioid, 10 serous, 4 clear cell and 12 mixed types) were studied.
Results 48 (39%) of the EAC showed surface epithelial changes: four with microglandular pattern simulating MGH; 16 with syncytial aggregates of eosinophilic cell, frequently with papillary or squamoid appearance, simulating EM or PSC; and 28 with mixed patterns. Surface epithelial change often demonstrated focal conjunction with a frankly malignant gland or a fibrovascular core and ranged from rare foci to diffuse surface involvement. Cytologic atypia was less pronounced than that of the underlying EAC and varied from mild to moderate. Mitoses were very rare. In some scanty curetting specimens, distinguishing these surface epithelial changes from benign changes was difficult, as was a diagnosis of EAC.
Conclusion In postmenopausal women with curettings showing features of EM, MGH, or PSC, caution should be exercised in making that diagnosis, particularly PSC, in the absence of evidence of benign shedding, and any associated cytologic atypicality should raise the possibility of the underlying EAC.

O-072

Pap smear cytology, visual inspection (VIA), cervicography, colposcopy and HPV testing by hybrid capture II as optional screening tools in Latin America*

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Objectives The performance of conventional diagnostic techniques (PAP smear cytology, screening colposcopy) is compared with 1) two optional screening tools (VIA, cervicography) and with 2) Hybrid Capture II test for Human papillomavirus (HPV), from a) conventional samples and b) using self-sampling, in women at different risk for cervical cancer in Brazil and Argentina.
Study design In this cross-sectional- and prospective cohort study, consecutive women attending the clinics during the first 12 months (expected n=12.000) are screened using the six (quality controlled) diagnostic tools, and women with biopsy-confirmed low-grade CIN are enrolled in the follow-up cohort. Data on the risk factors are collected, and cervical biopsies are analysed for biological prognostic markers using molecular detection techniques; PCR, IHC.
Results Until now, over 10.000 women have been examined by the clinics, randomised according to the 6 diagnostic arms. Significant differences are evident in the key demographics of the patients enrolled in the different clinics as are in their clinical findings and HPV detection data.
Expected outcome measures Apart from elaborating the information on the performance of the 6 diagnostic tools (necessary for designing cost-effective prevention strategies for cervical cancer in Latin America) this prospective follow-up of women in different regions permits the analysis of whether variations in cervical cancer incidence in these regions are due to a) different natural history of the precursor lesions, or b) due to different level of exposure to the known risk factors, e.g. HPV. *the LAMS Study supported by European Commission, INCO-DEV Programme: Contract # ICA4-CT-2001-10013.

O-073

Prevalence of cervical neoplastic lesions in Egypt: a national cervical cancer screening project. Final report

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Introduction Reducing morbidity and mortality is one of the main objectives of the health and population policies in Egypt. However, data from Egyptian studies provide widely varying estimates of the prevalence of pre-malignant and malignant cervical lesions. The main goal was (1) to define the prevalence of pre invasive and invasive cervical cancer among Egyptian women, and (2) to identify risk factors including HPVs and associated co-factors, as bacterial vaginosis, HSV, CMV, Chlamydia, schistosomiasis.
Methods and results The study was a community based full-scale cross sectional, household survey that included 5658 women aged between 35 and 60 years conducted between 2000 and 2002. However, 5453 women with complete data were included in the study (96%). The Bethesda classification was used for cervical cytology. Three percent of cases were unsatisfactory. Normal, metaplastic, infectious, and neoplastic lesions represented 28.9%, 0.06%, 63.3% and 7.8%, respectively. The most common type of infection was bacterial (48.98%). Neoplastic conditions, were categorized into ASCUS and AGCUS (3.1%), ASCUS (34.4%), AGCUC (15.3%), LGSL5.44% (41.0%), HSIL (5.2%) invasive squamous and invasive adenocarcinoma (0. 5% for each). Colposcopic-guided biopsy was performed on 274 cases representing 66.51% of the epithelial abnormalities. For standardization, a score of ten points including koilocytic atypia (4), binucleation (2), papillomatosis, dyskeratosis, acanthosis and basal cell hyperplasia (1 point for each), was applied to qualify a lesion as HPV-related (6points out of ten). Unsatisfactory biopsies were encountered in 13.86 %. Normal biopsies including inflammatory and metaplastic changes and atypical metaplasia represented 13.1% and 19.7%repectively. Cervical intraepithelial lesions (CIN) (53.7%), further categorized as CIN I (45.5%), CIN II (5.3%), CIN III/CIS (2.9%) were identified. Endocervical lesions represented 5.3% and were divided into endocervical glandular dysplasia (EGD) (4.9%) and adenocarcinoma in situ (AIS) (0.4%). Combined dysplasia was found in 7.8%, while invasive squamous cell carcinoma (SqCC) and lymphoma accounted for 0.4%of the biopsies. On histological basis, HPV was found in association with CIN I, CIN II, CIN III/CIS and SqCC in 93.6%, 92.3%, 85.7% and 100% respectively. Neither EGD nor AIS showed evidence of HPVs. In situ hybridization (ISH) using the broad spectrum HPV probe recognizing HPVs 6, 11, 16, 18, 30, 31, 35, 45, 51 and 52 was tested on 217 biopsy specimens starting from the atypical metaplastic changes and was found to be positive among 66.8%, negative in 28.6% and non significant in 4.6%. Interestingly, 16.7% of atypical metaplastic changes were positive. Further subtyping with probes 6/11, 16/18 and 31/33 revealed positivity in 11.5%, 31.33%, and 18.4% of cases respectively. Noteworthy, multiple infections with HPV 6/11 and 31/33 (4cases) and HPV 16/18 and 31/33 (7 cases) were identified. To examine the main determinants of cervical cancer, multiple regression analysis was conducted using high-grade lesions as the dependant variable and risk factors as independent variables. This showed that the only determinant was HPV infection. When the regression analysis was conducted without HPV as one of independent variables, history of treatment from schistosomiasis was found to have OR of 2.4 (95%CI 1.1-5.5) but the model was not statistically significant.
Conclusion In the Egyptian population, the prevalence of the precursor lesions of the invasive carcinoma is 3% while invasive lesions account for 0.8% of the population. The prevalence of HPV histologically is 2.2% and by ISH 2.6%. However, detection of HPVs by ISH in this study cannot be precise due to technical limitations and limited number of probes used. Evaluation of HPVs using PCR technology will be subject to another study.

O-074

Clinico-morphological characteristics of leiomyosarcomas of the cervix and uterine body

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Introduction Leiomyosarcomas represent mesenchymal tumors that are relatively frequent in the uterine body, but extremely rare in the cervix. Aims: We report 9 cases of leiomyosarcomas of the uterus: 2 of the cervix and 6 of the body. The macroscopical examination revealed fleshy tumoral masses of 3-6.5 cm in diameter (larger inside the uterine body).
Materials and methods The surgical specimens were routinely processed, stained with HE, PAS, Masson, silver impregnation and immunohistochemically for vimentin, actin, and desmin.
Results The microscopical examination revealed fascicles of smooth muscle fibers with anaplastic aspects, dyskariosis, and frequent mitoses. Leiomyosarcomas of the cervix did not extend to the body but were associated with leiomyomas of the uterine body. Leiomyosarcomas of the uterine body did not extend to the cervix. We analysed the following parameters: number of mitoses per microscopic field, size of cells and nuclei, degree of pleomorphism, level of nuclear hyperchromasia, intratumoral necrosis quantum, rate cellularity/connective matrix, and vascular invasion. The morphologic and immunophenotyping aspects confirmed the diagnosis of leiomyosarcomas stage I (5 cases of leiomyosarcomas of the uterine body), stage II (4 cases, 2 of the uterine body and 2 of the cervix).
Conclusion Although rare, beside leiomyosarcomas of the uterine body, leiomyosarcomas of the cervix may be diagnosed by pathologists, as separate unique location, even in the absence of clinical and cervico-vaginal cytological presumption of malignancy, as the surface epithelium remains intact. Both locations share morphologic, immunophenotypic and prognostic similarities, without evidence of extension to the neighboring counterpart.

O-075

Interferon Regulatory Factor 1 (IRF-1) suppression and derepression in endometrial carcinomas

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Interferon regulatory factor-1 (IRF-1) is a tumour supressor gene presumed to be involved in the control of cellular proliferation and transformation. Given that the IRF-1 is consistently expressed in the normally cycling endometrium, the question was raised of the

possible role of IRF-1 in the genesis of endometrial adenocarcinoma. A series of 86 such tumours was investigated using immunohistochemical techniques. Relatioships were sought with thymidine phosphorylase (TP), conventional histological features and prognosis. Malignant endometria were, by and large, deprived of IRF-1, as 81 of the 86 cases (94.2%) were negative for this antigen. Only 5 of the 86 cases were expressing IRF-1 in inner tumour areas. At the invading tumor front, however, IRF-1 was depressed in tumour cells in 35% of cases. This phenomenon was independent of the extent of lymphocytic response, but it was a strongly associated with TP expression. TP up-regulation and host's lymphocytic response in the area were directly associated. IRF-1 derepression by invading tumor cells was associated with poor prognosis. This prognostic parameter was independent of stage. It is concluded that down-regulation of IRF-1 is a constant finding in endometrial tumorigenesis. However, derepression of IRF-1 may occur in a subset of tumours, and this event is associated with TP up-regulation and aggressive tumor behaviour.

O-076

Morphometric study of GnRH analogue/ HMG/HCG effects on ultrastructure of human endometrial epithelium in the early luteal phase

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Introduction There is a little information about the effects of ovulation induction drugs on morphometry of human endometrium especially at electron microscopic level. The aim of the present study was to determine the effects of long protocol (GnRH analogue/HMG/HCG) on the ultrastructure of human endometrial epithelium at LH+4.
Material and methods Endometrial biopsies were obtained from fertile women (n=6) as well as infertile women (n=10) who had undergone this protocol at LH+4. Quantitative and qualitative studies on endometrial epithelium were performed by transmission electron microscopy (TEM) and morphometry and the results were statistically compared between the two groups.
Results Qualitative results revealed presence of nuclear channel system (NCS), sub vacuole of glycogen and giant mitochondria (GM) in both groups. Similarly, in quantitative analyses, the volume fractions (Vv) of glycogen, mitochondria and rough endoplasmic reticulum to cell and the also the Vv of euchromatin to nucleus were statistically not different between two groups ($P>0.05$).
Conclusion These results suggest that ovulation induction by long protocol is not associated with retarded or advanced endometrial epithelial development.

O-077

Is electron microscopy useful in the diagnosis of mitochondrial encephalomyopathies?

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Introduction Historically the diagnosis of mitochondrial encephalomyopathies relied, on the presence of ragged red fibres (RRF) as well as finding ultrastructural abnormalities in mitochondria. Currently accurate diagnosis requires a multidisciplinary approach but the usefulness of electron microscopy has been questioned. The aim of this study was to define the diagnostic role of electron microscopy in comparison to histology and histochemistry.
Patients and methods Thirty-five patients who fulfilled a clinical phenotype for mitochondrial encephalomyopathies, were separated into two groups. The paediatric group, which included 21 cases, with an age range of 6 days to 18 years old and the adult group which included 14 cases, with an age range of 19 to 75 years old. All patients underwent a muscle biopsy which was examined by histology, histochemistry and electron microscopy.
Results In the paediatric group, 4 cases exhibited RRFs, 8 cases showed RRFe and 16 cases had COX abnormalities. Electron microscopy revealed the presence of mitochondrial abnormalities in 10 cases and mitochondrial aggregates in 7 others. In 4 cases light microscopy was normal, but in 2 of these electron microscopy was abnormal. In the adult group all 14 patients, showed COX abnormalities, 12 cases had RRFe and 7 others had RRFs. Electron microscopy revealed the presence of mitochondrial aggregates in 12 cases and structural abnormalities in 7 cases.
Conclusions Electron microscopy examination is a useful independent diagnostic tool in the investigation of mitochondrial encephalomyopathies. It is particularly helpful, in the paediatric cases, where the classic light microscopical findings are often not well developed.

O-078

Eosinophil-tumour cell interaction in advanced gastric carcinomas: an ultrastructural study

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Introduction Tumour-associated tissue eosinophils (TATE) have been observed in human tumours and experimental tumour models, but the mechanism by which eosinophils induce tumour cell damage remains poorly understood. Aims: Electron microscopy is an essential technique in evaluating sublethal and lethal cellular injuries. Therefore, gastric carcinomas were studied by light and electron microscopy, focusing on the relationship between eosinophils and tumour cells and on the nature of tumour cell death.
Material and methods Fresh tumour tissue was obtained in the operating room for immediate fixation and processed for both light and electron microscopy examination from 1992 until 2002.
Results A light microscopy review of 107 cases of resected gastric carcinomas identified 5 cases (3 of intestinal-type and 2 of diffuse-type) with a massive TATE. In 3 cases of intestinal-type adenocarcinomas, eosinophils formed varied-sized aggregates in the tumour stroma. Some were seen passing through the neoplastic epithelium, and others were found lying within neoplastic tubules. In 2 cases of diffuse-type carcinoma, eosinophil clusters were seen to surround single tumour cells. With electron microscopy, performed in these 5 cases, tumour cells in intimate contact with eosinophils revealed sublethal and lethal cellular injuries. In particular, sublethal injuries, such as cytoplasmic vacuoles and indiscernible cell membrane, occurred at the contact region. Eosinophil “satellitosis” was associated with lethal damage of tumour cells including brush border disorganization, dilation of nuclear envelope, rough endoplasmic reticulum and Golgi apparatus forming empty spaces or vacuoles, small condensed chromatin particles, irregular distribution of nuclear pores. These

lethal injuries were compatible with non-apoptotic (non-lysosomal vesiculate) cell death. The adenocarcinoma cells, not contacted by eosinophils remained morphologically well preserved.
Conclusions These morphological data suggest the hypothesis of a direct relationship between eosinophil infiltration and induction of non-apoptotic tumour cell death in some cases of gastric adenocarcinoma. A deeper insight into the molecular mechanisms acting as cytokines that stimulate specific eosinophil functions may assist in the development of novel therapies to better treat human malignancies

O-079

Expression of p53 and c-erbB-2 oncoproteins in chronic schistosomal urinary bladder lesions

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This study was conducted in a trial to better understand the genetic mechanisms underlying the proliferative, premalignant and malignant changes frequently displayed in chronic schistosomal cystitis (Ch.Sch.C.) and schistosomal associated transitional cell carcinoma (Sch.TCC) of the bladder. Tissue exepression of P53 (tumor suppressor gene) and c-erbB-2 (oncogene) was assesed immunohistochemically in bladder urothelial specimens of a well characterized cohort of 55 male Egyptian patients (25 Ch.Sch.C; 20 Sch.TCC and 10 normal urothelial specimens as controls). Both P53 and c-erbB-2 oncoproteins were not expressed neither in morphologically normal nor in the hyperplastic epithelium. Overexpression of nuclear P53 and membranous c-erbB-2 was detected in 20% of Ch.Sch.C cases in whom premalignant changes (metaplasia and dysplasia) were displayed. While, in the Sch.TCC cases they were detected in 80% and 70% of cases respectively. On stratifying these patients according to the well recognized prognostic parameters (histopathological grade, stage, tumor size, multifocality and growth pattern) P53 was positively correlated with all the forementioned parameters. In contrast c-erbB-2 was only correlated with tumor size and growth pattern. In conclusion, the results of the current study may suggest the early involvement of both P53 and c-erbB-2 in the initiation of the premalignant changes occuring with Ch.Sch.C and the progression of these changes to malignancy. Moreover, the high incidence of P53 and c-erbB-2 expression among Sch.TCC cases with poor prognostic parameters (higher grade, stage; bigger tumor size; tumor multifocality and nonpapillary growth pattern) may point out to the possibility of using these biomarkers as early predictive markers in Ch.Sch.Cyst. cases displaying premalignant lesions and as poor prognostic indicators for Sch.TCC.

O-080

CD10 expression in a subset of chromophobe renal cell carcinoma

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Introduction CD10 is a neutral endopeptidase which participates in the peptide hidrolysis in the renal proximal tubules. Its immunohistochemical detection has been considered useful in the differential diagnosis of renal carcinomas because of its expression in 93-100% of clear cell renal and papillary renal cell carcinomas and its absence in chromophobe renal cell carcinoma (ChRCC), a histotype positive for parvalbumin, a protein of the distal nephron.
Material and methods We studied immunohistochemically 26 ChRCCs including four with aggressive features (two with sarcomatoid transformation, one with renal vein involvement and one with pancreatic metastasis), for CD10 and parvalbumin developing the reactions with different methods (ABC, Envision peroxidase detection and labeled polymer-AP Dako Envision systems) to prevent false-positive staining due to endogeneous biotin. Western blotting was done to confirm the results. As control we stained 75 clear renal cell carcinomas with both antibodies.
Results CD10 immunoreactivity was observed in 5 (20%) primary ChRCCs, three with aggressive features, using all the developing systems. All ChRCCs were positive for parvalbumin, but not the sarcomatoid areas. Western blotting confirmed the specificity of the immunoreactivities.
Conclusions 1) About 20 % of ChRCC express CD10 2) CD10 is not specific for clear cell and papillary carcinomas of the kidney; 3) A panel including both CD10 and parvalbumin is useful in the differential diagnosis between ChRCC and clear renal cell carcinoma; 3) Three out of four tumors with aggressive features stained for CD10, but further studies are needed to highligh the prognostic significance of CD10 in ChRCC.

O-081

Expression of tenascin in superficial and invasive bladder cancer

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Introduction Tenascin (TN) is an extracellular matrix glycoprotein, which is expressed during embryogenesis at the epithelial-stromal interface. TN is decreased in normal adult tissue but reexpressed in inflammatory, reparative and neoplastic processes. TN seems to be involved in neoangiogenesis, promotion of invasion and metastasis of malignant tumors. The aim of the study was to investigate the expression of TN in carcinoma in situ (CIS), invasive and superficial papillary bladder cancer.
Material and methods 33 cases of bladder cancer were selected (8 WHO grade I, 11 grade II, 9 grade III tumors, 5 CIS). We investigated the different staining patterns of TN by immunohisto-chemistry using a monoclonal antibody against TN (T2H5).
Results Immunhistochemical evaluation revealed stromal and cytoplasmic staining. Stromal staining was found at the borders of invasive tumors and in the lamina propria of most CIS. TN staining of blood vessels was located at the tips of the papillae or in invasive tumor. Cytoplasmic TN expression was present in all bladder cancer specimens. In CIS positive cells were present in all cell layers or distributed in a pagetoid pattern while adjacent normal urothelium showed staining of superficial cells. In WHO grade I papillary tumors TN staining was located in superficial cells, in grade II tumors scattered positive intermediate cells were present, while in grade III tumors TN was expressed focally in all cell layers. Most invasive tumor cell nests showed extensive TN staining.
Conclusion Expression of TN in bladder cancer seems to be associated with dedifferentiation and invasive potential.

O-082

Prognostic significance of cytokeratin 20, CD44v6, Bcl-2 and c-erb-b2 in stage T1, grade 3 urothelial carcinoma of the bladder (T1G3)

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Introduction Stage T1 Grade 3 (T1G3) urothelial carcinoma is a heterogeneous group of aggressive bladder tumours at higher risk of progression and death. Several prognostic factors including depth of invasion, DNA ploidy and cell-cycle protein status have been previously tested, but additional prognostic factors are needed. The aim of the study was to test the ability of cytokeratins (7, 20 and high molecular weight), CD44v6, c-erb-B2, LEA.135 and bcl-2 in predicting tumor behaviour in T1G3 tumors.
Material and methods Clinical and pathological data (age, gender, tumour size and associated carcinoma 'in situ') and the immunohistochemical expression of cytokeratins (7, 20 and high molecular weight), CD44v6, c-erb-B2, LEA.135 and bcl-2 were tested in order of determine their prognostic significance. Univariate and multivariate analysis of overall, disease-free and progression-free survival were done.
Results Tumour size, expression of cytokeratin 20, CD44v6 and bcl-2 were associated with a decreased overall ($p=0.0087$, 0.0001, 0.0002 and 0.0320) and progression-free survival ($p>0.0001$, $p=0.0059$, $p=0.0290$ and $p=0.0432$). Tumour size and c-erb-B2 expression were associated to decrease in disease-free survival ($p<0.0001$ and $p=0.0021$). Cox regression analysis selected cytokeratin 20, c-erb-B2 and CD44v6 as independent predictor of overall survival, cytokeratin 20, c-erb-B2 and CD44v6 for progression-free survival and c-erb-B2 for disease-free survival.
Conclusion The immunohistochemical expression of cytokeratin 20 and c-erb-B2, CD44v6 and bcl-2 may add some prognostic information in stage T1 grade 3 bladder cancer patients.

O-083

Second primary malignant tumors in patients with primary renal cell carcinoma

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Introduction Synchronous, antecedent or subsequent appearance of secondary primary malignancy (SPM) in patient with primary renal cell carcinoma (PRCC) is yet considered to be extremely rare. Recent surveys tend to confirm at least their higher if not even significant incidence. This study was performed to determine the incidence of SPM in the patients with PRCC as well as its localization and time of appearance in relation to PRCC.
Material and methods Of 447 patients who underwent surgery for renal malignancies at Department of Urology, Sestre milosrdnice University Hospital during the period from 1992 to 2000, data of 310 were available in Croatian National Cancer Registry. There were 297 patients with PRCC (193 males and 104 female patients) ranging in age from 24-91 years (median 59.9 years).
Results and conclusion In our series there were 24 (8.1%) patients with SPM. Secondary malignancies in descending order of frequency were cancer of prostate (2.1%), colon (1.0%), lungs (1.0%), bladder (1,0%) and skin (0.7%). There were 42.3% antecedent, 26.9% synchronous and 30,8% subsequent SPM. Only one patient had more than one synchronous second malignancy (urinary bladder, prostate and colon). SPM in patients with PRCC are of great importance for both the clinician and pathologist. Therefore, the clinician should maintain lifetime high-technique follow–up of the patients with PRCC. The knowledge of such an existence should raise an interest in pathologist for elucidating etiologic factors. Namely, it seems that the frequency of SPM in patients with PRCC is much higher that previously expected.

O-084

DNA damage repair in bladder urothelium after the Chernobyl accident in Ukraine

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Introduction In our recent studies we showed increased oxidative stress with p53 gene alterations in urinary bladder lesions in people living in radio-contaminated areas of Ukraine, after the Chernobyl accident. The aim of the study was to examined whether base and nucleotide excision repair is activated in bladder urothelium by chronic low doses of ionizing radiation in male patients with benign prostate hyperplasia and females with chronic cystitis living more than 17 years in 137Cs contaminated areas.
Materials and methods Bladder biopsies from 204 patients were submitted for histological examination and biopsies from 35 patients – for immunohistochemical study of 8-hydroxy-2'deoxyguanosine (8OHdG), 8-oxoguanine-DNA-glycosylase (OGG1-8), apurinic-apyrimidinic endonuclease (APE1) and xeroderma pigmentosum A (XPA).
Results Chronic proliferative atypical cystitis with multiple areas of dysplasia and carcinoma in situ was observed in 89% of patients from radio-contaminated areas with greatly elevated levels of 8OHdG, OGG1-8, APE1 and XPA in bladder urothelium.
Conclusion These findings support the hypothesis that significant activation of DNA damage repair (base and nucleotide excision repair) is induced by long-term low dose of ionizing radiation. The levels of DNA oxidative adducts pointing to mutagenic and carcinogenic potential were in line with the histopatologically diagnosed urothelial lesions.

O-085

Validation of combined use of Thin Prep monolayer preparation and multiprobe UroVysion FISH test for enchanced sensitivity of urinary cytology in detection of bladder cancer

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Introduction Urinary cytology (UC) and cystoscopy remain the standard methods for initial diagnosis and monitoring of recurrences in urothelial tumors. Low sensivity of UC and invasiveness of cystoscopy led to develop different ancillary methods to improve sensitivity of UC. Specific genetic alterations occurr in bladder cancers (BC), represented by aneuploidia of chromosome 3, 7, 17 and loss of chromosome 9. The aim of the study was to evaluate the use of thin layer techniques applied to UC diagnosis associated with multicolour interphase fluorescence in situ hybridization (FISH) for early identification of initial BC and recurrences.
Methods A total of 50 voided urine specimens were collected, 20 before resection of bladder cancer, 20 from patient with previous BC and 10 flogistic control patient. The samples were processed with Thin Prep (TP) monolayer technique and cytospin preparation (CS) and in cases with morphological alterations, was performed FISH UroVysion (Vysis) assay, consisting of four probes (3, 7, 17 and 9p21). Cytomorphology.
Results In general TP technique was better for cytomorphology, cleaner background little overlapping, with difficulties when only few urotelial cells are present and in cases of contamination with squamous cells. F I SH technique was positive in all carcinomas, with multiple gain of chromosomes 3, 7, 17, while none of negative controls was aneusomic. Only five cases in group of patients in follow-up with minimal atipia, showed FISH positity, with prevalence of loss of chromosome 9 (70%) compared to gain of chromosomes (30%).
Conclusions These preliminary findings suggest that the association of TP preparation with FISH anlysis can improve the efficience of UC to detect BC and predict its recurrence.

O-086

Chromophobe renal cell carcinoma: the variant with the best outcome?

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Introduction Few large series comparing conventional, papillary and chromophobe renal cell carcinomas (RCC) have been published. Most have been heterogeneous, including RCC subgroups of different stage and/or grade. The purpose of this study was to examine the clinico-pathologic features of a series of chromophobe RCCs comparing their outcome with that of conventional and papillary RCCs in a grade and stage matched fashion.
Materials and methods The clinico-pathologic features of 41 chromophobe RCCs, 40 papillary and 153 conventional RCCs were examined, focussing on the following parameters: patient age and sex, tumor size, stage (1997 pTNM), Fuhrman grade, and histology. Survival was analyzed using the Kaplan Meier method. The most important prognostic factors were identified using the Cox model.
Results For patients with localized disease at diagnosis; median, 5-year, and 10-year Disease Specific Survivals (DSS) were similar for chromophobe (120 mths, 5 and 10-yr DSS: 80%) and papillary (120 mths, 5 and 10-yr DSS: 79.5%) tumors. Conventional RCC patients had a significantly worse outcome (84 mths, 5-yr DSS: 55.8%; 10-yr DSS: 46.5%). The best outcome in low grade (G1+G2), low stage (pT1+pT2) tumors was observed in chromophobe RCCs. In multivariate analysis, conventional (nonpapillary) RCC histology was the major independent adverse prognostic factor whether or not stage and grade were included in the Cox models.
Conclusion Chromophobe RCC is a rare RCC variant of good prognosis. This favorable prognosis is maintained when low grade (G1+G2) / low stage (pT1+pT2) tumors are specifically examined.

O-087

C-kit expression in fetal, normal adult and neoplastic renal tissues

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Introduction C-kit is a transmembrane tyrosine kinase receptor, expressed at high levels in hematopoetic stem cells, mast cells, melanocytic cells, germ cells and the interstitial cells of Cajal. Recently, it has been shown that for certain tumors (especially gastrointestinal stromal tumors and mast cell diseases) c-kit mutation or activation is a major pathogenetic event. Only one previous study has been published regarding c-kit expression in normal renal tissue, and none regarding renal neoplasms. In this preliminary report of an ongoing study, we investigated the expression of c-kit protein via immunohistochemistry in various normal and neoplastic renal tissues.
Material and methods Our material included eight fetal kidneys (16-28 weeks of gestation), seven normal adult kidneys, two cases of renal dysplasia, and 20 renal tumors: 13 renal cell carcinomas, two nephroblastomas, three oncocytomas, one mesoblastic nephroma and one angiomyolipoma. Staining intensity was recorded in a three-grade scale.
Results All fetal kidneys showed cytoplasmic staining of proximal tubules (++). Henle loops were also positive (++), especially in older embryos. Cytoplasmic and membrane staining (++) of renal tubules was also observed in all normal adult kidneys. The two cases of renal dysplasia studied, had c-kit expression in their normal but not in their aberrant tubules. Of the renal cell carcinomas, all conventional type cases (n=9), one sarcomatoid and one papillary type case were negative, while one chromophobe and one granular type were positive (++ and + respectively). All oncocytomas were positive (+++ to ++), and quite interestingly the mesoblastic nephroma was also positive. The angiomyolipoma and the two nephroblastomas were negative.
Conclusion We conclude that c-kit is expressed in normal fetal and adult renal tubules, as well as in a subset of renal tumors. The expression of c-kit in the latter group may prove to have diagnostic significance and therapeutic implications.

O-088

Expression of B-cell markers in classical Hodgkin lymphoma: a tissue microarray analysis of 330 cases

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Introduction Hodgkin and Reed-Sternberg cells (HRSC) of classical Hodgkin lymphoma (cHL) arise from B-lymphocytes. However, classical markers of the B-cell phenotype, such as CD20, are present only in about 25% of the cases. The aim of the present study was to assess expression of the B-cell related antigens CD20, CD79a and CD138 in cHL using a tissue microarray (TMA) consisting of 330 cHL cases.
Materials and methods Expression of CD15, CD20, CD30, CD79a, CD138 and LMP1 of EBV was assessed by immunohistochemistry, and the methodology was validated by direct comparison of CD20 expression on the TMA cores with corresponding large sections. The influence of the number of arrayed sample cores on the obtained expression levels of CD20 was analyzed by comparing the results from single, duplicate and triplicate cores.
Results Two-hundred fifty-three (77%) of the 330 cases were morphologically representative. CD20 was expressed in 84 cases (33%), CD79a in 26 (10%) and CD138 in 2 (0.8%), respectively. CD20 and CD79a were co-expressed in 16 cases ($p<0.005$), and expression of CD20 correlated inversely with CD15 ($p<0.01$). Comparing the TMA results with those from conventional sections for expression of CD20 yielded a concordance of 94% (63/67). Examining 1, 2 and 3 cores from individual cases revealed positivity for CD20 at 24.1% (61/253), 32.4% (82/253), and 33.2% (84/253), respectively.
Conclusions We conclude that B-cell markers are expressed in 38% of cHL in the following rank order: CD20>CD79a>>CD138. The use of 2 cores per tissue sample renders the TMA technology effectively representative, and thus very useful for high-throughput evaluation of heterogeneously-expressed markers in cHL.

O-089

Increased proliferation due to enhanced activation of the RAF/MEK/ERK pathway in relapsed and refractory acute myeloid leukemia

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Background Relapsed/refractory acute myeloid leukemia (AML) is characterized by a deteriorating clinical course and resistance to therapy. Biological mechanisms responsible for resistance remain unclear, since consistent molecular characteristics of AML at relapse and refractory disease have not yet been reported.
Methods We explored paired bone marrow biopsy specimens of 18 patients (at diagnosis and relapsed/refractory AML after high dose chemotherapy) in an immunohistochemical study using Anti Human Ki-67 Antigen (MIB-1) antibody to determine the proliferation fraction of leukemic blasts. Gene expression was analyzed in a different sample set (15 at diagnosis and 11 relapsed/ refractory AML) using a "relapse specific" cDNA microarray covering 4128 genes, selected by literature and suppression subtractive hybridization. Microarray results were validated using quantitative real time PCR, immunohistochemistry and immunoblotting.
Results Recurrent and refractory AML blasts demonstrate a highly significantly increased ($P<0.0001$) proliferation fraction compared to primary diagnosis. mRNA levels of proliferation markers CD71 and PCNA were elevated 4.3-fold and 1.9-fold, respectively, in relapsed and refractory AML compared to diagnosis. Enhanced activation of the RAF/MEK/ERK mitogen-activated protein kinase cascade was indicated by increased mRNAs of B-RAF (1.7-fold), cyclin D1 (2.7-fold), MKP-1 (2.8-fold), c-fos (4-fold), and Egr-1 (5.6-fold). Additional immunohistochemical and immunoblotting analyses demonstrated an elevation of biphosphorylated ERK1/2 protein in recurrent AML, confirming enhanced activation of the RAF/MEK/ERK pathway.
Conclusion Our results provide for the first time evidence that high dose chemotherapy selects or induces leukemic blasts with increased proliferative potential via enhanced activation of RAF/MEK/ERK cascade.

O-090

Expression of VEGF, BFGF and VEGF receptors in diffuse large B cell lymphoma

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Introduction Diffuse large B-cell lymphoma (DLBCL) is the most common of the Non-Hodgkin's lymphomas (NHL), comprising approximately 35% of newly diagnosed cases. In order to define the role of angiogenesis in DLBCL, mRNA and protein expression of the two most important angiogenic growth factors, VEGF and bFGF, and two VEGF receptors, VEGFR-1/flt-1 and VEGFR-2/KDR were evaluated by real time quantitative PCR (RTQ-PCR) and immunohistochemistry in DLBCL.
Methods We analyzed 27 DLBCL cases, 3 low-grade follicle center lymphomas (FLS) and 2 cases with chronic lymphocytic leukemia (CLL) for expression of VEGF-A, bFGF, VEGFR-1/flt-1 and VEGFR-2/KDR. We used a real-time reverse transcription-polymerase chain reaction (qRT-PCR) method to quantify mRNA levels of these angiogenic markers using an ABI 7000 qRT-PCR system. Protein expression of these angiogenic markers was assessed by immunohistochemical staining.
Results and discussion The results of the current study show that both the mRNA and protein expression of VEGF and bFGF varied considerably among 27 DLBCL patients, which do not correlate to the microvessel density. VEGF mRNA expression showed significant correlation with bFGF mRNA expression. The VEGFR-2/KDR mRNA expression was higher than VEGFR-1/flt-1 mRNA expression in DLBCL but their expressions were strongly correlated. Histological staining of both VEGF receptors in DLBCL sections showed strong staining of both receptors on the microvessel endothelial cells but not on the tumor cells. Our data suggest that VEGF plays a role in the pathophysiology of DLBCL however autocrine/paracrine stimulation by VEGF does not seem to be relevant in DLBCL. The expression of VEGF and its receptors raises the possibility of using angiogenesis inhibitors as a novel therapeutic strategy.

O-091

Analysis of provisional extracellular matrix in lymph nodes - loss of the laminin gamma2-chain indicates follicular lymphoma

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Introduction ECM regulates cellular differentiation, life span, migration and contributes to lymph node compartmentalisation. The aim of this study was analysis of occurrence and distribution of embryonal fibronectin (Fn), tenascin (Tn) isoforms and laminin chains (Ln) in reactive lymph nodes (RLN) and follicular lymphoma (FL).
Material and methods Shock frozen tissue of 10 RLN and routine material of 10 FL and RLN. Primary antibodies: all Fn variants (IST-4), ED-A Fn (IST 9), (BC-1), all Tn-C variants (BC-4), Tn-CL (BC-2), Ln-alpha2- (5H2), Ln-alpha3- (BM 165), Ln-alpha5- (4C7), Ln-beta1- (4E10), Ln-beta2- (C4), Ln-gamma1- (2E8), Ln-gamma2-chain (GB3 and D4B5). APAAP-technique.
Results RLN: all Fn's, ED-A Fn and Tn-CL were demonstrated in lymphatic / blood vessels and as reticular depositions in the stroma; spot-like depositions in the follicle center and ribbon-like at the border follicle centre / mantle zone. Ln-alpha3, -alpha5, -beta1, -beta2 and -gamma1-chains were positive mainly in lymphatic and blood vessels with depositions in the stroma. Ln-gamma2-chain was restricted to follicular dendritic cells and ribbon-like co-deposited with Tn-CL at the border follicle centre / mantle zone. FL: in contrast to normal follicles, a total loss of Ln-gamma2-chain is regular observed in neoplastic follicles.
Conclusions 1. A provisional ED-B Fn / Tn-CL / Ln-gamma2-chain matrix membrane controls the traffic follicle centre/mantle zone. 2. In analogy to thymic T cell maturation, a role of Ln-gamma2-chain (Ln-5) can be assumed for B cell maturation mediated by Ln-gamma2-chain (Ln-5) positive follicular reticulum cells. 3. The loss of Ln-gamma2-chain indicates neoplastic follicular structures in follicular lymphoma.

O-092

Follicular lymphoma grading by Mann and Berard are predictive of overall survival and time to treatment failure, however, only grade 1 has favorable OS and TTF

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Follicular lymphomas (FL) are a heterogeneous group of tumors, varying in clinical features, immunophenotype, and cytogenetics. WHO recommends grading of FLs by using the method of Mann and Berard, which recognizes three grades. The aim of this study was to examine whether Mann and Berard's grading has prognostic significance in patients with follicular lymphoma who were not uniformly treated. Samples from 73 patients with follicular lymphoma with follow up ranging from 7–223 months (median 59 months) were included in the study. Forty-three patients (59%) achieved a complete response (CR), 19 (26%) partial response (PR), while 11 (15%) were considered non-responders during the follow up period of 223 months. Twenty-seven (37%) patients died, 11 patients (15 %) from dissemination, 11 from transformation to other lymphoma type and 5 (6, 8%) patients died from non-lymphoma related causes. OS rate was significantly worse in patients with Ann Arbor stage IV than patients Ann Arbor stage I-III (p=0.07). Patients with lower IPI score did significantly better than those with a higher IPI score for OS (p<0.0001) and TTF (p<0.0001). Grade 1 FL patients had longer OS (p=0.02) than grade 2 and 3 patients. A trend for longer TTF was also found (p=0.05). Cox regression analysis showed that these results were independent of stage, but were not independent of IPI score. Higher grade was also associated with the presence of B symptoms (p=0.04) and larger size of the primary tumor (p=0.03).

O-093

BCL-6 and CD10 protein expressions are prognostic factors of overall survival and time to treatment failure in follicular lymphoma

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Follicular lymphomas (FL) are a heterogeneous group of tumors, varying in clinical features, immunophenotype, and cytogenetics. The aim of this study was to examine interrelationship and prognostic significance of BCL6 and CD10 protein expression for overall survival (OS), and time to treatment failure (TTF). Samples from 73 patients with follicular lymphoma were evaluated by immunohistochemistry. Bcl-6 and CD10 expression levels were determined semiquantitatively. Since almost all cases expressed Bcl-6, FLs were designated either "low expression level-group" (LLG 0 and 1+, 29 patients) or "high expression level group" (HLG, 2+ and 3+, 44 patients). Based on CD10 expression, FLs were designated either "negative" (13 patients) or "positive" (58 patients). 44 patients with high levels of Bcl-6 expression had favorable overall survival (OS) (p=0.005) and TTF (p=0.01) compared to 29 patients with low levels of Bcl-6 expression. Cox regression analysis showed that the results for OS, but not TTF were independent of IPI score. Patients with CD10-negative follicular lymphomas also had worse OS (p=0.01) and TTF (0.005). Our results suggest that expression of CD10 and strong expression of Bcl-6 protein could be indicators of well-differentiated follicular lymphoma since such cases were associated with lower histological grade and better clinical outcome.

O-094

CD30+ primary cutaneous lymphoproliferative disorders (PCLDs): TCR-gamma gene analysis by PCR on DNA extracted from CD30+ microdissected cells

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Introduction CD30+ PCLDs spectrum includes lymphomatoid papulosis (LyP), anaplastic large cell lymphoma (ALCL) and

"borderline lesions" with clinicopathologic features in between LyP and ALCLs. LyP nature (neoplastic vs reactive) is matter of debate because of its benign clinical course and variable molecular findings (T-cellular clonality in less than 50% of cases). Borderline lesions have a favourable outcome similar to LyP but, due to their rarity, limited data are available on their biologic features and clonal status. The aim of this study was to compare possible divergences in the search for clonality in the same individual skin lesions using respectively DNA extracted from laser microdissected CD30+ cells and DNA from whole paraffin sections.
Materials and methods 4 LyP and 2 borderline skin lesional biopsies were tested for TCR-gamma rearrangements by means of PCR on DNA extracted respectively from whole-tissue and a pool (50-100 cells) of CD30+ cells microdissected using the ZEISS-PALM Robot-MicroBeam System.
Results and conclusions PCR analysis of whole-tissue DNA documented TCR-gamma monoclonal rearrangement in 2/6 cases (2/2 borderline, 0/4 LyP); TCR analysis on DNA extracted from microdissected CD30+ cells showed monoclonal rearrangement in 5/6 cases (2/2 borderline, 3/4 LyP). We confirm that PCR analyses on DNA extracted by microdissected CD30+ cells enhance the rate of monoclonality detection mainly in LyP lesions, in which the limited number of atypical CD30+ cells and predominant reactive cellular background may be responsible for negative or ambiguous signalling when DNA extracted by the whole lesion is tested.

O-095

Malignant testicular lymphoma accompanied with complete AV block – case report

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A 43-year-old male patient was admitted, with the signs of complete AV block and bradycardia accompanied by syncopes. A complete lab examination with US, CT and MRI was done which all confirmed a massive tumorous infiltration into the root of mesenterium, presence of lymphadenomegaly around it, as well as nonhomogeneous and unclearly demarcated infiltrative lesion of myocardial septum. The patient completed his anamnesis by imparting that his right testicle was slightly enlarged and periodically painful. Semicastration was indicated and the removed tissue was prepared for pathohistologic analysis. The testicle had only peripherally preserved normal tissue, whereas the tumor was clearly demarcated, 4.5 cm in diameter. Five-micrometre-thick tissue sections from the paraffin-embedded tissue were stained with H&E. The cells were quite variable in their morpholoric features and size. The nuclei were generally hypochromatic, with irregular chromatin distribution. The nuclear morphologic properties varied and included pleomorphic mono- and multinucleated giant tumour cells with multiple nuclear lobulations. Nucleoli were conspicuous. Also, numerous benign-appearing lymphocytes and plasmacytoid elements were scattered throughout the background. This histologic picture could correspond either to spermatocytic seminoma or lymphoma. Since the patient needed an urgent therapy, histochemically (PAS-) and immunohistochemically (LCA +, CD3 +, CD20 +), the diagnosis of lymphoma was confirmed. The patient received CT (CHOP protocol), which resulted in a satisfactory clinical response. In this case, it is interesting to point to the most probable infiltration of the myocardium by the diagnosed lymphoma which elicited the cardiologic symptoms and from which the diagnosing of the patient's disease was to start.

O-096

Diffuse large B-cell lymphoma of bone: an analysis of differentiation-associated antigens with clinical correlation

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Twenty-nine patients with diffuse large B-cell lymphomas (DLBCL) presenting with bone involvement, including 18 localized primary bone lymphomas (group I), 2 multifocal primary bone lymphomas (group II) and 9 patients with extraskeletal disease at diagnosis (group III) were studied. The tumors were subclassified according to the criteria of the WHO classification and evaluated by immunohistochemistry for expression of antigens associated with germinal center (GC) and non-GC stages of B-cell differentiation (bcl-6, CD10, MUM-1, VS38c, CD138, bcl-2 and CD44). The presence of a BCL-2/IgH gene rearrangement was investigated by PCR. All cases were characterized by similar clinicopathologic and morphologic features, and had similarly good overall outcome. The patients (23 M, 6F, median 44 y) had tumors in long bones (14), axial skeleton (8), and limb girdles (3) and multiple sites (4). Most tumors (24) were centroblastic, with multilobated cells in 12 cases. Almost half of the tumors (14/29, 48%) were bcl-6+CD10+ («GC-like»), 9/29 cases (31%) were bcl-6+CD10- («indeterminate» phenotype) and 6/29 cases (21%) were CD10-bcl-6- («post-GC like»). The «indeterminate» phenotype was seen only in primary bone lymphoma. MUM-1 was frequently expressed in GC-like and non-GC-like categories. We found no evidence of plasmacytic differentiation by CD138 and VS38c immunoreactivity was distinctly rare (2/29 cases). CD44 was detected in 6 tumors, all CD10-. Bcl-2 was expressed by 70% of the tumors but only 1/23 cases tested had a Bcl-2/JH rearrangement by PCR. A survival analysis showed that GC-like tumors had a longer overall survival duration compared to non-GC-like tumors (p=0.0046).
In conclusion, a "GC-like" immunophenotype, characterizes roughly half of large B-cell lymphomas of bone and is associated with an improved survival.

O-097

Ultrastructural characteristics of liver-associated lymphocytes in chronic delta hepatitis

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Liver sinusoids contain a heterogenous population of lymphocytes including 'pit cells' discovered by Wisse in 1970. However, ultrastructural patterns of liver-associated lymphocytes (LAL) in delta hepatitis are still not well identified. In present work we investigated LAL in chronic delta hepatitis (CHD) with electron microscopy. Liver biopsies from 16 with CHD were fixed in 2,5% glutar aldehyde, postfixed with 1% osmium teroxide and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead

citrate. Ultrastructural study revealed migration of lymphocytes between hepatocytes in CHD. They have heterogenous population of lymphocytes varying in morphology. Some of them were large granular lymphocytes and could be natural killer cells. Some of LAL could correspond to a T lymphocyte. In electron microscopic study of LAL we also observed presence of plasma cells and the pattern of intracellular membrane systems common to cells that secrete protein: well developed granular endoplasmic reticulum, large juxtanuclear Golgi complex. A centriole occupies a position just marginal to the Golgi. The chromatin in the nucleus of these cells is characteristically condensed into large masses just within the envelope. Our results demonstrate a heterogenity of LAL in chronic delta hepatitis including the presence of B cells.

O-098

Hepatic steatosis in relation to serum apolipoprotein level in egyptian patients with HCV and HCV- associated HCC: histopathological, biochemical and clinico-sonographic study

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Chronic hepatitis C is a known risk factor for cirrhosis and hepatocellular carcinoma (HCC). Liver steatosis is common in HCV patients suggesting that specific mechanisms might be involved. The aim of our study was to assess variability in serum apolipoproteins in relation to hepatic steatosis in HCV patients of genotype 4 with or without HCC. Material included 73 Egyptian subjects; 25 (HCV), 25 (HCV-associated HCC), and 23 controls. Clinical examination for stigmata of chronic hepatic affection and ultrasonography for assessment of liver size, surface, echogenicity and the presence or absence of a focal lesion were done. Histopathological examination of liver biopsies was done for assessment of hepatitis activity index (HAI) and stage as well as the grade of hepatocytic steatosis (HS). Biochemical study included liver function tests, lipid profile and assessment of apolipoproteins. Results showed a significant decline in apo-A, apo-B, apo-CII, apo-CIII and apo–E lipoprotein in HCV and HCV-associated HCC groups compared to the control group. Analysis of HAI in HCV patients showed significant association of HS with periportal necrosis, but not portal inflammation or lobular degeneration. In patients with HCV associated HCC, HS was significantly associated with portal inflammation and lobular degeneration, suggesting a correlation between virus activity and hepatocytic neoplastic changes. We concluded that steatosis should be searched for in patients with chronic HCV, being an important cofactor in accelerating the development of hepatic fibrosis and in increasing necroinflammatory activity. A correct therapeutic approach to steatosis would slow down the evolution of chronic hepatitis.

O-099

Steatohepatitis – shortcut to diagnosis

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Introduction Nonalcoholic fatty liver disease (NAFLD) is defined as a constellation of clinical conditions characterized by predominantly macro vesicular steatosis. It can be extremely difficult to reach the diagnosis of NASH in the absence of correct clinical data. Exclusion of excess alcohol intake is notoriously difficult and requires a desialylated transferin (the method rarely present in laboratories). Aim: Histomorphologic investigation of liver biopsies from patients with mild laboratory test abnormalities and steatosis complicated by necroinflamatory changes. A pilot study of morphometric characteristics of lipid droplets in NASH and alcoholic hepatitis were also performed.
Material and methods Forty liver biopsies with steatosis and inflammation were examined using the method of Dixon. Ten of these biopsies were morphometrically analysed regarding the lipid droplet features. Following morphometric parameters were estimated: 1) diameter, 2) perimeter and 3) area. We used Olympus Micro Image- Image Analysis Software v.3.0.1.
Results The presence and distribution of Mallory hyaline was the most variable parameter. Morphometric analyses confirm a statistically significant differences for all parameters tested in NASH and alcoholic hepatitis, and were more pronounced in NASH.
Conclusion In the cases of fatty change with necro-inflammatory changes and pericellular fibrosis in liver biopsy and presence or absence of Mallory hyaline, morphometric analysis of lipid droplets can help in reaching the diagnosis of NASH, if testing of desialilated transferring is not possible.

O-100

Mutations of the BRAF gene in cholangiocarcinoma, but not in hepatocellular carcinoma

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Introduction The Raf/MEK/ERK (MAPK) signal transduction cascade is an important mediator of a number of cellular fates including growth, proliferation and survival. The BRAF gene, one of the human isoforms of RAF, is activated by oncogenic Ras, leading to cooperative effects in cells responding to growth factor signals. This study was performed to elucidate a possible function of BRAF in liver tumours.
Methods Mutations of BRAF and KRAS were evaluated in 25 hepatocellular carcinomas (HCC) and in 69 cholangiocarcinomas (CC) by direct DNA sequencing analyses after microdissection. The presence of active intermediates of the MAPK pathway was assessed immunohistochemically. The results obtained were correlated with histopathological variables and patient survival.
Results Activating BRAF missense mutations were identified in 15/69 CC (22%) and in one case of tumour surrounding liver. KRAS mutations were found in 31 out of 69 (45%) CC examined and in two cases of tumour-surrounding non-neoplastic liver tissue. In HCC, neither BRAF nor KRAS mutations were detected. All 31 CC with KRAS mutations had an intact BRAF gene. We failed to observe a correlation between BRAF or KRAS mutations and histopathological factors or prognosis of the patients.
Conclusions Our data indicate, that BRAF gene mutations are a relatively common event in CC, but not in HCC. Disruption of the Raf/MEK/ERK (MAPK) kinase pathway - either by RAS or BRAF mutation - was detected in about 65% of all CC and is therefore one of the most frequent defects in cholangiocellular carcinogenesis.

O-101

Prognosis and treatment of cholangitis after cholecystectomy

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Introduction The purpose of our research was to study prognostic tests of cholangitis after cholecystectomy (CE) for prophylaxis and optimal treatment.
Materials and methods For 10 years 134 patients with so called "Postcholecystectomic Syndrome" (PCES) were treated, 39 (29%) had a clinical picture of acute cholangitis (AC), which was shown by pains in the right hypochondrium, yellowness of skin and scleras, shivering, high hyperthermia and leukocytosis. Patients (25 women and 14 men) were 42-76 years old, 12 of them more than 60 years. Initial operation (CE) was made in various hospitals in terms from 4 months to 2 years ago. The diagnosis was verified by: clinical, laboratory and immunological researches, peroxidation of lipids (POL), ultrasonic research, computerized tomography, fibrogastroduodenoscopy, endoscopic retrograde pancreatocholangiography, transdermic and transhepatic cholangiography, fibrocholangiograpgy, bacteriological researches of bile and blood.
Results According to investigations, in 21 patients the reason of infringement of bile flow was choledocholithiasis (in a combination with stenosis of big duodenal papilla (BDP) or without it, in 10-stenosis of BDP, in 6-cicatricial stricture of hepatocholedochus, in 2 extended stenosis of BDP and a terminal part of the common bile duct (CBD). Laboratory prognostic tests (risk factor) AC concern: hyperbilirubinemia, increase of activity of alanine transpeptidase, aspartate transpeptidase, hypoproteinemia, dysproteinemia, decrease of a level of protrombin lower than 70%, leukocytosis, anemia, increase of the contents of urea in blood, bacteriocholia, concentration of molecule of average weight were higher than 0.6 unit, decrease of level of vitamin E, high contents of immunoglobulin M on a background of a low parameter immunoglobulin G.
Conclusions At the presence of 4 risk factors prognosis was frequently in contrary to expectations and mortality reached to 50-80%. All patients with acute cholangitis after cholecystectomy represented a complicated problem and demanded a complex approach. Revealed prognostic tests of the development of acute cholangitis are necessary for taking into account for its prophylaxis and optimum treatment.

O-102

Study of c-myc oncogene in hepatocellular carcinoma

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Structural alterations in c-myc oncogene, copy number and expression have been implicated in the pathogenesis and progression of several human neoplastic diseases. However, the biologic significance of c-myc gene in human hepatocellular carcinoma (HCC) is unconfirmed. In the present study we correlated c-myc gene amplification and protein expression with the clinicopathologic features. In 20 HCC cases c-myc amplification in tumour tissue was determined using a differential PCR, a procedure for evaluation of gene amplification in comparison with a dopamine D2 receptor gene. The c-myc gene was amplified in 6 out of 20 tumor specimens (30%). Amplification of c-myc was more frequent in younger male patients with HBV infection and in less differentiated tumors. All cases demonstrated positive staining using anti-c-myc monoclonal antibody with increasing percentage of immunoreactive cells in less differentiated tumors. However, the high protein expression was not statistically correlated with c-myc amplification.

O-103

Histological sectioning of brush bristles allows improved diagnosis of biliary tract and bronchial lesions

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Brushing of thin ducts such as those of the biliary tract, pancreas and bronchial tree is often used by endoscopists in order to obtain diagnostic material proving the nature of related lesion. The material is spread on slides. Cytological examination folows. Preservation of the material so obtained is often sub-obtimal, partly because of the presence of fluids such as bile affecting cell preservation, partly because spreading of delicate cells may result in crushing artifacts. To circumvent such problems, we devised an original procedure, where brushes were not smeared, but instead immediately embedded in immersed Methanol. Then the tube with alcool and brush was sent to the laboratory. In order to induce formation of a glue surrounding the brush, this was immersed while wet into egg albumen (a mixture 1:1 of egg albumen and glycerol), then returned to methanol. For paraffin embedding, the metal handling was cut away and the brush was introduced as such into a cassette. Sections parallel to the long axis were cut until the metal wire was almost reached, then the block was rotated and new section was obtained from the opposite side. Sections were stained in Haematoxilin-Eosin and with the Papanicolau procedure. Small fragments of the mucosa, of inflammatory cell aggregates or of related carcinomas were observed. These resulted optimally fixed and allowed a definite histological diagnosis which proved mandatory for proper therapy. Advantages and prospects of this novel procedure will be illustrated and discussed.

O-104

Is telomerase catalytic subunit gene re-expression an early event in gallbladder carcinogenesis?

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Introduction Telomerase catalytic subunit (hTERT) is a rate limiting step for telomerase activity, a key enzyme implicated in cellular immortalisation and transformation. The aims of this study

were to determine whether a.) differences in the levels of hTERT exist among different grades of gallbladder epithelial abnormalities, and b.) hTERT gene re-expression has any role in gallbladder carcinogenesis.
Material and methods The expression of hTERT was analysed in 89 gallbladder tissue samples: 16 normal epithelia, 14 reactive hyperplasias, 15 low grade dysplasias, 16 high grade dysplasias and in 28 adenocarcinomas by immunohistochemistry. At least 200 nuclei were quantified per slide and the number of positive signals per nucleus was expressed as a hTERT index.
Results The mean hTERT index increased progressively with the degree of gallbladder epithelial abnormalities: from 0.03 (standard deviation=0.05) in normal epithelia, 0.04 (SD=0.05) in hyperplastic epithelia, 0.25 (SD=0.14) in low grade dysplasia, 0.82 (SD=0.20) in high grade dysplasia to 0.93 (SD=0.27) in adenocarcinoma. According to the hTERT expression, three groups of gallbladder epithelial changes were found: 1.) normal and hyperplastic gallbladder mucosa, 2. low grade dysplasia, and 3.) high grade dysplasia and adenocarcinoma of the gallbladder. The differences between the groups were statistically significant ($p<0.001$).
Conclusions We believe that occasional presence of hTERT in normal and reactively hyperplastic gallbladder mucosa merely reflects its regenerative capacity. Nevertheless, significantly higher hTERT indices in low grade as well as high grade dysplastic epithelium and gallbladder adenocarcinoma are most probably the consequence of hTERT re-expression - an early event in the multistep process of gallbladder carcinogenesis.

O-105

Spontaneous intestinal perforation vs. necrotizing enterocolitis: 2 different entities clinically and pathologically

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Introduction Necrotizing enterocolitis (NEC) is the most common neonatal surgical emergency. However, during recent years an increasing number of laparotomies has been performed for spontaneous intestinal perforation (SIP) in premature infants. The aims of this study were to compare the clinical and pathological features of SIP and NEC and to identify parameters useful to make the differential diagnosis.
Materials and methods We reviewed the clinical files and biopsies from 40 infants indexed as “necrotizing enterocolitis” or “intestinal perforation” between 1991 and 2002.
Results Twenty-six patients had SIP and 14 had NEC. Patients with SIP had lower gestational age and birth weight. They presented early with abdominal distension. Frequent radiological signs were dilatation of bowel loops and pneumoperitoneum. Perforation was in the small bowel in all cases. 77 % survived. Patients with NEC presented later with more systemic symptoms. Pneumatosis intestinalis was present in more than half. The colon was involved more frequently than the small bowel. 50 % survived. Cases of NEC were characterized by necrosis, inflammation and reparative tissue changes with or without pneumatosis intestinalis. In contrast, the bowel in cases of SIP was not necrotic and showed little inflammation. Its most distinguishing characteristic was the presence of one or more defects in the internal layer of the muscularis propia. The myenteric nerve plexus and interstitial cells of Cajal had developed normally.
Conclusions Spontaneous intestinal perforation and necrotizing enterocolitis differ in their clinical and histopathological characteristics. In our cases, SIP was always associated with segmental absence of the intestinal musculature.

O-106

Impact of epitope retrieval in detection of CD117 (c-kit) in gastrointestinal stromal tumors (GISTs) using manual and automated immunohistochemical (IHC) procedures

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Introduction Gastrointestinal stromal tumours are solid tumours associated with c-kit and PDGF-R expressions. The recent introduction of Gleevec/Glivec, an effective inhibitor of the activated c-kit and PDGF-R has stimulated interest in standardizing the immunohistochemical detection of c-kit in GIST-tissues, assuring an appropriate selection of patients for therapy with Gleevec. Aims: The DakoCytomation anti-Human c-kit antibody, code A 4502, was investigated in four different set-ups: 1) without heat-induced epitope-retrieval (HIER), 2) with HIER, 3) preabsorbing the primary antibody with the synthetic peptide used for generation of the primary antibody and 4) testing on fresh versus archival tissue sections.
Materials and methods Tissue sections were obtained from 35 different cases of GIST, confirmed by morphology and IHC-characterisation (c-kit, CD34, S100, SMA, desmin). The IHC was performed manually as well as on automated platforms including the TechMate™ and the Autostainer instruments.
Results The use of HIER consistently displayed a stronger and more uniform staining pattern as compared to an IHC protocol without HIER. In few cases the expression of CD117 was found faintly positive in IHC without HIER. Automated and manual IHC methods displayed similar staining patterns. The specificity of detecting CD117 in a protocol using HIER was confirmed by preabsorption of the primary antibody with immunogen. Storing of fresh cut sections for more than 10 months demonstrated a decrease in IHC CD117 staining intensity.
Conclusion Epitope retrieval enhances the CD117 staining intensity in GIST-tissue. The specificity of the CD117 detection in GIST’s can be confirmed by complete blocking of the positive staining in a parallel set-up, using preabsorption of the antibody by a synthetic c-kit peptide.

O-107

Loss of heterozygosity and microsatellite instability in early onset gastric carcinomas

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Introduction 5-16% of gastric cancer patients worldwide are under 45 and the clinicopathological features are different from the older age group. Here we characterise some of the genetic changes in 40 cases of early onset gastric cancer (EOGC).
Methods Using DNA isolated from microdissected material, a panel of 19 polymorphic microsatellite markers near known or presumed tumour suppresser genes was used to evaluate loss of

heterozygosity (LOH). Microsatellite instability (MSI) status was ascertained using 3 polymorphic microsatellite markers in 40 cases of EOGC and in an additional 24 biopsy cases. Corresponding immunohistochemistry was performed for p53, SMAD4, E-cadherin, MLH1, MSH2 and MSH6. EBV status of the carcinomas was determined using EBER1-mRNA in-situ hybridisation.
Results The most common losses were at loci adjacent to CDH1(17%), TP53(15-23%), RUNX3(22-29%) and SMAD4/DPC4(14-17%). Less frequently, losses were detected at 3p21, 14q24, and loci adjacent to APC, p16INK4a, PTEN, and TFF. No MSI was detected in the 64 cases examined. Immunohistochemistry revealed abnormalities for E-Cadherin in 55% and p53 in 34%. All tumours examined were EBV negative.
Conclusion Common events in EOCG are genetic alterations in CDH1, TP53 and RUNX3 whereas other LOH findings were infrequent. Protein abnormalities of E-Cadherin and p53 were commoner than losses near the corresponding gene. In contrast to gastric cancer in older patients, MSI does not appear to occur in young patients. These findings confirm that unique genetic alterations lead to gastric cancer in the young and highlight the need for further investigation to elucidate the mechanisms by which they occur.

O-108

A place of foveolar hyperplasia in benign epithelial gastric polyps development and classification: Ki-67 LI analysis

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Introduction A place of Foveolar Hyperplasia (FH) in benign epithelial gastric polyps (BEGP) classification, as well as its role in a development of Hyperplastic Polyps (HP) is still a matter of controversy. Aims: Two questions are to be studied: 1. does FH, HP, Adenoma (A) and Carcinoma (Ca) represent a continuum in terms of Ki-67 proliferative activity ? 2. are there any differences of the means of Ki-67 labelling index (LI) in hyperplastic areas of HP when comparison is made between HPs without other lesions (HP-NOS) and with HPs with different focal lesions such as intestinal metaplasia (HP-IM), dysplasia (HP-D) and/or carcinoma (HP-Ca)?
Materials and methods 135 BEGP from 110 patients were selected to perform Ki-67 immunohistochemical analysis. There were 40 FH, 72 HP (including 21 HP–NOS and 51 HP-IM, HP-D and/or HP-Ca) and 23 A. As controls 16 cases of normal mucosa (N), 9 cases of regenerative FH (FH-R) and 7 cases of Ca were used. Paraffin sections were studied immunohistochemically for Ki-67 LI with application of MIB-1 antibody. The data were analysed using one-way ANOVA models.
Results It has been found that the means of Ki-67 LI between all the groups studied differed significantly ($p<0.001$). Ki-67 LI for FH was high (38.8) and did not differ significantly from FH-R (47.6), A (43.3), and areas of low grade dysplasia in HP-D (42.5), but was significantly higher from N (24.4) and HP (22.8).
Conclusions 1.We hypothesize that FH may probably represent an early event in BEGP formation. 2.There were no significant differences of Ki-67 LI results for hyperplastic areas in HP (19.2) independently if there were focal IM, D and/or Ca simultaneously, or not.

O-109

Histopathological and immunohistochemical evaluation of gastric carcinoma investigated by endobiopsy

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Introduction Among the malignant tumors that occur in stomach, carcinoma is overwhelmingly the most important and common (90-95%). The aims of this study were to report how we histologically evaluate gastric carcinoma (GC) on endobiopsies, using WHO and Lauren classifications. Additionally, we tried to detect the rhabdoid features in specimens with diffuse GC.
Material and methods The study included 2424 gastric endobiopsies, GC being diagnosed in 451 cases (19%), mostly in men (311 cases – 69%). Endobiopsies, routinely embedded, were stained by hematoxylin-eosin, Giemsa, PAS and Alcian blue (pH 2.5). Immunohistochemical studies were performed using vimentin and cytokeratin antibodies for detection of rhabdoid features.
Results and conclusions The highest incidence of GC was in 60-69-year-old aged patients (34%). Using Lauren classification, 279 cases were categorised as diffuse type (62%), 167 intestinal type (37%) and 5 (1%) mixed type GC. Each of these three types were also histologically analyzed according to WHO classification: intestinal type GC (formed by recognizable well/moderately differentiated glands) and diffuse type GC (consisting in poorly cohesive cells diffusely infiltrating the gastric wall with little or no gland formation). In diffuse type GC with rhabdoid features we evidentiated vimentin and cytokeratin in cells with an eccentric nucleus and a paranuclear inclusion. Finally, we pointed out that is very easy to diagnose GC by Lauren and WHO classifications, but is difficult to evidence the mixed type GC due to reduced size of endobiopsies. Recognition of the rhabdoid phenotype in diffuse type GC is important because this feature is associated with poor prognosis and unresponsiveness to conventional therapy.

O-110

Von Recklinghausen's disease: gastrointestinal manifestations of type 1 neurofibromatosis

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Introduction Gastrointestinal involvement in von Recklinghausen's disease occurs in three forms: hyperplasia of the submucous and myenteric nerve plexuses which leads to disordered gut motility; GISTomas showing varying degrees of neural or smooth differentiation; and a distinctive glandular somatostatinoma of the duodenum. This relatively uncommon disorder and late autopsy, diagnosis, are the reasons for this report.
Materials and methods A 42-year-old man reffered to Surgical Clinic for menagement of the profuse bleeding from the upper

gastrointestinal tract. The patient had a 4-year history of dyspepsia, diarrhorea, mild diabetes mellitus and cholelithiasis. One hour after the admission, he died in hypovolemic shock. Taken tissues from all organs were fixed in 10% formaldehyde and embedded in paraffin. Laboratory sections were stained with: HE, AB-PAS, Van Gieson, Grimelius, Masson's argentaffine reaction and BSP methods, using antibody to somatostatin.

Results Cafe au lait spots, multiple skin neurofibromas, mental retardation, skeletal deformities and a variety of tumours arising in the brain (Craniopharyngioma), follicular thyroid adenoma and duodenal somatostatinoma, were found during autopsy. Duodenal bleeding ulcer of gigantic size, was covered with coagula. In the bottom of the duodenal ulcer was discovered duodenal carcinoid of the glandular architecture, intermingled with solid acinar and trabecular growth pattern; its cells contained somatostatin. Marked increase in number and thickness of nerve fibres also was seen, leading to disordered motility and to striking hypertrophy of the muscularis propria.

Conclusion having in mind that the patient has the sister, and that this disease has hereditary character, we will invite the sister as soon as possible and she will be in the future the target of our investigation.

O-111

Increased expression of muc1 and muc6 and decreased expression of muc2 in colorectal adenoma-carcinoma spectrum: A tissue array study

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Introduction Mucin-type glycoproteins are widely expressed in the gastrointestinal tract. Little is known concerning the relationship of altered mucin antigens in colorectal polyps. The aims of this study were to determine the different patterns of mucin distribution of MUC1, MUC2, MUC5AC and MUC6 and compare their patterns of expression with respect to histopathologic criteria of malignant potential in colorectal carcinogenesis by tissue array using immunohistochemistry.

Materials and methods Core tissue biopsies of 4 mm in diameter taken from paraffin-embedded tissue blocks of 97 colorectal adenomas [19 tubular, 40 tubulovillous, 38 villous] among which 16 contain intramucosal carcinoma (P-IMC) were examined for MUC1, MUC2, MUC5AC and MUC6 expression. MUC expression was defined as “markedly reduced expression (MRE)”, “reduced expression (RE)” and “strong expression (SE)”.

Results MUC1 expression significantly increased in P-IMC when compared with adenomas with mild (MRE: 75% vs 100%; RE: 25% vs no adenoma) and moderate dysplasia (MRE: 75% vs 96.7%; RE: 25% vs 3.3%). MUC6 expression increased as lesions proceeded from mild to moderate to severe to IMC. When the polyps were categorized as benign and malignant; MUC1 (MRE: 75% vs 96.2%; RE: 25% vs 3.8%) and MUC6 (MRE: 87.5% vs 98.8%; RE: 12.5% vs 1.3%) expression increased in malignant group compared with benign group. However MUC2 expression (MRE: 22.5% vs 43.8%; RE: 28.7% vs 31.3%; SE: 48.8% vs 25%) increased in benign adenomas compared with malignant ones.

Conclusion Increased expression of MUC1 and MUC6 and decreased expression of MUC2 play a role in the progression of colorectal adenomatous change.

O-112

Immunophenotypic analysis of submesothelial fibroblasts during peritoneal dialysis-related fibrosis

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Introduction Peritoneal dialysis (PD) is an alternative to hemodialysis for the treatment of end-stage renal disease. The long term exposure to dialysis solutions causes progressive peritoneal fibrosis and functional decline of the peritoneum. The pathologic process that leads to fibrosis is not well understood and most studies have focused on mesothelial cells, probably because they are easy to obtain from peritoneal effluents. The aims of this study were to establish diagnostic markers of peritoneal fibrosis and pathogenic clues to explain the fibrogenic process. The study was focused on submesothelial fibroblasts from normal controls and PD patients.

Materials and methods Parietal peritoneal biopsies were collected from three patient groups: normal controls (n=15), non-PD uremic patients (n=17) and uremic patients on PD (n=27). In order to study myofibroblastic differentiation and mesothelial-to-mesenchymal transition, alpha-smooth muscle actin, desmin, cytokeratins, and E-cadherin were analyzed. We also evaluated CD34 expression by submesothelial fibroblasts.

Results Fibroblasts from normal controls and non-PD, uremic patients showed no myofibroblastic nor mesothelial markers, but an intense expression of CD34. The opposite immunophenotypic pattern was present during PD-related fibrosis. Expression of mesothelial markers (cytokeratins and E-cadherin) by fibroblast-like cells and alpha-smooth muscle actin by mesothelial and modified mesothelial cells are evidence that mesothelial-to-mesenchymal transition occurs during PD. Loss of CD34 expression was another helpful indicator of fibrosis.

Conclusion The immunophenotype of submesothelial fibroblasts varies depending on the existence of a fibrogenic condition. This information results useful for the differentiation between quiescent and activated fibroblasts. In addition, it shows interesting information regarding the origin of this cell population.

O-113

Immunohistochemical and genetic high-throughput analysis of gastrointestinal stromal tumors

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Introduction Gastrointestinal stromal tumors (GIST) are among the most common primary mesenchymal tumors of gastrointestinal

tract. They are characterized by diffuse immunohistochemical positivity for c-KIT protein (CD117). The aims of this study were to assess the immunohistochemical and genotypic features of a large series of GIST using tissue microarray technique.
Methods 100 GIST were retrieved from the files of Dpt of Pathology of our Institutions and a tissue microarray (TMA) was built. Immunohistochemistry for CD117, Actin, Desmin, S100, NSE, Mib1 was performed. Interfase FISH analysis was carried out for the following genes: mdm2, EGFR, C-myc, CCD1 and Her-2.
Results 84% of the tumors resulted positive for CD34, 7% for Desmin, 48% for Actin, 10% for S100, 51% for NSE, 25% for Mib1. A significant difference between gastric and non-gastric GIST was observed for CD34, Desmin and S100. Age, size, necrosis and NSE positivity significantly correlated with aggressive behavior of the tumors. Moreover, an amplification of c-myc, mdm2, EGFR, CCD1 in 19 tumors was found. The tumors were all but two borderline or high risk tumors. Cases of co-amplification were also detected in clinically malignant or histologically high risk tumors.
Conclusion Useful parameters for prognostic evaluation of GIST are age, size and necrosis. Furthermore, the observed amplification of c-myc, mdm2, EGFR suggests a role of Rb-CCD1 pathway in the acquisition of malignant phenotype in a subset of GIST.

O-114

Digital slide and virtual microscopy based routine and telepathology evaluation of routine gastrointestinal biopsy specimen

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Introduction The aim of the study was the evaluation of a recently developed digital slide and virtual microscope system, its comparison with optical microscopy on routine gastric biopsy specimen in local and remote access mode.
Materials and methods H/E stained routine gastric, duodenal and colon (altogether 61) biopsy specimen were selected, scanned and evaluated by two specialists on an optical (OM) and virtual microscope (VM).
Results The overall concordance of VM to a consensus diagnosis (CON) was 95.1% and 97% of the OM to CON. The clinically important concordance was 100% and 100% respectively. The two methods showed concordance in 92% and clinically important concordance in 94.1%. The reasons of the discordance was image quality (one case), interpretation difference (3 cases). The overall hard disc place for a gastric biopsy was between 30 to 50 megabytes. The scanning time was between 20 to 40 minutes on a commercial microscope (Axioplan 2, Carl Zeiss, Germany) and 2 to 5 minutes on an automated slide scanner (Hi-Scope, 3DHISTECH Ltd., Budapest)
Conclusions Remote evaluation of the digital slides through Internet shows the advantages of the previously used static and dinamic telepathology methods. The digital slide and the virtual microscope are real alternative techniques in the computerization of the histology laboratory and in teleconsultation services.

O-115

MUC4 neoexpression in intraductal papillary mucinous carcinomas and pancreatic ductal adenocarcinomas

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Introduction The expression pattern of the MUC glycoproteins MUC1, MUC2 and MUC5AC may be useful in classifying the various types of intraductal pancreatic neoplasms (IPMNs). Recent studies have revealed that MUC4 is expressed in ductal pancreatic carcinomas (PDAC) only. This study investigates whether MUC4 is expressed in IPMNs and in particular in those with invasive components. In addition, MUC4 reactivity was studied in PanINs of both IPMN and PDAC.
Methods Paraffin blocks from resection specimens from 20 PDACs and 9 IPMNs (6 invasive) were investigated immunohistochemically ([IHC] antibody clone IG8) and by in-situ hybridization ([ISH] digoxigenin labeled 48 oligomer nucleotide specific for sequences corresponding to repeat domains of MUC4).
Results The normal pancreas is MUC4 negative. 17 of 20 PDACs were positive for MUC4 in both IHC and ISH. Only 3 IPMNs were MUC4 positive, and these cases were of the oncocytic type. Invasive IPMN components of the DAC type (3/3) and one of muconodular type (1/3) showed MUC4 expression at both the protein and the RNA level. In IPMNs associated PanIN lesions of all grades were negative, whereas in PDACs a few PanIN3 lesions were positive.
Conclusions MUC4 neoexpression in the pancreas seems to be a feature of invasive ductal neoplasms (IPMN, PDAC), with the exception of oncocytic IPMNs. The aberrant expression, in particular the shift from the negative intraductal to the positive invasive component in IPMNs, is so far unexplained, but it may be related to the genetic alterations known in IPMNs and PDACs.

O-116

Hypermethylation of hMLH1 and HPP1 in adenocarcinomas of the upper gastrointestinal tract

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Introduction Hypermethylation of CpG islands represents an important epigenetic mechanism for silencing of tumor suppressor genes during carcinogenesis. In this investigation, the prevalence of hypermethylation of the DNA mismatch repair gene hMLH1 and of the recently identified HPP1 gene was investigated in adenocarcinomas of the upper gastrointestinal tract.
Materials and methods Using methylation-specific real-time PCR and fluorescence-labeled DNA-probes, hypermethylation of hMLH1 and HPP1 was investigated in 50 Barrett's-esophagus-associated adenocarcinomas, 50 cardiac carcinomas and in 50 gastric carcinomas. Additionally, hMLH1 protein expression was investigated by means of immunohistochemistry. In a subset of cases the presence of DNA-microsatellite instability was tested.

Results Hypermethylation of hMLH1 was found in 14% of Barrett's carcinomas, 28% of cardiac carcinomas and in 32% of gastric carcinomas, whereas hypermethylation of HPP1 was found more frequently in all 3 tumor types (64% vs. 38% vs. 54%). In gastric cancer, HPP1 hypermethylation was found more frequently in tumors with concomitant hMLH1 hypermethylation (81%), than in those without hMLH1 hypermethylation (41%, p=0.008). Complete loss of hMLH1 protein expression which was present in 10 carcinomas (5 cardiac and 5 gastric) was invariably correlated with hMLH1 hypermethylation and with microsatellite instability.
Conclusion Our data indicate that microsatellite instability and loss of the mismatch repair protein hMLH1, which is mainly caused by hMLH1 gene hypermethylation, is more prevalent in the carcinogenesis of the stomach and the cardia than in the esophagus. Moreover, in gastric cancer hMLH1 hypermethylation is correlated with hypermethylation of the HPP1 gene.

O-117

Antral G-cells in sporadic fundic gland polyps patients: an immunohistochemical study

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Introduction Fundic gland polyps (FGPs) are tiny (2-5mm), often multiple polyps of the acid secreting gastric mucosa, described in a sporadic form prevalently in middle-aged females or in association with Familiar Adenomatous Polyposis (FAP) and attenuated (AFAP) variants, and recently in association with Zollinger-Ellison syndrome. This recently described association prompted us to evaluate the antral G-cell population in a case series of sporadic FGPs.
Materials and methods We studied prospectively a group of 26 patients with sporadic FGPs who had an antral biopsy available. Prior therapy with PPI was taken in account. The 26 antral samples, fixed in Bouin's fluid, were stained with hematoxylin-eosin, Giemsa and Alcian blue (pH2.5)-PAS and anti-gastrin polyclonal antiserum (Dako, Dakopatts, Denmark), diluted 1:300 for 30' at room temperature, using a standard ABC method. Four antral normal biopsies were used as controls. The results were scored as follows: normal: 1-2 G-cells per-gland or foveola, with uneven distribution; simple hyperplasia: more than four-five G-cells per gland; linear hyperplasia: continuous lining of G-cells.
Results All samples showed a normal appearing antrum, with slight hyperemia, but without significant inflammation, intestinal metaplasia and Helicobacter pylori (Hp) colonization. Two out of 26 samples showed a normal G-cell population (rare positive cells with uneven distribution) whereas 10 samples showed a simple, 14 a linear hyperplasia. Only 5 patients (1 with normal G-cell population, 4 with hyperplasia) had a previous long-term treatment with PPI.
Conclusions Our preliminary study of 26 sporadic FGPs with available antral biopsies, showed that morphologically normal, Hp-free antral mucosa harboured in fact a significant G-cell hyperplasia (24/26). This hyperplasia was correlated with an assumption of PPI in only 4 patients, being apparently primitive in 20 patients. The present results raise the possibility that primary antral G-cell hyperplasia may have a role in the genesis of sporadic FGPs, in close similarity with the occurrence of FGPs in the Zollinger-Ellison syndrome.

O-118

Activation of caspases could contribute to degradation of AP-2a and c-kit during melanoma progression

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Introduction Malignant melanoma is an increasing neoplasm in the western world. Advanced tumors gain metastatic potential and are responsible for high mortality rates. During tumor progression a downregulation of the transcription factor AP-2a and the receptor thyrosine kinase c-kit was observed. However, mechanisms leading to a loss of both proteins are unknown. Besides genetic changes post-transcriptional downregulation by caspase-6 and -3 is recently discussed. Aims were: i) to investigate malignant melanoma for AP-2a and c-kit genetically and by protein expression, ii) to study the implication of activated caspases.
Methods 10 SSMs, 10 NMs and 30 metastases were analyzed by direct sequencing analysis for all exons of AP-2a and c-kit genes and by immunohistochemistry for the respective proteins and cleaved (activated) caspase-6 and -3.
Results Four tumors showed point mutations in the activation motif of AP-2a (exon 2). No genetic alterations were detected in the c-kit sequence. Immunohistochemistry revealed a progressive loss of dermal AP-2a and c-kit expression when comparing SSMs with NMs and Ms which was paralleled by a strong expression of caspase-6 in the primary melanomas and Ms, the later were also characterized by caspase-3 expression.
Conclusion Tumor progression in melanoma is characterized by a loss of AP-2a and c-kit. Caspase-6 probably contributes to this down-regulation, as it was recently shown in vitro. The activation of caspases in melanoma might not only induce apoptosis but could be substantial for tumor growth.

O-119

VEGF-A induced micronodular transformation as a novel mechanism of melanoma metastasis

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Introduction In cancer patients, tumor-expression of the angiogenic factor Vascular Endothelial Growth Factor-A (VEGF-A) correlates with high tumor vascularity and increased metastatis, but how these latter two relate is unclear. We investigated the mechanisms of VEGF-A –enhanced metastasis of the human melanoma cell line Mel57.
Methods Stably transfected Mel57 cells, expressing VEGF-A or EGFP, were injected into the internal carotid artery or tail vein of nude mice to establish brain or lung metastases (colonization models). Subcutaneous tumors were grown to examine spontaneous metastasis to lung. Tumors and metastases were examined by (immuno)-histochemistry.
Results In colonization models, VEGF-A expression did not give tumor cells a colonization advantage. Metastases originated predominantly by clonal expansion of single cells. In contrast, spontaneous metastasis from subcutaneous tumors was enhanced in

tumors with VEGF-A expression, this enhanced metastatic potential was induced by a distinct architectural transformation of the s.c. tumors: while Mel57-EGFP and parental Mel57 tumors showed extensive necrosis, Mel57-VEGF-A tumors presented a micronodular growth pattern, with nodules protruding into sinusoidal dilated vasculature. These micronodules proved to be polyclonal, had tissue characteristics and caused metastatic embolism in the pulmonary artery with subsequent metastatic outgrowth.

Conclusion VEGF-A may enhance spontaneous metastasis not simply by increased single cell shedding into the circulation by an increased tumor vascularity but by induction of a micronodular transformation of a tumor resulting in enhanced shedding of (polyclonal)tissue fragments into the circulation. This step of tumorintravasation is probably a rate limiting factor in development of metastasis.

O-120

Correction of cytogenetic disturbances in patients with relapsing genital herpes

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Introduction Serious forms of relapses of genital herpes (GH) are accompanied by disturbances of chromosomal apparatus of lymphoid cells (L.A. Lebedinskya, 2001). Correction of the given infringements is the important problem of practical public health service. The aim of our research was development of a complex method of correction of cytogenetic infringements in patients with GH.

Materials and methods We had investigated and treated 45 patients (complex treatment) with GH in the age of 18-50 years, with frequency of relapses of one time in 1.5-2 months. The control group was made by 30 healthy persons, same age group. Cytogenetic disturbances in T-lymphocytes of peripheral blood surveyed determined by metaphase method. Complex method of treatment of patients with GH included acyclovir 200 mg 5 times per day before clinical recovery and Phytopreparation “Bacplan”, received by an original way from Quercus cortex. Phytopreparation “Bacplan” shows immunomodulation, anti-inflammatory and antimutagen properties. Preparation “Bacplan” appointed per Os 1.0 g per day within 20 days. External –2% solution of Bacplan in the form of lotion on affected body parts 5 time in day.

Results Average frequency of cells with aberrations of chromosomes (AC) in T-lymphocytes of peripheral blood of patients with GH before treatment were much higher in comparison with parameters of control group (accordingly 2.3±0.13 and 1.47±0.14% $P<0.05$). In patients were marked disturbance of chromosomal and chrmotoid types. In 51.8% cases aberration of chromosomes were registered in 1-5 chromosome pairs. After complex treatment of average frequency of cells with AC in patients has decreases to 20 days to1.6±0.11% ($P<0.05$) and practically come nearer to control parameter (1.47±0.14%).

Conclusions Thus, use of viral specific preparation acyclovir in combination with phytopreparation “Bacplan” reduces frequency of structural infringements of chromosomes in T-lymphocytes of patients with GH.

O-121

Different expression patterns of S-100 versus S-100B in primary skin melanoma

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Differential diagnosis of melanoma involves immunohisto-chemistry where S-100 is a traditional marker which is recently completed by gp100/HMB45, MART-1 and tyrosinase. The S-100 family of Ca-binding proteins comprises the A (1-6) and B isoforms. Recent microarray analysis identified S-100B as the melanocyte lineage marker. In this work we have compared the expression patterns of S-100 proteins using antibody recognizing both isoforms versus that which is specific for S-100B in 68 human skin primary melanoma samples. There were 3 expression patterns observed: focal, herogenous and diffuse for both antibodies. The most frequent expression pattern was the diffuse one (36-45%) while the least frequent was the focal pattern (10-14%) in case of both antibodies. When the individual cases have been analysed for the expression patterns for the two antibody we found 49% discrepancy. In about half of these cases (22%) the discrepancy was minor (heterogenous versus diffuse staining pattern), but in almost a third of the cases (27%) it was found to be considerable (focal pattern versus heterogenous or diffuse). We have concluded that the expression patterns of S-100 and S-100B proteins in human melanoma are frequently different. Since gene expression studies identified S-100B as linage marker, it is highly recommended to use monospecific antibody for routine diagnosis. Furthermore, S-100B is validated as sensitive serum marker for melanoma progression accordingly it is another indication for the use of anti-S-100B antibody in the routine diagnosis.

O-122

Primary cutaneous diffuse large B-cell lymphoma

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Introduction Primary cutaneous B-cell lymphomas represent subtypes of extranodal B-cell lymphomas with distinct clinico-pathologic features. They account for approximately 25% of primary cutaneous lymphomas. The most common forms include follicle cell lymphoma, marginal zone lymphoma, immunocytoma, and diffuse large B-cell lymphoma. We report 2 cases of primary cutaneous diffuse large B-cell lymphomas, one in the left gluteal region (4/5 cm in diameter), and the other in the left leg (5/3 cm in diameter), both appearing clinically ulcerated.

Materials and methods Routine paraffin embedding and usual stainings, followed by immunohistochemistry were used.

Results Microscopy evidentiated in the papillary and reticular dermis a diffuse pattern of growth of cells with a moderate degree of morphologic variation, with moderate, slightly basophilic cytoplasm and round-ovalar nuclei, with vesicular aspect owing to

margination of chromatin at the nuclear membrane, 1-2 nucleoli, and frequent mitoses. Gomory staining revealed a network of reticulin with reduced intervening spaces. Immunohistochemistry evidentiated a diffuse positivity of vimentin and CD20 (L26), S100 positivity in few cells, desmin negativity, MIC 2 negativity, CD30 (BERH2/Ki-1) negativity, actin negativity in tumor, but positivity in vessels, EMA negativity and PCNA positivity in almost 50% of the cells.

Conclusion Final diagnoses of primary cutaneous diffuse large B-cell lymphomas were considered correlating the clinical, morphologic, and immunophenotyping features. Although prognosis of most primary cutaneous B-cell lymphomas is favorable, with 5-year survival rates over 90%, diffuse large B-cell lymphoma is more aggressive. We appreciated an unfavorable prognosis of both cases, but especially in the patient where the lymphoma occurred in the lower leg, as this location is associated with a more aggressive behaviour.

O-123

In malignant melanoma CD10 protein expression is associated with metastatic tumors, higher Clark levels and tumor thickness according to Breslow

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CD10 antigen is a 100 kD a cell surface zinc metalloendopeptidase and is expressed by a variety of normal and neoplastic lymphoid and non-lymphoid tissue including melanomas. Kanitakis et al. (Melanoma Res 2002;12:241-244) have recently demonstrated that CD10 expression is significantly more common in metastatic than primary melanomas. We have evaluated biopsies from 70 patients with primary and 27 patients with metastatic melanoma. Formalin-fixed/paraffin-embedded tissues were studied by immunohistochemistry using mAb 56C6 from NovoCastra Laboratories and EnVision+® from DakoCytomation. Overall, CD10 was expressed in 34/97 samples, but the percentage of CD10-positive cell was significantly higher in metastatic tumors (p=0.002) and also in primary tumors with higher Clark level (p=0.01) and tumor thickness according to Breslow (p=0.008). Even though the intensity of expression was associated with depth of invasion (Clack levels p=0.007, Breslow p=0.03), there was no definitive difference between primary and metastatic tumors (p=0.058). We conclude that CD10 expression in malignant melanoma is associated with tumor progression.

O-124

Sclerosing adenosis of the skin

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Sclerosing adenosis is a lesion that occurs in the breast; rare cases have also been reported in other organs such as prostate and skin. We report a case of sclerosing adenosis of the skin incidentally found at the periphery of a basal cell carcinoma. A 72-year-old man had a polypoid lesion of the scalp measuring 3.5 cm in diameter. The lesion was surgically removed and, at histology, it was composed of a nodular basal cell carcinoma. Below the basal cell carcinoma, there was a well circumscribed but not encapsulated lesion constituted by small, packed glands separated by cellular stroma, reminiscent of sclerosing adenosis of the breast. Some glands showed open lumina bordered by cells with eosinophilic, granular cytoplasm while other glands were arranged in solid clusters or elongated cords. Two distinct layers of cells were seen bordering the glands: a luminal layer of cuboidal cells and an outer layer of basal cells with scanty cytoplasm and spindle nuclei. At immunohistochemistry the outer cell layer was positive for actin, calponin, caldesmon and keratin 14 antibodies showing therefore myoepithelial cell differentiation. The luminal layer was positive with low weight keratin; moreover luminal cells were decorated by GCDFP-15 antibody, a marker of apocrine differentiation. To the best of our knowledge this is the first case of sclerosing adenosis of the skin showing apocrine metaplasia of luminal cells. The case here reported is an additional example of the existence of occasional similar lesions between skin and mammary gland.

O-125

Immunolocalization of human sperm protein 17 in skin cancer

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Introduction A variety of cancer\testis (C\T) antigens has been found to be expressed in healthy testis and in a wide range of human malignancies, including melanoma. The effectiveness of these antigens as target for active specific immunotherapy has been largely demonstrated. Recently a new human C\T, called sperm protein 17 (HSp17) has been investigated in multiple myeloma and ovarian cancer. The aim of this study was to investigate the expression of HSp17 in healthy skin and melanocytic skin lesions, in order to evaluate the usefulness of this antigen to develop effective specific immunotherapeutic procedures.

Methods The authors assessed the immunolocalization of HSp17 in paraffin embedded formalin fixed specimens of healthy skin (n=3) and tissues representing cutaneous melanocytic lesions [common nevi (n=15), Spitz nevi (n=5), blue nevi (n=7), atypical nevi (n=5) and melanomas (n=19)].

Results HSp17 was not detected in healthy skin. It was recognized with a high frequency in cutaneous melanocytic lesions in some cells of the dermal stroma, often containing melanin, compatible with melanophages. A higher expression of HSp17 was observed in hyperpigmented nevi.

Conclusion The emergent complex function of HSp17 suggests the necessity of further studies to investigate the relation between melanophages and HSp17. Although HSp17 was not detected in healthy skin, its limited detection in resident melanophages recognized in cutaneous lesions, and not in tumoral cells precludes its applicability as target in skin cancer immunotherapy.

O-126

Histopathological findings in autopsy tissues of Crimean Congo haemorrhagic fever - a case report

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Introduction Crimean Congo haemorrhagic fever (CCHF) is endemic in many countries in Asia, Africa and Europe. The virus, which causes CCHF, is a Nirovirus from the Bunyaviridae family. Although primarily a zoonosis, sporadic cases and outbraces of CCHF affect humans.
Case report A 43-year-old female patient was hospitalized due to sudden onset of fever, headache, vomiting and abdominal pain. During the next days, her condition became worse, she was unconscious, anuric, cyanotic with multiple edema on extremities, large vaginal coagulums and with appearance of haemorrhagic secretion in aspired fluid from the respiratory tract. Three days after admission, due to sudden onset of cardiac arrest, patient died. Pathologic findings: severe bleeding from the mucous membranes and internal organs were found, particularly emphasized in the liver, kidneys, heart, brain and other organs. Widespread hepatocellular necrosis with focal microvesicular steatosis and multiple intracytoplasmatic microvesicular inclusions were the most striking morphological findings. Diffuse necrosis of renal tissue was also found. Recent haemorrhages in lungs, cardiac muscle, suprarenal gland, spleen, and gastric mucosa were found. Lymphoid depletion was detected in lymph nodes. The RT- PCR method for detection CCHF virus has been applied in analysing serum and tissue samples. Serological testing and testing from paraffin embedded tissue were positive.
Conclusion Autopsy pathologic findings of CCHF include widespread haemorrhages of the skin, mucous membranes, pleura, peritoneum. Necrosis is frequent in all organs and tissues, often of ishaemic nature. Autopsy results of our patient showed similar pathologic findings and by PCR method we confirmed etiopathogenesis of this disease.

O-127

The modern morphological characteristic of placenta in syphilis

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Material and methods We investigated the placentas in three cases of intrauterine death caused by inherited syphilis. Grossly, they were small and medium lobular, showed a marked sclerosis of large vessels and they looked white-muddy. The umbilical cord was also sclerotic and showed obliteration of vessels. Their weight corresponded to term of gestation, whereas their area was smaller and their thickness greater than in normally proceeding pregnancy, but these differences were not significant ($p>0.05$).
Results Light microscopic investigation revealed avascularized stroma and sclerotic villi with vessels showing obliterating angiopathy. These changes were combined with processes of compensatory character, such as sharply extended subsyntitially located capillaries and large number of small vesels.
Conclusion In the morphological study of placenta, the attributes of damaged placental tissue and development of compensatory reactions directed towards the maintenance of necessary balance in the mother-placenta-fetus system were revealed. In placenta, contrary to the descriptions of the last years, we found no significant increase of its weight and change in this connection fetus-placenta of factor. The basic changes in placenta were characterized by the development of productive vasculitis with obliteration of the vascular lumina and presence of avascularized terminal villi. The thickening of vessel walls of the main villi as a result of proliferative endarteriitis can be considered as a characteristic attribute for syphilitic infection.

O-128

Immunohistochemical detection of deleted LMP1 in EBV associated lesions

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Introduction The latent membrane protein 1 (LMP1) of Epstein-Barr virus is one of two postulated viral oncogenic proteins. Previous reports have suggested that sequence variations, and in particular a 30 base pair deletion variant called CAO, may define different disease populations.
Material and methods We developed a cocktail of rat monoclonal antibodies that were specific for the non-wild type LMP1 and compared the presence of the antibody staining with LMP1 DNA sequence analysis on clinical samples of nasopharyngeal carcinoma, peripheral T-cell lymphoma, Hodgkin's disease and lymphoblastoid cell lines from normal volunteers and patients with NPC.
Results and conclusion Our results demonstrate specificity of the monoclonal cocktail for detecting the wild-type LMP1 and the ability to sub-differentiate between the Mediterranean type of LMP1 and the CAO-LMP1.

O-129

Visceral leishmaniasis primarily diagnosed in duodenal biopsy: report of three cases

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Introduction Visceral leishmaniasis (kala-azar) is a zoonosis caused by a flagellate protozoan, of the genus Leishmania, which is transmitted to man from reservoir animals (like a dog), by a vector of the genus Phlebotomus. Generally it affects the mononuclear phagocytic system of the bone marrow, liver and spleen. The gastrointestinal system is rarely involved. Diagnosis of the infection depends on microscopic identification of leishmanias amastigotes in tissues or blood cultures or by serologic means. Aims: Visceral leishmaniasis is a rare disease in patients without immunodeficiency, the authors report three cases diagnosed primarily in duodenal and gastric biopsy from immunocompetent patients.
Materials and methods From the files of Pathology Department of S. Marcos Hospital five cases of visceral leishmaniasis have been collected in a 12 years period, all from immunocompetent patients, namely without HIV infections. The authors describe three of the five cases, which were primarily diagnosed by gastro-duodenal biopsy. Lately, was noted the presence of amastigotes in other tissues.

Results After histologic diagnosis resulted a full clinic diagnosis of systemic leishmaniasis with advanced stage of disease.
Conclusions Pathology Department files reveal a significant percentage of visceral leishmaniasis diagnosed by gastroduodenal biopsy, three of five cases. Thus, in no endemic countries for this opportunistic infection and in immunocompetent patients, gastrointestinal biopsy can, eventually be a rapid and sensitive method of diagnosis, since most of the patients present at an advanced stage of disease.

O-130

Impact of the Sudden Acute Respiratory Syndrome (SARS) on the pathology laboratory: a Singapore experience

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The sudden acute respiratory syndrome (SARS) is a new global health problem that has received unprecedented attention. The predominant route of infection is by droplets and close contact with an infected person poses the highest risk of being infected with the SARS virus. The majority of cases among health care workers have occurred in doctors and nurses who have cared for SARS patients. Pathologists and laboratory personnel are regarded as low risk for infection because they are infrequently in direct contact with patients. However, they receive specimens, including sputa, bronchiolo-alveolar lavage samples, excised tissues, blood and body fluids from infectious cases. Two further possible sources of infection are during autopsies and the onsite processing of fine needle aspiration specimens for immediate diagnosis. Not uncommonly, few clinical details are attached with a specimen to alert the laboratory so universal precaution must be in place and all specimens regarded as infectious. But with a new infection such as SARS where information on its natural history, infectiousness and mode of transmission are still evolving, greater vigilance and a pro-active approach are needed. During the period March - May 2003 Singapore was faced with a potentially serious SARS epidemic and to minimize the chance of personnel in the Pathology Department, National University Hospital being infected and thus compromise the diagnostic service a contingency plan was implemented. This report describes the impact of the plan on the department. SARS is a global disease and pathologists in countries who have not encountered it may find the information useful for preparing their laboratory for the eventual arrival of SARS.

O-131

Angiotropic large cell lymphoma of the central nervous system - report of four cases

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Introduction Angiotropic large cell lymphoma (ALCL), or intravascular lymphoma, is a rare form of high grade non-Hodgkin's lymphoma, predominantly of B-cell lineage. It is characterized by extensive proliferation of lymphoma cells within the lumina of small blood vessels particularly of the CNS and skin with tendency to disseminate into multiple organs. Because of nonspecific manifestations the clinical diagnosis of ALCL is difficult as well as the morphological diagnosis, which may be problematic and subtile.
Methods We studied four cases of ALCL with dominant neurologic symptoms in the form of vasculitis, dementia and cauda equina syndrome. The diagnosis was made by autopsy in three cases and by brain biopsy in one case. In one autopsy case, the ALCL was associated with AIDS in a 12-years old hemophilic boy.
Results Microscopically, in all cases, small blood vessels of the CNS were distended by collections of large atypical lymphoid cells which obstructed the vascular lumina. Tumor cells cytoplasm was amphophilic, the nuclei were large, irregular with prominent nucleoli. Mitoses were numerous. In the vicinity, there were small zones of spongy edema, reactive gliosis and foci of total or selective necrosis. In an autopsy case with diagnosis 'vasculitis' (female 52-years) there was dissemination into multiple organs. Immunohistochemistry shows strong positivity for CD45 and CD20, and the negative reaction for CD3, CD45RO and Factor VIII-related antigen. Based on the above findings the diagnosis of angiotropic large B-cell lymphoma was made.
Conclusion The antemortem diagnosis of ALCL of the CNS may be established by brain biopsy with application of immunohistochemistry.

O-132

Evaluation of cell death in animal model of brain contusion treated by hyperbaric oxygen therapy

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Cerebral contusion (CC) remains the most important consequence of traumatic brain injury and has unexplained unique evolving clinical course.The aim of this study was to evaluate the extent of apoptosis in perilesional area of CC and to examine the effects of hypoxia and hyperbaric oxygenation therapy (HBOT).The model of CC was based on the technique of cortical dynamic deformation (Shreiber et al.,1999) on Sprague-Dawley rats. One group of animals afterwards was placed in closed chamber and exposed to mild hypoxemia, simulating posttraumatic hypoxemia. Both groups of animals (hypoxia-exposed and non-exposed) were treated by HBOT. Brains were cut through produced lesion and paraffin sections were stained by TUNEL method and for active caspase-3. Apoptotic cells (AC) were counted in five successive perilesional layers of 0.5 mm each. It was shown that in the group of rats that were exposed to hypoxia, the width of perilesional area and the number of AC were significantly higher than in non-hypoxic animals, raising from 12.2% to 31.8% at 0.5 mm ($p<0.001$).One treatment by HBOT induced significant decrease both in the radius of perilesional area and the number of AC in both groups of animals (in hypoxia-exposed animals this effect was even more pronounced).The results of our study prove the role of perilesional area in the 'growth' of CC. Hypoxemia caused profound increase in cell death extension. HBOT shows marked positive effect in both hypoxic and nonhypoxic animals. We used quantative pathologic monitoring of number of AC to develop the optimal regimen of HBOT in animal model of CC.

O-133

Surgery for seizures. A pathological review between 1997 and 2003 at Jimenez Diaz foundation, Madrid, Spain

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Introduction Surgery for seizures consists of surgical procedures made in the central nervous system to treat drug-resistant epilepsy. Different lesions may be the cause. Aims: We review the cases diagnosed between the years 1997 and 2003 in our hospital.
Materials and methods The surgycal specimens were stained with hematoxilin-eosin and neural or glial immunostains after paraffin embeding.
Results The most frequent lesion is Mesial Temporal Sclerosis (47-70% of the cases). Other causes are tumors; Dysembrioplastic Neuroepithelial Tumor (DNT), neuronal tumors and glial tumors. Less frequent lesions are phacomatosis, malformative lesions like cortical dysplasias or vascular malformations, vascular or trauma lesions and inflammatory or unspecified lesions. Mesial Temporal Sclerosis consists of neuronal loss and gliosis in sectors CA1, CA3, CA4 and dentate gyrus. Between 1997 and 2003 we diagnosed 43 cases; 24 were women and 19 were males. DNT is a very infrequent, intracortical tumor composed of glia and neurons, usually associated with cortical dysplasia. In our experience we diagnosed 6 cases; 4 women and 2 men. We have found other tumors associated with epilepsy; 7 cases of ganglioglioma (3 women and 4 men), 3 cases of gangliocytoma (1women and 2 men), and astrocytic tumors (6 Protoplasmic Astrocytomas, 1 Mixed Astrocytoma and 2 Fibrillary Astrocytomas). We also diagnosed other processes associated with epilepsy: 2 cases of cortical dysplasia, 9 of glial scar, 7 of gliosis or unspecified changes and 2 cases of vascular malformation.
Conclusion Pathology associated with seizures is very diverse; a multidisciplinary and experienced team is needed.

O-134

Expression of sperm protein 17, a novel cancer-testis antigen in human nervous system tumours

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Introduction Human sperm protein 17 (HSp17) is a highly conserved protein originally isolated from a rabbit epididymal sperm membrane and testis membrane pellet. Recently, HSp17 has been included in the cancer/testis antigens (CT) family and showed to be expressed in multiple myeloma and ovarian cancer. The aim of the study was to investigate the immunolocalization of HSp17 in specimens of nervous system malignancies, in order to establish the usefulness of this antigen as target for tumour-vaccine strategies for nervous system tumours.
Methods The authors assessed the expression pattern of HSp17 in formalin-fixed and paraffin embedded surgical specimens of nervous system malignancies, including 25 neuroectodermal primary tumors (8 astrocytomas, 13 glioblastomas multiforme, 3 oligodendrogliomas, and 1 ependymoma); 19 meningeal tumours; and 5 peripheral nerve sheath tumours (4 schwannoma, and 1 neurofibroma), using a standardized immunohistochemical procedure.
Results HSp17 was found to be expressed in a low number of tumoral cases (nearly 14%), with a major expression in astrocytomas histological group (25%). No case among peripheral nerve sheath tumours was found to be immunopositive for HSp17. The expression pattern was found heterogeneous in all of the positive samples and it does not correlate with the degree of malignancy.
Conclusions The low frequency and the heterogeneous distribution of HSp17 suggest the useless of this antigen as unique target for immunotherapeutic strategies. However, the present study shows firstly the immunolocalization of HSp17 in some cells of nervous system tumors tissues. The emergent complex function of HSp17 renders necessary further studies to understand the link between positive-cells and this protein.

O-135

In vitro FDDNP labeling of pathological protein aggregates in the CNS of selected "conformational" disease cases

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Introduction A molecular imaging probe 2-(1-{6-[(2-fluoroethyl)(methyl)amino]-2-naphtyl}ethylidene)malononitrile (FDDNP), is a highly hydrophobic, fluorescent substance, known to label senile plaques and neurofibrillary tangles (NFT) in the brain of Alzheimer disease (AD) patients. The aims of this study were to evaluate in vitro FDDNP labeling of protein aggregates in selected "conformational diseases" of the brain and to determine if they fulfill the tinctorial and polarizing optics criteria for amyloid.
Material and methods Brain sections of patients with confirmed AD, variant AD (vAD), amyloid angiopathy (AA), sporadic Creutzfeldt-Jakob disease (CJD), variant CJD (vCJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), Pick disease (PiD) and progressive supranuclear palsy (PSP) were labeled with FDDNP, Congo red (CR) and PAS. Analysis was performed using fluorescent microscope (FDDNP), and light microscope (CR and PAS) with polarizing optics (CR).
Results In addition to senile plaques and NFT in AD and vAD FDDNP reliably labeled: prion plaques in CJD, vCJD and GSS, Lewy bodies in vAD and vascular amyloid in AA. It did not label Pick bodies in PiD, and globoid NFT in PSP. With the exception of FDDNP positive Hirano bodies in AD and Lewy bodies in vAD, CR and PAS stained all the FDDNP positive deposits. All CR positive aggregates also showed birefringence.
Conclusion In our study, FDDNP labeled all protein aggregates that were shown to posses tinctorial and birefringent characteristics of amyloid, but also some that cannot be classified as amyloid. Aside from its potential for in vivo diagnostics of some neurodegenerative diseases, FDDNP may prove useful in labeling certain pathological protein aggregates in vitro. Work supported by MŠZŠ grants 0381-518 and L3-3435.

O-136

Remodelling in vascularization: an important step in the development of peripheral adenocarcinomas of lung?

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Aim To investigate the development and clinical significance of vascularization in adenomatous alveolar hyperplasia (AAH) and peripherally located adenocarcinomas of the lung.
Material and methods Histological slides obtained from 70 patients with AAH, and from 190 patients with potentially curatively operated lung adenocarcinomas including normal lung parenchyma are immunohistochemically stained with an anti CD34 antibody, and subject to quantitative image analysis. Syntactic structure analysis measures the absolute and relative features of vessels including the vessel-associated cellular densities. These data are compared in between and associated with tumor volume, post surgical TNM stage, and the patients' survival.
Results AAH displays an increased level of vascularization characterized by regular size and increased number of newly formed vessels. Spatial agglutination (clusters formation) of vessels is common in AAH. The tumor vascularization (volume fraction Vv) amounts to 3% in AAH and to 7% in lung carcinomas, and increases in advanced tumor stages (pT4, pN3). AAH displays the lowest, advanced tumor stages the highest numerical vascular density. Minimum diameter and circumference of vessels maintain. The density of epithelial cells decreases with increasing distance from the vascular surface in both AAH and adenocarcinomas. It is the highest in advanced tumor stages. According to multivariate statistical analysis the patients' survival is closely associated with the pN-stage, tumor volume, cell type and the numerical density of tumor cells within a distance < 20m to the nearest neighboring vessel.
Conclusion AAH is characterized by probably non-reversible changes in vascular density and spatial vessel clustering. Vascularization of pulmonary adenocarcinomas becomes altered in advanced tumor stages. Of prognostic significance is the distribution of tumor cells in relation to the nearest neighboring vessel only. Changes in vascularization probably play an important role in genesis and development of pulmonary adenocarcinomas.

O-137

Cellular and connective elements between bronchial glands in sarcoidosis: a quantitative analysis

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Objective Objective of the study was to quantify the inflammatory and connective cells, as well as connective fibres between the bronchial glands in pulmonary sarcoidosis (S).
Material The study included the bronchobiopsy samples obtained from 31 sarcoid patients. The control group (CG) included 25 young healthy subjects who lost their lives in an accident, having the bronchus sampled at autopsy. The quantity of inflammatory and connective cells was measured by the stereometric numerical density method and the quantity of the connective fibres by the volume density method.
Results In the examined group the total quantity of all cellular elements between the bronchial glands in S was estimated to range from 11.231/mm^3 to 101.739/mm^3 or 37.129/mm^3 on the average. In the CG, the quantity ranged from 10.653/mm^3 to 39.020/mm^3, or 24.205/mm^3 on the mean.

Table 1 reviews the mean quantities of certain cell types per mm^3.

Cells type	Sarcoidosis		Control group	
	No. cells	%	No. cells	%
Lymphocytes	26.909	72.5	15.417	63.7
Plasma cells	1.900	5.1	1954	8.1
Macrophages	227	0.6	25	0.1
Neutrophils	6	0.02	5	0.02
Fibroblasts	1.924	5.2	397	1.6
Fibrocytes	6.143	16.5	6.407	26.5

Besides cellular elements, connective fibres were also registered between the bronchial glands. In S, the connective fibres made 10% of the interstitium volume and only 5% of it in the CG. This difference is statistically significant.
Conclusion In sarcoidosis, the total quantity of all cellular elements between the bronchial glands exceeds their quantity in the CG for 1.5 times. The most numerous are lymphocytes in both groups, but in S their quantity is 1.7 times as high as their quantity in the CG. The quantity of the connective fibres is 2 times as high as their quantity in CG. These differences are statistically significant.

O-138

Cytogenetic variants of dysplastic epithelial lesions and their malignant potential in honeycomb lung in usual (UIP) and desquamative interstitial pneumonia (DIP)

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Introduction Honeycomb lung is characterized by the development of numerous dysplastic epithelial lesions: adenomatous hyperplasia, atypical adenomatous hyperplasia, goblet cell hyperplasia, squamous metaplasia and dysplasia. The aim of our study was to analyze morphology, cytogenesis and malignant potential of these lesions in accordance with clinical, functional, high resolution CT data in UIP and DIP patients at early and late stages of the disease.
Materials and methods 72 UIP patients were studied with open lung biopsy, which were performed by formal thoracotomy (47 cases), video-assisted thoracoscopy (6 cases), and transbronchial biopsy (19). Histologic classification was based on criteria for idiopathic interstitial pneumonias (Katzenstein A., Myers J., 1998). CD34, bTGF, FRF, IGF family proteins, p53, Ki-67, PCNA, bcl-2, c-myc were detected immunohistochemically in paraffin sections.
Results We obtained development of early interstitial sclerosis with honeycombing in UIP patients. Wide spectrum of epithelial changes was found, including new entities, such as atrophy of bronchial and alveolar epithelium and neuroendocrine hyperplasia in oval structures with background sclerosis.
Conclusion Obtained data may prove significant plasticity of bronchiolar stem cells in honeycomb lung. These morphological data correlates with predicted survival rate and progressive deterioration in lung function in patients with idiopathic interstitial

pneumonias. The dramatic progresion of UIP comparing with DIP is based on progressive interstitial fibrosis and prominent reconstr uction of pulmonary tissue.

O-139

Intrapulmonary lymph nodes in South African miners - An autopsy survey

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Introduction Progress in imaging technique has succeeded in visualizing intrapulmonary lymph nodes (IPLN), yielding a differential diagnostic challenge. There has been no systematic pathologic study on IPLN in the literature. The aim of this study was to describe the prevalence and histopathology of IPLN with special reference to dust-related lesions.
Materials and methods Histopathologic slides of lungs from 1984 consecutive autopsies of South African miners registered at National Centre for Occupational Health were reviewed to describe the pathology of IPLN. Special attention was paid to compare lung parenchyma and IPLN regarding dust-related lesions.
Results IPLN were seen in 73 of 1984 cases (3.7%). 9 cases (12.3%) had multiple IPLN. Silicotic nodules were seen in 28 cases (38.4%), of which only 2 (7.1%) cases showed co-existing parenchymal silicosis. In case of advanced IPLN silicosis, lymphoid tissue was almost totally replaced by confluent silicotic nodules, which contrasted with surrounding lung with no silicosis. Tuberculosis was not found in IPLN in 5 cases which had evidence of pulmonary tuberculosis. Metastatic deposits were seen in IPLN in 2 out of 4 cases which had both primary lung cancer and IPLN.
Discussion and conclusions Intrapulmonary lymph nodes (IPLN) are not rare. IPLN shares the general pathology of lymph nodes including metastatic malignancy. Silicotic nodules are commonly seen in IPLN in the absence of parenchymal silicosis in occupationally exposed individuals. IPLN that are completely replaced by silicotic fibrosis may mimick parenchymal disease, leading to an erroneous diagnosis of a pulmonary silicosis.

O-140

Karyometric analysis of small cell carcinoma and non-small cell lung carcinoma

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Introduction Making a histologic distinction between small cell carcinoma (SCLC) and non-small cell carcinoma of the lung (NSCLC) on routine biopsies obtained via bronchoscopy sometimes presents diagnostic problem. In difficult cases additional classification methods may be helpful, particularly karyometry. The aim of this study was to estimate karyometric variables of SCLC and NSCLC.
Materials and methods At Institute of Pathology, University of Niš, formalin-fixed, paraffin-embedded bronchoscopic mucosal samples from 41 patients with SCLC and 83 patients with NSCLC were retrieved from pulmonary pathology archives. Serial histologic sections of 5 μm thickness were prepared for staining with hematoxilin and eosin and analyzed by image analyzer Lucia M 3.51 ab (Nikon, Tokio, Japan), using objective x40 (NA=0.65). The binary images were manually edited. Seven nuclear variables were estimated: nuclear area, equivalent diameter, volume of equivalent sphere, perimeter, mean chord, circularity and integrated optical density. In each case a hundred nuclei were measured. A statistical analysis was performed using Student's t-test.
Results All measured nuclear variables were found to be significantly different between these two major histological types of lung carcinoma. The values of nuclear variables (except circularity) of NSCLC were significantly larger than in SCLC, $p<0.001$.
Conclusions Histologic classification of lung cancer is subjective and often difficult to reproduce. Karyometric analysis, in which the morphology of the nucleus is described by a number of mathematical parameters, may be helpful in distinguishing SCLC from NSCLC.

O-141

Use of automated quantitative cytology sputum test for detection of early lung cancer

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We have been using a fully automated quantitative sputum test (Automated Quantitative Cytology sputum test, AQC sputum test) based on a fully automated, high resolution image cytometer, for detection of early lung cancer. The test involves measurements of several thousand cell nuclei per sputum sample, stained with a DNA specific and stoichiometric stain. The digital images of the nuclei are captured at the limit of optical (spatial and photometric) resolution with the cytometer, and over one hundred nuclear features are then calculated from each nucleus. Selected nuclear texture features are then used to calculate the probability of the presence or absence of lung cancer. In this study, a total of 382 sputum samples were collected of which 178 patients were subsequently confirmed by histopathology diagnoses of lung cancers. Approximately half of the lung cancers were of the early stage (stage 0 or stage I) and 204 subjects presents matching (high risk) population with no known malignancy. The results of the quantitative sputum test (sensitivity and specificity), as analyzed by AQC sputum test, were then compared to those derived by conventional cytology. The results of the AQC test from sputum cells show a several fold increase of sensitivity (35% -60% depending on the lung cancer stage and type vs. 1 - 25% by conventional cytology) at an acceptably reduced specificity (90% vs. 98%, for AQC and conventional cytology, respectively). The role of the AQC cytology test in a comprehensive approach to detection of early (non-invasive and minimally invasive) lung cancers will also be discussed.

O-142

Carcinogenesis in bronchial epithelium - an immunohistochemical evaluation of preneoplastic lesions

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Introduction Characterization of bronchial preneoplastic lesions is based upon cellular atypia and disturbed tissue architecture. Depending on the individual experience, the grading of such lesions varies considerably. We evaluated immunohistochemical staining for p53 and Mib-1 in regards of their value in routine diagnostics of bronchial biopsies.
Material and methods 40 bronchial biopsies ranging from normal bronchial epithelium to invasive squamous cell carcinoma have been stained with antibodies against p53 and Mib-1. The biopsies have been divided into 3 groups: squamous cell metaplasia with basal cell proliferation to low grade dysplasia, high grade dysplasia, and carcinoma in situ. For comparison, normal bronchial epithelium and invasive carcinoma have been included.
Results In normal epithelium 2% of the cells showed positivity for p53. With increasing degree of atypia, the number of posititive cells per 100 nuclei increased to 53% in carcinoma in situ. Almost linear progression has been found in the proliferation activity assessed by immunohistochemical staining with Mib-1. In normal epithelium the activity was <1.5%, and the number of positive cells increased to 42% in carcinoma in situ.
Discussion and conclusions In bronchial preneoplastic lesions an increasing accumulation of p53 and Mib-1 can be found in accordance to the degree of atypia. This provides additional and valuable information to facilitate differentiation between low grade and high grade lesions. In regards of an increase in less invasive diagnostic and therapeutic methods, immunohistochemical staining with p53 and Mib-1 adds valuable information for a correct grading of these lesions, and is therefore of considerable clinical importance.

O-143

Pitfalls in diagnosis and assessment of lymph node metastases in lung cancer - a morphometrical analysis of pulmonary and mediastinal lymphnodes

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Introduction Besides histological subtype and tumor size, metastatic spread in pulmonary and mediastinal lymphnodes in lung cancer is also an important factor concerning therapeutic strategies. Prior to resection of the primary tumor a thorough staging of pulmonary and mediastinal lymphnodes is performed using computer tomography – but lymphnodes will be judged by size only.
Material and methods In an autopsy study of ten cases with advanced lung cancer, 345 lymphnodes have been dissected. Lungs have been resected en bloc, and were cut in horizontal sections according to computer tomographic layers. Lymphnodes have been measured and histologically examined. Lymphnodes were judged as normal with a transversal diameter of not more than 10mm.
Results In the histological examination metastases were found in 45% of normal-sized lymphnodes, while 18% of enlarged lymphnodes presented as negative. Using other parameters did not yield better specifity or sensitivity. Discussion: There is a wide range of what maybe the normal size of a lymphnode which is influenced especially by the study collective. We found a variety of size-influencing factors (pneumoconioses, silicosis, previous infections). Furthermore, there are differences in size of lymphnodes in collectives from rural and industrial populations. The high number of metastases in normal-sized lymphnodes is explained by the highly advanced stage of disease in our study collective.
Conclusions For imaging diagnostics, sufficient size parameters for lymphnodes are not existing. We are convinced that pre-operative staging of lymphnode status is important. In many cases the radiological evaluation on the lymphnode stations is not sufficient to differentiate between reactive enlargement and metastatic involvement. Mediastinoscopy is still needed in pre-operative diagnostics, because it allows taking biopsies with following histological examination.

O-144

Expert system dedicated on diagnosis in thyroid pathology

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Introduction Encoutering diagnostically difficult cases, pathologists usually asks consultations from more experienced colleagues or uses materials in form of journal articles, textbooks, atlases etc. These sources can provide the information in a passive way. Usage of expert systems (special computer programs) let pathologists obtain the information in an interactive way and have more accuracy in the diagnosis. Our purpose was to create an expert system capable to improve histological diagnostics in area of thyroid tumors and tumor-like conditions.
Materials and methods Materials for our work consisted of more than 400 cases of thyroid tumors and tumor-like conditions (near 1200 pictures), representing 15 thyroid diseases. Every case was described by two experts according to 33 histologic features and then entered in PC data base.
Results Our computer program can act in two principal ways. These are 1) diagnostics – expert system propely, 2) learning – virtual textbook. 1) Using the expert system, pathologist enters new data in PC and marks so many features as he can. Then he obtains the information about all the possibilities of probable diagnosis and some other results. Entering more features can help to narrow the borders of probable diagnosis. 2) Applying our program as a virtual textbook, it is possible to learn better the known significance of pathological features, to take a look on the descriptions the experts made etc. The program can also function as a virtual trainer and examiner.
Conclusion Our computer program increases the diagnostic quality in the area of thyroid pathology by means of high capability of information integration and improving pathologist's qualification during his learning.

O-145

Vascular 3D features in normal and pathological thyroid tissue

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Introduction Neoangiogenesis has been demonstrated to be an important feature of numerous tumours. Three dimensional (3D) reconstruction has given important insights in breast neoplastic conditions. Purpose of the current study is to investigate the 3D features of vessels in normal and pathological thyroid tissue.
Materials and methods Twenty-three cases were considered: 6 post-mortem normal thyroid, 6 nodular goiter, 6 papillary carcinomas (4 classic, 1 follicular, 1 diffuse sclerosing), 3 follicular adenomas (1 oncocytic) 1 minimally invasive follicular carcinoma and 1 Basedow-Grave's disease. Two patients underwent preoperative eco-color-doppler (ECD) examination. Thin sections (thickness of 3 to 5 mm) were obtained from the surgical specimen, dehydrated for 3 D examination. Parallel sections were paraffin embedded for histology.
Results Normal tissue: thyroid follicles are surrounded by a network of small vessels which originate from main vessels located inside the capsule. Benign nodular lesions: show vascular pattern identical to that of normal thyroid tissue with an increased network at the periphery of the nodules. Vascular spots highlighted by ECD corresponded to these vessels. Carcinomas: in addition to the peripheral vessels, glomeruloid, anastomosing, small vessels were present within the lesion. No difference was seen between follicular and papillary tumours.
Conclusion The present data suggest that malignant thyroid lesions display a characteristic vascular pattern.

O-146

The peptide xenin: from the identification of the cellular source in neuroendocrine cells of the upper intestine to endocrine pathology

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Introduction Xenin is a biologically active 25-amino-acid peptide extractable from the gastro-entero-pancreatic system. The aim of our study was to identify the unknown cellular source of xenin and to analyze the expression of xenin in neuroendocrine tumors.
Methods Normal tissue of humans, rhesus monkeys and dogs and a large series of 72 neuroendocrine foregut and midgut tumors were investigated by immunohistochemistry, confocal laser scanning microscopy, immuno-electronmicroscopy and high pressure liquid chromatography, assessed by radioimmunoassay.
Results In all three species, xenin was expressed in a subpopulation of gastric inhibitory (GIP)-cells of the upper intestine. Immuno-electronmicroscopy demonstrated presence of xenin in secretory granules (187±19 nm diameter). Xenin immunoreactivity and extractable xenin was found in 23 of 26 duodenal neuroendocrine tumors, including gastrinomas, somatostatinomas, non-functioning and enterochromaffin cell tumors. In contrast, xenin was absent from gastric, pancreatic, ileal and bronchial neuroendocrine tumors.
Conclusions The localization of xenin in ultrastructurally characterized secretory granules supports the concept that xenin represents a further endocrine regulatory peptide. The finding that xenin expression distinguishes between endocrine tumors arising in the duodenum from those of nonduodenal origin may be useful for topographical tumor classification and the differential diagnosis of neuroendocrine tumors of the upper gut. (Supported by DFG)

O-147

Tall cell variant is frequent in all types of papillary thyroid carcinomas in 405 investigated cases

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Introduction The tall-cell variant (TCV) is known as a rare but aggressive form of papillary thyroid carcinoma (PTC) with increased incidence of local recurrence and mortality. It is defined as papillary thyroid carcinoma with at least 30% of specific cellular changes within the tumor cells and occurs in 5-10% of all PTCs.
Materials and methods In our series of paraffin embedded and H&E stained tumor tissues, tall cell morphology was exactly defined for the first time and then 405 cases of PTCs, including all stages and variants, were investigated concerning tall cell morphology and associated changes within the tumors.
Results In contrast to previous reports, 163 cases (40,25%) were classified as TCV in our series. Taken together with minor tumor parts, 268 cases (66,17%) presented with TC morphology. These parts were in a high percentage associated with fibrosis and lymphocyte rich areas and showed a higher mitotic activity than usual PTCs.
Discussion The differences in the occurrence of TCV and TC-morphology between the presented series and previously reported cases might result from until now not clearly defined tall cell morphology as well as from similarities to PTCs, such as the oxyphilic variant, which is extremely rare in our series, and maybe also from often described squamous changes within PTCs. Due to these data it is not clear which tumor parts have relevance for prognosis and which tumors should be treated more aggressively than others. In accordance with previous reports concerning the prognostical impact of this variant, we think that the occurrence of TC morphology plays a major role in both development of extrathyroidal growth and metastatic disease.

O-148

Myocardial extensions around venae cavae as a potential substrate of atrial fibrillation

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Introduction Thoracic veins have been recognised as the important sites housing arrhythmogenic foci that trigger atrial fibrillation (AF). However, only limited data are available for the presence and characteristics of myocardial sleeves around venae cavae (VC). Aims: We studied VC to evaluate the presence, the length, the thickness, and the arrangement of myocardial fibres.
Materials and methods We studied microscopically 23 human autopsied hearts (13 males, age range 39-78 years, 6 subjects had a history of AF).
Results Myocardial extensions were found in 17 superior VC: the maximum length reached 47 mm, the average length was 17.5 mm. The maximum thickness was 4 mm, the average thickness was 1.5 mm in the whole group. In the AF group, the average length was 7.7 mm and the average thickness 1 mm. The most prevailed pattern was circular one. Myocardial sleeves in 19 inferior VC reached maximum length of 61 mm (the average length was 19.2 mm in the whole group and 21.8 mm in AF group). The maximum thickness was 4 mm (the average thickness was 1.5 mm in the whole group and 1.4 mm in AF group). The most prevailed pattern was circular one too.
Conclusions There is inter-individual variability in the presence, length, thickness, and pattern of myocardial fibres around VC. The results will have a clinical implication for the catheter ablation treatment of AF.

O-149

Altered endothelial permeability secondary to ischemia-reperfusion

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Introduction During open heart surgery some damages to myocardial capillary endothelium occur, with the consequence to the regarding structure and function of cardiomyocytes as well. Cardioplegia (CPL), long term hypoxia and reperfusion are strong contributing factors for these lesions. The aim of the study is to assess ultrastructural changes and permeability of endothelium of myocardial capillaries due to hypoxia and reperfusion during open heart surgery.
Patients and methods The study included 68 consecutive patients (pts) who underwent open heart surgery. 290 samples were taken from right ventricle anterior wall and apcal region. Biopsies were taken at the end of cardioplegic period, during ischemic period and during period of reperfusion. All patients were divided in two groups depending on duration of period of ischemia or cardioplegia-group 1 (CLP<65 min) and group 2 (CLP-66-120 min). All samples were analyzed by electron microscope using quantative stereological analysis. Volume fraction of the pinocyte vesicles (PV), mitochondria (M) and endoplasmatic reticulum (EPR) were calculated.
Results Endothelium ultrastructure analysis showed increase in permeability of the capillary wall. The worse damage was found after 65 min. of CPL usage: edema of the endothelial cells, widening of the cell junction and other degenerative changes of cardiomyocytes. Analyses of result confirmed that the more significant damages were found during reperfusion in group 2.
Conclusion Our study showed significant changes in endothelial permeability and function after ischemia and reperfusion. Disturbances of transendothelial transport may play certain role in the development of heart failure after open heart surgery.

O-150

Comparative clinico-pathological and immunohistochemical study of cases with postinfarction myocardial rupture

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Introduction The authors compared the clinico-pathological and immunohistochemical features of 16 acute postinfarction cardiac rupture and 16 control (with near the same clinical age without rupture) cases.
Material and methods Cryostat sections of both groups from infarction/rupture and controls were stained with Oil red O, Sudan black B (BDH Chemicals) and were reacted to anti-C-reactive protein (CRP), to complement C5a and C5b-9 (Dakopatts).
Clinico-pathological findings Myocardial rupture occured in elder women with relative smaller weight of heart, thrombosis of one main coronary artery, location in the anterior wall at the first infarction within the first week. Microscopically the main observation is the large amount of granular lipid deposition in the area of myocardial rupture even in the cytoplasm of the necrotic myocardial fibers using Oil red O and Sudan black B staining. Immunohistochemistry: The anti-CRP and C5b-9 antibodies showed intense staining around the area of the rupture. The inflammatory cells were neutrophils in high numbers in both group. Eosinophils were represented in low percentage (below 10%).
Conclusion The postulation is, that the granular myocardial lipids in the area of infarction/rupture are phospholipids (mainly mitochondrial cardiolipin), which through binding CRP induce pathologic complement activation. In experimental heart infarctions complement target (recombinant etc.) molecules could reduce the neutrophil chemotaxis to the area of necrosis and the size of the infarction.

O-151

Nuclear factor-κB regulates apoptosis of intimal smooth muscle cells

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Introduction Apoptosis contributes to reduce intimal smooth muscle cell (SMC) hyperplasia following angioplasty or during atherogenesis. Nuclear factor (NF)-kB is involved in the regulation of many SMC functions. Aim of the work was to verify the role of NF-kB in bax-dependent SMC apoptotic cascade and its possible targeting for novel biologically based therapies of vascular hyperplasia.

Methods Apoptotic proteins were evaluated by immunohistochemistry and Western Blotting and NF-kB using a anti-p65 (RelA) antibody recognizing its active form. Apoptosis was induced in rat aortic neointimal SMCs after endothelial injury in vivo and in vitro by propyonil-l-carnitine (PLC).
Results In vivo, expression of constitutive NF-kB activity of SMCs of neointima was higher compared to underlying and normal media. Treatment with PLC associated to reduced neointimal relative volume and increased apoptotic rate of intimal SMCs compared to controls. Increased apoptotic rate associated to bax overexpression and RelA reduced immunopositivity. In vitro, PLC reduced G1 to S phase progression of intimal but not of medial SMCs. Hoechst staining, flow cytometry and ligation-mediated PCR showed increased apoptotic rate of intimal cells treated with PLC together with bax overexpression and the release of cytochrome c. SN50, an inhibitor of NF-kB nuclear translocation, induced an increase of apoptotic rate in intimal but not in medial SMC cultures, prevented the apoptotic effect of PLC and upregulated the cytoplasmic bax expression and the release of cytochrome c.
Conclusions Our studies with inhibitors of NF-kB strongly suggest that this nuclear factor plays a crucial role in the control of intimal SMC apoptosis.

O-152

Alpha-actin isoform distribution in normal and failing human heart: a morphological, morphometrical and biochemical study

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Introduction Two sarcomeric actins, α-cardiac (α-CA) and α-skeletal (α-SKA), are coexpressed in adult myocardium and represent the preponderant thin filament actin isoforms of cardiomyocyte contractile units. In the human heart, the relative amount of α-SKA mRNA increases steadily during development and after birth and becomes the predominant isoform in adults but data in the literature concerning hypertrophy and failure are scarce and controversial. Aim of the work was to determine how and where α-SKA isoform accumulates during heart development, ageing and with disease.
Methods By using monospecific antibodies, we investigated by immunohistochemistry and Western Blotting the distribution of α-SKA, α-CA and α-smooth muscle actin (α-SMA) isoforms.
Results At 20 weeks of fetal life, α-SKA was localized in a small proportion of subendocardial and papillary muscle cardiomyocytes, whereas a diffuse α-CA staining was observed, associated with a focal expression of α-SMA. In normal adult subjects, α-SKA positive cardiomyocytes were distributed in a transmural gradient with the highest proportion located subendocardially. In myocardial hypertrophy and cardiomyopathies, the amount of α-SKA was increased and a diffuse staining was seen in all layers of ventricular myocardium, with the exception of idiopathic dilated cardiomyopathies. Cardiomyocytes were always negative for α-SMA. As expected, fibroblasts in post-infarct scars expressed α-SMA and TGF-β1, but were negative for these proteins in interstitial fibrosis.
Conclusions Changes in the distribution of actin isoforms in hypertrophy and failure provide new insight into the mechanisms by which the heart adapts to work overload and help to define their role in these pathological situations.

O-153

Arrhythmogenic right ventricle dysplasia and sudden cardiac death. Controversial aspects

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Introduction Arrhythmogenic right ventricular dysplasia (ARVD) has received much interest, since it may cause sudden cardiac death (SCD). Yet, pathogenesis remains controversial, not at least because adipose and fibroadipose types are considered different entities by some. The present study has looked into the histopathologic features of hearts with ARVD with a particular eye for a clue to pathogenesis.
Material and Methods Clinical history review and heart macroscopic/microscopic examination (with histochemical/immunohistochemical techniques) were performed on 29 patients dying suddenly with ARVD. Twenty-three, 15M/8F were young (13-35years) and 6, 4M/2F adults (37-73years).
Results 1)One young had family history of SCD. 2)One adult had cardiovascular antecedents. 3)Seven patients had died during exercise. 4)Both young and adults presented adipose (n=9vs4) and fibroadipose (n=14vs2) types, whilst each variant basically showed similar overall architectural arrangements. 5) Occasional inflammatory infiltrates were present in fibroadipose, but not in adipose type. 6) Left ventricular involvement occurred in 5 young and 1 adult heart. 7) Coexistent left ventricular pathology was occasionally identified.
Conclusions 1) ARVD can be manifest in young and older persons, either as adipose or fibroadipose variants. 2) Both types are morphologically identical, except that some may have additional fibrosis, occasionally accompanied by small inflammatory aggregates. Subtle differences hard to interpret as features distinguishing different entities. 3) Preferential and almost selective involvement of the right ventricular wall suggests a congenital nature of the disease, particularly since recent insights in right ventricular development have showed “regionalization” of genetic expression in the formation of cardiac chambers. Fatty/fibrofatty tissues may appear ab initium through an alternative mesenchymal pathway.

O-154

A new method of quantitative functional morphology for systemic examination of the cardiac muscle

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Introduction Determination of the vital capacities of heart muscle has special importance for cardiac surgery. Morphological methods could be the most trustworthy in this respect. But it was not possible yet to extract this information from myocardial specimens.

The aim of this study was to elaborate a method, which will be able to determine the volume of vital reserve of myocardium taking into account all known morphological changes and alterations.

Material and methods The investigation was carried out on heart muscle tissue of healthy people, who died accidentally and of patients who died from myocardial infarction. The myocardium of white rats (normal, physically trained, during pancreonecrotic shock) was used.

Methods of histology, electron microscopy, cytoenzymochemistry, morphometry, micromechanography, statistics and mathematical modelling were used.

Results Most informative parameters were chosen for characterisation of the microcirculatory bed and of the paths of ultracirculation. The property of contractility has been taken as a system-forming criterion. A mathematical model was elaborated, which allows to unite the most important structural changes and alterations from tissue, cellular, ultracellular and macromolecular levels of the heart muscle. The basic variant of the model in a simplified version is the following:

$$fmcd.=K\bullet Vv_{1mfb.}\bullet N_{ATPI}\bullet\varphi= K\bullet Vv_{1mfb.}\bullet\{(1/Vv^{II}_{cap.}) + \\ +[1/(0{,}000625\bullet Sv^{II}_{cap.})] + (Vv^{II}_{int/}0{,}00550) + \\ +[1/(0{,}000219\bullet Sv^{II}_{mcl.})] + (Vv^{II}_{mcl./}0{,}04296) + \\ +[1/(Sv^{II}_{crst.}\bullet 0{,}0000245)] + [1/(Sv^{II}_{mit./mfb.}\bullet 0{,}0000551)] + \\ +(Vv^{II}_{spl./}0{,}00341) + [1/(\alpha\bullet Sv^{II}_{mfb./mit.}\bullet 0{,}0000551)] + \\ +(Vv^{II}_{mfb./}0{,}02299)\}^{-1}\bullet(\varphi\bullet vl).$$

The equation has a modular construction and an open architecture. All parameters are measurable. The indices (φ) and (v1) are considered as standard values.

Conclusion The examination of the contractility of myocardial specimens from same hearts by two different methods confirmed the functional adequacy and efficiency of the method of quantitative functional morphology. Supported by grant 0777 of RA.

O-155

The role of endomyocardial biopsy in end-stage heart failure

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Introduction Endomyocardial biopsy is considered to be an important diagnostic procedure for the evaluation of patients with end-stage heart failure. The aim of this retrospective study was to evaluate the role of apoptosis, proliferation markers, volume density of interstitium, and myofibril volume fraction for the prognosis in patients with end-stage dilated cardiomyopathy (DCM).

Methods Endomyocardial biopsy was performed during open-heart surgery in 56 pts with end-stage DCM. Patients were divided into 2 groups, one with shorter survival (24 ± 9 months, mean ± standard deviation) and another with survival of more than 7 years after operation. The TUNEL method was used for the detection of apoptosis, and immunohistochemical methods were used for the evaluation of inhibitor of apoptosis (bcl-2), and proliferation markers (PCNA, Ki-67).

Results The increased percentage of apoptotic myocytes, decreased expression of bcl-2, PCNA and Ki-67 antigen, but lower myofibril volume fraction and lower volume density of interstitium was found in the group with early mortality compared to that with longer survival.

Conclusion Morphometrical analysis of the endomyocardial specimens of the patients with terminal heart failure due to DCM may enable us to get access to crucial information about the status of the myocardium, which may help us to predict the prognosis and optimal treatment of patients with end-stage heart failure due to DCM.

O-156

Detection of GFP positive dendritic cells in tissues of bone marrow transplanted mice

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Introduction Trafficking studies in bone marrow transplantation (BMT) more often use fluorescent reporter molecules like the green fluorescent protein (GFP). Dendritic cells (DC) represent an interesting population for trafficking analysis, because they undergo steps of maturation, which seem to be related to their localization in the tissues. The aim of this study was detection of ex vivo expanded DCs, transduced to express GFP, in tissues of mice that had received an allogeneic BMT.

Material and methods Tissues of recipient mice were pretreated by fixation in 4% formalin followed by 20% sucrose, before embedding in OCT and freezing on dry ice. In addition tissues were also fresh frozen and exposed to formaldehyde vapor as cryostate sections. The GFP signals were validated using an anti GFP antibody. Additional markers investigated were CD11c, CD4, CD8, H-2Kb and H-2Kd.

Results Vapor fixation of slides showed equal properties in containing GFP in comparison with the immersion method. GFP+ DCs were detected in small bowel, lymph nodes, thymus and spleen for up to 42 days after i.v. injection. Many of these GFP+ cells were positive for CD11c. The GFP signals could be validated on fluorescence and light microscopic level using anti GFP antibodies.

Conclusions Allogeneic DCs survive in BMT recipients for up to 42 days. They migrate into small bowel, mesenterial lymph nodes, thymus and spleen without causing GvHD. The visualization of survival and migration in BMT settings will provide new insights into the immunological role of DCs in vivo and might contribute to the optimization of DC based treatment strategies.

P-001

Breast hamartoma: cytologic, pathologic and immunohistochemistry study of 36 cases

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Introduction Breast hamartomas enter the differential diagnosis of breast masses, necessitating an accurate diagnosis. It is still not quite clear into which group of lesions these masses should be categorized. The aim of our study was to investigate the cytologic, histologic and immunohistochemical profile of breast hamartoma in order to obtain a detailed picture of this somewhat unusual lesion.
Materials and methods This study presents cytological, pathological and immunohistochemical analysis of 36 patients with breast hamartoma. The immunohistochemical panel included: estrogen and progesterone receptors, Her 2-neu protein, P-53, Ki-67, CKMNF116, vimentin, alpha-SMA.
Results The patients ranged in age between 16 and 69 years. All patients presented with a palpable unilateral mass. The aspirates contained sheets of both bland ductal cells and lobular units. Adipose tissue was present in varying amounts. Typical macroscopical and microscopical features were noted. Immuno-histochemical studies showed estrogen and progesterone receptor positivity in epithelial cells as well as in the stromal cells, in all 36 hamartomas. Her 2-neu protein over expression was noted, P-53 expression was not observed. Ki-67 showed a 2-3% positivity in epithelial cells in most cases.
Conclusions The finding of intact lobular units and a relative paucity of stroma in an aspirate from a well-circumscribed breast lesion, may suggest the diagnosis of hamartoma. Typical macroscopical and histological features of breast hamartoma were noted. The immunohistochemical profile appears to be similar to that of normal and fibrocystic breast tissue, raising the possibility of a spectrum of lesions.

P-002

Basal cell characteristics in breast carcinoma with medullary features (cmf)

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Introduction CK-pattern in NOS breast carcinoma has been studied extensively, including high molecular weight types, such as CK-14, present in rare cases. Published data on CK-14-expression in less common subtypes of breast carcinoma are limited The aim of this study was to learn more about the immunohistochemical profile of CK-14 in cmf, a systematic analysis on CK-14-reaction in a consecutive series of this variant of breast carcinoma was undertaken, including correlation with some traditionally recorded tumor characteristics.
Materials and methods Forty-three consecutive cases of cmf were examined for CK-14. Extent of staining was assessed semiquantitatively and reported as a percentage; its correlation to tumor size and receptor status was calculated.
Results Sixteen (37,2%) of the 43 cases comprised CK-14-labelled tumor cells. Though CK-14+ tumors were less commonly receptor+ and tended to be larger than CK-14- cases, statistically significant differences were not obtained (p=0.14 and p=0.07, respectively).
Conclusion No specific immunohistochemical profile typifying cmf has been identified. Nevertheless, most cmf seem prone to exhibit immunophenotypic aberrations with higher incidence of vimentin+, c-erb-B2+, and P-53+, lower incidence of Her2+ and ER/PgR+. We here demonstrate that CK-14-expression can be added to this list. Though not statistically significant, a trend towards an inverse relation between basal cell characteristics and receptor expression and a positive correlation between CK-14+ and tumor size was demonstrated.

P-003

Evaluation of margin status in lumpectomy specimens and residual breast carcinoma

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Introduction Residual disease leads to most local recurrences, especially in patients treated with breast conserving therapy. This study was undertaken to identify whether assessment of excisional biopsy margins accurately predicts the presence or absence of residual tumor in the lumpectomy bed.
Material and method The margin status of 166 consecutive lumpectomy specimens followed by re-excision, taken from an equal number of patients with primary breast carcinomas (148 infiltrating,18 in situ), were evaluated microscopically and classified as 'positive' if the tumor was found at the inked margin, 'negative' if the entire tumor was found in >0.1cm from the inked margins, 'close' if it was within 0.1cm of the inked margins, but did not transected them and 'indeterminate' if biopsy specimen was not inked or the tissue was fragmented. The tumor size and grade of the initial biopsy were also analyzed, as potential predictors for residual disease.
Results Residual tumor was found in 42% of the patients (69/166): in 16%(10/61) of the cases with negative, in 64%(41/64) with positive, in 32% (8/25) with close and in 62.5%(10/16) with indeterminate margin status. In 36%(23/64) of the 'positive margins' and in 68%(17/25) of the 'close margins' cases no tumor was found in re-excised specimens. In 23%(16/69) of the cases the residual disease was composed entirely of an in situ component of the same histologic type as the initial biopsy. No relationship was found between tumor size or grade and residual disease.
Conclusions For breast tumors histologically negative margins in biopsy specimens do not guarantee complete excision. 'Close' margins may be considered almost equal to 'positive' ones. Residual in situ carcinoma in the immediate vicinity of the previous biopsy site seems to represent the advanced edges of the tumor, apparently difficult to be excised completely and most likely responsible for the locally recurrent disease.

P-004

Syringomatous adenoma of the breast

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Introduction Syringomatous adenoma of the breast is a benign lesion of the nipple, which may be confused with an infiltrating carcinoma. The presence of the myopithelial cells and the immunohistochemical profile is still controversy in the literature. We present the case of an 22 years old patient, with an left nipple mass.
Materials and methods After excision, the excised tissue was fixed and paraffin embedded and H-E stained. We performed also immunohistochemical staining: with cytokeratin, actin, ER, PR, p53.
Results The excised tumor measured 4 cm, had ill-defined margins and yellow color.Microscopically, the lesion consisted of tubules and strands, composed of small and uniform basophilic cells, diffusely infiltrating the stroma of the nipple. The tubules were lined by two layers of cells, had a comma-like appearance and their lumen was usually open and round. The tumor cells lack pleomorphism. Squamous differentiation resulted in keratotic cysts. Immunohistochemically, the luminal layer of epithelial tumor cells was cytokeratin-positive and the peripheral myoepithelial cells were actin-positive. The tumor cells were negative for ER, PR, p53.
Conclusion Syringomatous adenoma of the breast is a benign rare lesion. The pathologist must not confuse it with an tubular carcinoma or with an adenosquamous carcinoma. We demonstrated in this case the presence of the myoepithelial cells.

P-005

The role of smooth muscle myosin heavy chain in diagnosing sclerosing adenosis of the breast with an infiltrative pattern

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Introduction Sclerosing adenosis (SA) of the breast can produce an infiltrative pattern mimicking invasive carcinoma. Immunohistochemical markers for myoepithelial cells, such as smooth muscle myosin heavy chain (SMMHC), have been shown to stain basal myoepithelial cells in normal mammary glands and fibrocystic changes. Absence of myoepithelial cells is associated with invasive carcinoma; however, we have observed occasional attenuation of SMMHC staining in SA. Studies evaluating the role of SMMHC in sclerosing adenosis have not been performed. The intent of this study is to further evaluate the staining pattern of SMMHC in SA with an infiltrative pattern.
Materials and methods Twenty cases of SA with an infiltrative pattern were identified, H&E stained, and immunohistochemistry performed for SMMHC (M3558, Dako Corp), 4µg/ml, as primary antibody, using a polymer based Envision+ detection system (Dako Corp). Normal-breast tissue positive controls and negative isotype matched IgG1 controls were used. Cases were then classified into one of three categories: positive (staining >95% of myoepithelial cells), intermediate (51% – 94%), or attenuated (<50%).
Results Three of the first ten cases (30%) showed attenuated staining with SMMHC. The remaining seven cases demonstrated positive staining of myoepithelial cells. All cases exhibited positive staining within normal lobules, serving as an internal control for each case. Ten additional cases of SA have been identified and are being stained with SMMHC for evaluation.
Conclusions Preliminary findings show that SMMHC yields inconsistent results when applied to SA with an infiltrative pattern. Such lesions can have features similar to invasive carcinoma on H&E, and application of SMMHC could produce misleading results.

P-006

Co-expression of ErbB family members in human breast cancer: Her-2/neu is the preferred dimerization candidate in nodal-positive tumors

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Introduction Overexpression of members of the ErbB receptor family has been asssociated with malignant transformation and the amplification of Her-2/neu in tumor tissue is now an established prognostic factor in breast cancer. In order to initiate signal transduction, ErbB receptor monomers need to form homo- or hetero- dimers. The composition of these dimers is thought to greatly influence both quality and quantity of downstream signalling pathways, and to ultimately determine the elicited biological response.
Material and methods The protein expression pattern of all four ErbB receptors EGF-R, Her-2/neu, Her-3 and Her-4, and expression of their putative ligands EGF, TGF-a and HRG was investigated in 74 women with invasive breast cancer by membrane isolation and Western blot analysis.
Results The co-expression of all four ErbB family members was detected in 79,7% of cases, and of all of the three investigated ligands in 82,4%. While we did not observe a correlation between EGFR and Her-2/neu or Her-4 protein expression, EGFR and Her-3 ($p=0.005$), and Her-3 and Her-4 ($p=0.05$) were clearly co-expressed. The strongest overall correlation, however, was found between Her-2/neu and Her-3 ($p=0.001$) and between Her-2/neu and Her-4 ($p=0.001$). This was particularly true in nodal-positive tumors ($p<0.001$ and $p=0.002$, respectively) whereas in nodal-negative tumors the co-expression was either less significant (Her-2/neu and Her-3: $p=0.01$) or not significant at all (Her-2/neu and Her-4). The co-expression of EGFR/Her-3 was associated with the expression of all three ligands, whereas the Her-2/neu/Her-3 was correlated with HRG ($p=0.002$), thereby indicating a functional relation between specific receptor-dimer combinations and putative ligands.

Conclusion Taken together, we have performed a comprehensive survey of ErbB system expression in breast cancer, and have demonstrated the presence of a co-regulated receptor/ligand system in vivo. Her-2/neu appears to function as the preferred co-expression partner in nodal-positive tumors, thus rendering him the most likely dimerization candidate in malignant breast tumors.

P-007

The role of the AP-1 family members c-Fos, FosB, Fra-1 and Fra-2 in the invasion process of breast cancer cells

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Introduction Members of the AP-1 family of transcription factors (c-Jun, JunB, JunD, c-Fos, FosB, Fra-1 and Fra-2) are able to form Jun-Jun or Jun-Fos dimers which bind to the regulatory sequences of target genes. In a prior study on mammary carcinomas, we found that high Fra-1 and reduced FosB expression are associated with indicators of poor prognosis. As most of the proteases involved in degradation of the extracellular matrix and basal membrane (matrix-metalloproteinases, members of the uPA/PAI-system) are regulated by AP-1 complexes, we assumed that the expression of single AP-1 proteins might also be important for the process of invasion of breast cancer cells.
Method In order to analyze the influence of the single Fos family members (c-Fos, FosB, Fra-1 and Fra-2) on the invasive behavior of mammary cancer, we performed Western Blot analysis of MMP1 and MMP9 and of the members of the plasminogen activator system PAI-1, uPAR, and PAI-1-uPA-complexes in 75 breast cancer samples, and correlated the expression of these proteins with that of the AP-1 proteins.
Results Interestingly, c-Fos, Fra-1 (phosphorylated form), and Fra-2 correlated with MMP9 expression levels. High expression of Fra-2 was also significantly associated with that of PAI-1 and the SDS-insoluble uPA/PAI-1 complex. On the other hand, the expression of FosB was significantly associated with high levels of MMP1.
Conclusion Our results indicate that a shift in AP-1 protein expression, especially high expression of Fra-1 and Fra-2, might be involved in invasion of breast cancer cells. (supported by Deutsche Krebshilfe, No. 10-1867-Ba2)

P-008

Immunohistochemical study of estrogen receptors and progesteron receptors in primary breast carcinomas and their axillary lymph node metastases

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Estrogen receptors (ER) and progesteron receptors (PR) was investigated in 57 primary breast carcinomas and their axillary lymph node metastases to determine if the malignant cells retained or changed this phenotypic feature during the metastatic process. The data were also compared with the tumour size, histologic type, grade and age. Immunohistochemistry with the ER (DAKO-1D5-N1575) and PR (DAKO-PgR 636-N1630) on formalin-fixed paraffin embedded tissue was used. The ER status in primary tumours and lymph node metastases was concordant in 45 patients (78,9%) and discordant in 12 (21,1%). Six of these eleven patients had ER-positive primary tumours and ER-negative lymph node metastases. The PR status in primary and lymph node metastases was concordant in 43 patients (75,4%) and discordant in 14 patients (24,5%). Ten of these twenty-four patients had PR-negative primary tumours and PR-positive lymph node metastases. We observed higher PR positivity in axillary lymph node metastases. The parameters, tumour histology, grade and age showed no statistically significant relation with receptor status. There was a significant correlation between the tumour size and the number of axillary lymh node metastases. The discordance of hormone receptors status between the primary tumour and lymph node metastases might be due to the heterogenity of tumour cells or loss of the phenotypic features of metastatic cells.

P-009

Breast carcinoma in a cancer hospital

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A total of 1302 cases of infiltrating breast Ca, diagnosed in our hospital during the last decade, was studied. The material concerned mastectomies with ispilateral lymph nodes dissection specimens. The patients were classified according the TNM staging system, based upon the primary tumor size (T), the presence of regional axillary nodal involvement (N) and distant metastases (M). This categorization revealed a preponderance of patients with T2 tumors. The overall decade rate for T1, T2 and T3 tumors was 51, 0,1%±2,77%, 31,86%±2,58% and 17,13%± 1,9%, respectively. There was a statistically significant difference between the three groups ($p<0,0001$). The annual distribution of these groups revealed that since 1992 the mean group rate had a tendency to approach the corresponding decade rate, a finding suggesting some degree of stabilization of these proportions. On the other hand, the lymph node metastases were more common in the T3 tumors. The mean rate for T3N+, T2N+ and T1N+ was 76,53%±5,9%, 66,01%±3,01% and 42,09%±-4,88%, respectively; the difference is statistically significant for the T3N+ and T2N+ ($p<0,004$) as well as for T2N+ and T1N+ ($p<0,0001$). The annual overall rate of patients with lymph nodes metastases, independently of T (TN+) was 60,09%± 2,73%. In conclusion, there is no tendency of early patient presentation and a more careful intimate medical care. The infiltrating breast cancer in our material, is represented mainly by theT2 tumors and the great majority of patients come to the hospital in an advanced stage regarding the T and N.

P-010

Aberrant copies of chromosome 16 and X are related with an aggressive phenotype and impaired prognosis of invasive breast tumors

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Introduction Breast cancer is a genetically complex disease, which involves the accumulation of various structural and numerical aberrations. In the present study, we assessed the numerical status of chromosomes 16 and X in 114 primary invasive breast carcinomas in relation to clinicopathological parameters, patients' overall survival, and indices of cell growth (c-erbB-2, topoisomerase IIa) and survival (caspase-3, bcl-2).
Material and methods The number of copies of both chromosomes was detected by interphase chromogenic in situ hybridization with pericentromeric probes on paraffin embedded sections. ER, PR, cerbB-2, topoIIa, caspase-3 and bcl-2 expression was immunohistochemically detected (ABC/HRP). The results were statistically assessed by univariate and multivariate analysis.
Results Polysomy of chromosomes 16 and X was detected as the predominant aberration (73.7% and 57.9% respectively). Gain of chromosome 16 copies was associated with high nuclear grade (p=0.009), increased tumor size (p=0.041), advanced stage (p=0.002), the expression of topoIIa proliferation index (p=0.005), and worse overall survival by multivariate analysis (p=0.0317). The levels of chromosome X polysomy were elevated in ductal carcinomas of high histological grade (p=0.008), in high nuclear grade tumors (p=0.001), and were associated with the expression of topoIIa (p=0.005) and loss of caspase-3 protein (p=0.036). In addition, gain of chromosome X copies was an independent predictor of impaired prognosis of ductal carcinomas (p=0.0414).
Conclusion Polysomy of chromosomes 16 and X was observed as the predominant alteration in phenotypically aggressive breast tumors, characterized by poor differentiation, increased growth potential and impaired prognosis, whereas gain of chromosome X in particular, is probably implicated in cell survival.

P-011

Expression of survivin, bcl-2, p53 and bax in breast carcinoma and ductal intraepithelial neoplasia (DIN 1a)

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Introduction Survivin is a recently discovered member of the family of proteins that inhibits apoptosis. This anti-apoptotic compound can be detected in most types of cancer and expression is associated with a poor prognosis. We immunohistochemically investigated the expression of survivin in breast carcinomas and intraductal epithelial neoplasia of breast to determine whether expression of this protein is associated with clinicopathological parameters such as grade, stage, mitotic rate.
Materials and methods The study involved 43 patients with invasive ductal carcinoma (IDC) and 62 patients with intraductal epithelial neoplasia with mild, moderate and severe, florid ductal epithelial cell hyperplasia. Survivin, bax, bcl-2, and p53 monoclonal antibodies were administered by immunohistochemical method. Findings were analyzed with statistical methods.
Results In 34 of 43 cases (79.1%) of breast carcinomas and 22 of 62 cases (35.4%) of intraductal epithelial neoplasia with mild, moderate and severe ductal epithelial cell hyperplasia stained positively for survivin. None of the histological parameters analyzed were significantly correlated with survivin expression in breast carcinomas. In the carcinoma cases, survivin expression was positively correlated expression of bcl-2, but was not correlated with expression of p53, bax, c-erbB-2 and estrogen, or progesterone receptors. Some of the intraductal epithelial neoplasia cases with moderate or severe ductal epithelial hyperplasia stained positively for both survivin and p53. The breast carcinomas exhibited significantly increased expression of survivin, p53, and bcl-2 than the breast with intraductal epithelial neoplasia.
Conclusion Survivin was not correlated with any of the clinicopathological parameters studied, however, it could be a useful tool in early carcinomas and florid, severe ductal epithelial hyperplasia.

P-012

Non malignant and pre malignant lesions in large needle core biopsy of the breast (LNCB)

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Introduction Certain non-malignant and pre-malignant lesions cause dilemmas for the most appropriate management following LNCB. The aim of this study was to determine the accuracy of LNCB in diagnosising the most frequent non-conclusive findings: benign papillary lesions (BPL), radial scars (RS), fibroepithelial lesions with stromal hypercellularity (FLSH), atypical ductal hyperplasia (ADH) and intraductal carcinoma (DCIS).
Material and methods In a retrospective review of 2,052 imaging guided LNCBs (1993-2001), we found: 19 (0.9%) BPL - 9 with surgical correlation (SC) and 10 with mammographic follow-up (MF); 21 RS (1%) with SC in 6 and MF in 15; 15 (0.7%) FLSH all with SC; 54 (2.6%) ADH - with SC in 40, and 174 (8.5%) DCIS - with SC in 118.
Results 1) BPL: 10 patients without changes in MF. At surgery: 5 BPL - 1 with ADH and 4 invasive carcinomas (3 with mammographic histologic discordance and 1 diagnosed 4 years post LNCB). 2) RS: 15 without mammographical changes and 6 RS (3 with ADH). 3) FLSH: at surgery, 3 fibroadenomas, 11 benign and 1 malignant phyllodes tumours. 4) ADH: at surgery, 19 (47.5%) ADH, 12 (30%) DCIS, 9 (22.5%) invasive carcinomas - 2 of which microinvasive. 5) DCIS: at surgery, 64 (54.2%) DCIS; 12 (10.1%) microinvasive, and 42 (35.6%) invasive carcinomas.
Conclusions 1) Diagnosis of BPL at LNCB seems accurate when

correlating with image findings. 2) RS at LNCB can be followed mammographically. 3) FLSH at LNCB always requires surgical excision. 4) ADH and DCIS diagnosed at LNCB need complete removal of lesion, due to frequent underdiagnosis.

P-013

Estrogen receptor beta protein expression in invasive breast cancer

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Introduction Estrogen receptor beta (ERb) is known to be present in breast tumors; its prognostic and pathophysiological roles, however, remain to be established. The aim of this study was to investigate ERb protein expression in a well documented series of 181 invasive breast carcinomas.
Materials and methods Standard immunohistochemistry with a specific monoclonal antibody (clone PPG5/10, Serotec) was performed on paraffin sections; 10% of strongly immunostained carcinoma cells was used as a cutoff point to classify tumors as ERb-positive. Statistical correlations were sought with clinico-pathological variables (including hormone receptor status) and patients' disease-free and overall survival. Cell proliferation was immunohistochemically assessed by topoisomerase IIa (topoIIa) index. Statistical analysis was univariate and multivariate.
Results ERb immunoreactivity was detected in the majority of specimens (71.2%)and was positively linked with ERa immunoreactivity ($p<0.001$) and increased topoIIa index ($p=0.045$). A significant favorable impact of ERb immunopositivity emerged with regard to disease-free and overall survival in both univariate ($p=0.0002$, $p=0.0002$, respectively) and multivariate ($p=0.001$ and $p=0.011$ respectively) statistical analysis; progesterone receptor expression was also found to exert an independent favorable influence on patient's survival with less statistical significance though ($p=0.045$ and $p=0.041$). On the contrary, patients' survival was not significantly impacted by ERa status.
Conclusions Because of the positive association between ERb immunoreactivity and topoIIa expression, the presence of ERb in breast cancer cells may be considered an indication of increased proliferation. Nevertheless, ERb immunoreactivity emerges as a valuable, independent indicator of favorable prognosis.

P-014

Large needle core biopsy (LNCB) in invasive lobular carcinoma of the breast (ILC) including immunohistochemical expression of E-cadherin (EC) and high molecular weight cytokeratin (CK34ssE12): analysis of results

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Aims To determine diagnostic value of LNCB in patients with ILC using EC and CK34ssE12.
Material and methods Retrospective review of 50 cases diagnosed as ILC either at LNCB or at surgical biopsy (SB), between 1990 and 2000. Immunohistochemical (IHQ) study with EC and CK34ssE12 was performed at both LNCB and SB. Three immunoprofiles were established: EC- CK34ssE12 + (IHQ of ILC); EC + CK34ssE12 - (IHQ of ductal carcinoma - IDC) and EC + CK34ssE12 + (IHQ of mixed ILC-IDC). Initial diagnosis at LNCB was correlated with final diagnosis (morphologically and immunoprofile). Discordant cases were analysed in order to determine usefulness of IHQ at LNCB.
Results In LNCBs initially diagnosed as: 1) ILC (19 patients), final diagnosis was ILC in 18 cases (EC- CK34ssE12+) and IDC in 1 case (EC+ CK34ssE12 -); 2) Invasive carcinoma probably lobular type (ILCp) (26 patients), final diagnosis was ILC in 20 cases (EC- CK34ssE12+), IDC in 4 (EC+ CK34ssE12 -) and mixed ILC-IDC in 2 (EC+ CK34ssE12+); 3) IDC (5 patients), final diagnosis was ILC in all cases (EC- CK34ssE12+), 3 of them ILC pleomorphic-type. In all discordant cases, immunoprofile at LNCB was concordant with final diagnosis
Conclusion 23% of patients diagnosed as ILCp at LNCB corresponded finally to IDC or mixed IDC-ILC. 11.6% of patients finally diagnosed as ILC were initially interpreted as IDC at LNCB. Our findings suggest that EC and CK34ssE12 are of great value to help characterise breast carcinomas at LNCB, and improve management of patients.

P-015

Recurrence of ductal carcinoma in situ of the breast (DCIS) in a series of 371 patients. A multiinstitutional study

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Aims To assess recurrence rate of DCIS, evaluating initial treatment and histological criteria.
Material and methods 371 patients with DCIS were identified between 1990-2001. DCIS type, nuclear grade, lesion size, margin clearance (Van Nuys Score), initial treatment and recurrence type (DCIS vs invasive carcinoma) were analysed. Median follow-up: 45 months (range 1-146).
Results 12 cases of recurrence appeared in 11 patients (3.2 %). 4.1% (6/146) after mastectomy and 2.7% (6/225) after breast-conserving surgery. Actuarial rate of 5-year recurrence was 5.2% (Kaplan-Meier). 4 cases (36%) recurred as invasive carcinoma or as metastasis.

1st treatment	Recurrence (months)	Grade	Size	Margins	Recurrence type
		(Van Nuys Score)			
T(1)- M+AD(2)	7 and 31	2	1	3	DCIS and DCIS
M	13	3	2	-	DCIS
T - M	15	3	2	-	Axillary MTX
T + RT	17	2	1	1	DCIS
T + AD + RT	21	3	1	1	DCIS
T – M + AD	22	1	2	-	Invasive carc.
M + AD	24	3	2	-	Invasive carc.
T + RT	47	3	2	3	DCIS
T + AD	51	1	2	3	DCIS
M + AD	58	3	3	-	DCIS
T – M + AD	66	2	1	-	Systemic MTX

(T: Tumorectomy / M: Mastectomy / AD: Axillary Dissection / RT: Radiotherapy / MTX: Metastasis)

Conclusions 1) In our series, higher recurrence occurred in patients treated with mastectomy. Recurrence may occur despite radical treatment. 2) No significant differences in grade, size or margin status were observed in patients with recurrence comparing to whole DCIS series.

P-016

HER2 status in breast cancer determined by IHC and FISH: comparison of the results

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Background HER-2 status became an important prognostic and predictive factor in breast carcinoma. There are two main techniques of evaluation HER2 status: immunohistochemistry (IHC) for the protein expression and fluorescence in situ hybridisation (FISH) for amplification of HER2 gene. The aim of the study was to compare the results obtained by IHC and FISH methods in determination of HER2 status in breast cancer.
Materials and methods Two hundred breast cancer specimens were examined. IHC and FISH were performed in every case. IHC was performed with DAKO HercepTest, FISH with Oncor-QBiogene reagents. IHC results were classed into 4 groups, accordingly to the intensity of membrane staining (0,1+, 2+, 3+). FISH results were divided into three main categories: NA - no amplification, LA - low amplification and HA - high amplification. The number of copies of chromosome 17 was also assessed.
Results Over 87% of cases described by IHC as 3+ exhibited also amplification of HER2/neu gene. Remaining cases were positive with IHC, but presented no gene amplification. This might be due to the subjective assessment of the membrane staining or to the procedure of material fixation. Another possibility is that overexpression of the protein was caused by mRNA stability or disorders in receptor circulation. The majority of cases classed by IHC as 2+ were also negative by FISH (over 75%). Nearly one fifth of IHC 2+ were found to exhibit gene amplification. Remaining cases showed no amplification of HER2/neu gene, combined with aneuploidy of chromosome 17. All cases described by IHC as 0/1+ showed no amplification.
Conclusions IHC is well-established method of assessing HER2 status in breast cancer. Nonetheless, group of cases described as 2+ should be additionally examined using FISH method because the results are more reliable. In order to improve accuracy and gain the highest quality of HER2 status evaluation, in those cases both methods should be applied.

P-017

DCIS of the breast with excessive cancerization of the lobules simulating invasive carcinoma

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A 33-years old woman had a right sided mastectomy with axillary dissection performed, because of suspicious mammografic microcalcifications and finding of malignant cells by fine needle aspiration. Grossly, an 9 cm large suspicious area with accentuaded consistency and multiple pale-yellow flecks, representing comedo-necrosis, were found. Multiple sections representing the suspicious area showed DCIS. The ducts were dilated and contained malignant cells and central necrosis. In areas classical cancerization of the lobules were present. Furthermore, in multiple microscopic areas the stroma of the lobules were oedematous and infiltrated by lymphocytes. The acini were slightly dilated and covered by one or two layers of malignant epithelial cells, but the central lumen was preserved. The morphology greatly simulated invasive ductal carcinoma. However, by immunohistochemistry the myoepithelial cells were showed to be preserved and the lobular unit were well demarcated with preserved circumscription. The axillary fat contained 13 lymph nodes all without metastasis. It was concluded that the alterations represented a variant of lobular cancerization, earlier described by Tavassoli (1992).

P-018

Spindle cell tumors of the breast; difficulties in the differential diagnosis

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Spindle cell tumors of the breast are rare entities with difficult differential diagnosis. We report four spindle cell tumors: a metaplastic carcinoma, a fibromatosis, a malignant spindle cell myoepithelioma and an inflammatory pseudotumor. The metaplastic carcinoma occurred in a 66-year-old woman as a 2.5x2.2x2 cm nodule with scarce bland clear cells proliferation and very important desmoplastic reaction; immunohistochemical stains revealed positivity for cytokeratins (MNF116) and vimentin and negativity for actin and desmin. The fibromatosis occurred in a 36-year-old man as an 8x6x5 cm tumor with bland spindle cell morphology; very little atypia and rare mitoses were identified and immunohistochemical positivity for vimentin was demonstrated. The malignant spindle cell myoepithelioma occurred in a 52-year-old woman as 4x3x2 cm nodule with central necrosis; histopathologic examination revealed a malignant spindle cell proliferation with many mitoses and atypical cells; focal positivity for MNF 116, vimentin, actin and S 100 was identified. The inflammatory pseudotumor occurred in a 35-year-old woman as a highly cellular spindle cell proliferation with fascicular pattern admixed with myxoid areas and inflammatory cells – type I-II mammary inflammatory pseudotumor. One important criterion

which should be kept in mind in the process of evaluation of a spindle cell tumor of the breast is that these tumors may have deceptive histopathologic appearance – some of them have bland looking morphology despite of their biological behavior (our example of metaplastic carcinoma) and others with worrisome morphology were diagnosed as benign or intermediate malignancy entities (inflammatory pseudotumor).

P-019

Calcitonin receptor down-regulation in breast cancer. Quantitative analysis of gene expression with laser capture microdissection

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Introduction Calcitonin receptor (CTR) belongs to the G protein-coupled receptors and it binds calcitonin (CT), which acts on bone and kidneys to maintain calcium homeostasis. However, both of CT and CTR have also been identified in a large number of tissue and cell, suggesting it might has any unrecognized roles. Although CTR expression has been found in several breast cancer cell lines and in primary breast cancer, quantitative analysis of CTR expression in matched normal breast tissue and primary breast cancer has not been explored up to date. Here we present the detection and quantitation of CTR gene expression in matched normal breast tissue and breast cancers, by using laser capture microdissection.
Materials and methods Fourteen breast cancers and matched normal epithelium from the same patient were laser capture microdissected to obtain pure cell groups and to submit to quantitative analysis by using one step real time RT-PCR.
Results Both normal breast epithelium and cancer cells expressed CTR. As compared with paired normal epithelium, decreased CTR expression was found in 9 of 14 cases breast cancer (64.28%) while increased CTR expression in 2 cases (14.29%) and no change in 3 cases (21.43%).
Conclusion Our investigation revealed that CTR expression was constantly expressed in normal breast tissue and breast cancers. To the best of our knowledge, this is the first report, which revealed that CTR RNA expressed in normal breast epithelium. Down-regulation of CTR is a common phenomenon in the breast cancer, suggesting that it may have important roles on the development of breast cancer.

P-020

Effect of neoadjuvant chemotherapy on HER 2 status in patients with breast carcinoma

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Introduction Neoadjuvant chemotherapy is the standard of care for patients with locally advanced breast cancer and is being evaluated in patients with earlier-stage operable disease. The effects of chemotherapy on biological markers such as an oncoprotein expression are not well known. The aim of the study was to determine whether Her 2 expression was modified by neoadjuvant chemotherapy.
Methods Forty-nine patients with T2-4N0-2M0 breast cancer were treated in a prospective trial utilizing FEC regimen. Matched pairs of pre and post-chemo therapy breast tissue were evaluated immunohistochemically for HER 2 status using HercepTest Kit (DAKO).
Results Her 2 overexpression was observed in 12/49 patients (24%). The Her 2 status of 41 (83.6%) patients was unchanged after chemotherapy. Modification was found in 6 cases- decreasing in 2, increasing in 3 and loss of expression in 1 patient. Her 2 status was not changed significantly following primary chemotherapy.
Conclusions Our results showed that, in most patients HER 2 status is stable parameter in patients with breast carcinoma after primary treatment with neoadjuvant chemotherapy.

P-021

The microvessel density in female breast cancer among younger and older age groups

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Introduction Breast cancer remains one of the most important oncologic problems worldwide. It seems that breast cancer first detected by the age of 20-40 years follows more aggressive clinical course than tumors developing later. Young females with breast cancer more frequently present with axillary lymph nodes metastases. Expression of the estrogene receptors is less frequent, and the histological grade is usually higher among this group. The main purpose of our study was the evaluation, quantification, and comparison of the angiogenesis in breast cancer among young women, below the age of 35 years and older patients, above 60 years.
Material and methods Tissue samples of breast cancer from patients aged 35 years and less (50 cases), and from patients older than 60 (20 cases) were studied. Ten specimens of non-neoplastic breast tissue from the normal breast in the tumor vicinity (younger patients) served as a control. Microvessels were visualized immunohistochemically with use of CD-34 antibody and counted within the areas of the highest density („hot spots"). Six separate high power fields (HPF), 0.216 mm2 each, were studied in each case.
Results and conclusion The average microvessels count per HPF in the breast cancer tissue in younger group was higher than in the older age group and non-neoplastic tissue. The differences were statistically significant. The increased density of proliferating microvessels seems to play a role in the aggressive clinical behavior.

P-022

Assessment of telepathological frozen section diagnosis of sentinel lymph node of breast cancer

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Introduction Telepathology has now become widely used in Japan. After we introduced telepathology equipment on November 2000,

total 178 frozen section diagnoses have been performed mainly on sentinel lymph node of the mammary gland.The aim of the study was to analyze our result, and estimate whether the telepathology is of practical use or not.
Material Total 178 cases including 244 lymph nodes diagnosed using telepathology.
Results and conclusion We failed to find carcinoma metastasis in 1 out of 244 sentinel lymph nodes of breast. It was very minute metastasis, and was not contained in the transmitted pictures. Our transmission speed is 64 Kb that takes about 8 seconds for each picture. There exists a limitation about the transmittable pictures within a restricted time. Improvement of transmission speed may solve this problem. In two cases, while telepathologically negative of sentinel node, we found metastasis in the other dissected nodes by permanent section examination. The method of sentinel node identification should be improved. There was a confounding of the specimens that occurred between two patients on the same day. It was a case that happened at the beginning of telepathology. Since the surgeon and the technicians are far away from the pathologist, and communication is done by the telephone, it is important for each party to keep in close contact with each other. Our result is fairly good considering the limitation of the telepathology. Although some issues are still remaining, we conclude that telepathology is now of practical use.

P-023

Pigmented mammary Paget disease in a 65 year old man- a case report

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Mammary Paget disease results from intraductal mammary Paget carcinoma that extends to the epidermis of the nipple and areola through a lactiferous duct or from invasive breast carcinoma that reaches the epidermis via direct extension from the dermis. Pigmented mammary Paget disease is a rare clinicopathologic variant of mammary Paget disease. It may mimic malignant melanoma clinically and histophatologically. It seems that involvement of the dermo-epidermal junction by neoplastic cells of the mammary Paget carcinoma seems to be a prerequisite for the development of the clinical pigmentation. We report a case of pigmented mammary Paget disease. The patient was a 65-year-old male who complained of an erythematous, pigmented scaly plaque around his right nipple measuring 8x6 cm with mild fissuring of two year duration. The histopathology revealed pagetoid spread of large cells in the epidermis with pigmentation of basal cells and melanocytes. The IHC confirmed the mammary Paget disease and excluded malignant melanoma and squamous cell carcinoma.

P-024

Frozen section evaluation of sentinel lymph nodes in breast cancer

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Introduction The use of Sentinel Concept as the newest approach in surgical treatment of breast cancer, apart from tumorectomy, means the extirpation and analysis of one or more regional lymph nodes which receives lymph drainage from primary tumor (guardian lymph nodes - sentinel nodes). The presence or the absence of secondary deposit in them is considered the most important predictor of the positive secondary deposit status of the other axillary lymph nodes. Depending on histological analysis of sentinel node, the operation is finished with or without the axillary lymph node dissection.
Materials and methods Sentinel concept in surgical treatment of the breast cancer is applied only in the cases where the largest dimension of the tumor is not more than 2cm (pT1). Marking of the sentinel node is conducted before the operation by a combined method, the usage of Patent Blau color and Technetium 99m as a radio marker. Sentinel lymph nodes identity, before sending to a pathohistological analysis, is checked by gamma detection probe. Two groups of prospective tests (A and B) were formed with 50 cases each. Sentinel lymph nodes from group A in the first stage of the testing were treated by pathohistological method (one hematoxylin and eosin preparation) and then microscoped. The cases in which secondary deposit was not noted, were subjected to a serial cutting and immunohistochemical staining by the EMA markers, specific for intraductal epithelium of a breast. Sentinel nodes from group B were submitted fresh for frozen section investigation, where they were approached with a series of cuttings and immunohistochemical analysis by the EMA markers. The nodes without secondary deposits were fixed in formalin, paraffin embedded and, with the application immunohistochemistry (EMA), submitted to serial cutting (similar to the second phase in group A) as a precaution.
Results In the first stage 50 paraffin samples (group A) were first examined by H&E method, and additionally with immunohistochemistry (using EMA). With this additional examination secondary deposits were found in 6 cases (12 %). This can only prove already known fact about statistically significant higher sensitivity and specifity of immunohistochemistry compared to H&E. In the second stage (group B) 50 new samples of SN, first investigated by frozen section investigation, were examined by immunohistochemichal method (using EMA). In addition, these samples were serially cut and treated like paraffin samples by immunohistochemistry method. The results of these two analysis were identical. Facts mentioned above show that sensitivity and specifity of the frozen section analysis using immunohistochemical method (EMA) is 100% (high percentage is probably the consequence of a relatively small number of samples).
Discussion Acquired data suggested that implementation of one of the used methods (serial frozen section analysis with immunohistochemistry) from the Sentinel Concept is an efficient histo-morphological method in the evaluation of sentinel lymph nodes that make valid intraoperative staging of breast carcinoma possible. The authors came to significant data in both groups of tests and determined sensitivity, particularity and the efficiency of the applied methods.
Conclusion Application of the suggested method would spare a considerable number of patients with pT1 breast cancer of subsequent axillary lymph node dissection and thus reduce the number of postoperative complications and long term morbidity.

P-025

Correlation of histologic prognostic factors in core biopsies and in surgical excisions of breast lesions.

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Introduction Needle core biopsy (NCB) of palpable lesion and stereotactic biopsy (SMB) of non-palpable mass are the major preoperative methods of breast tumor diagnosis. The aim of this study was to evaluate methods accuracy and to emphasize the main detectable prognostic factors.
Materials and methods 809 core biopsies (398 NCBs and 411 SMBs) were performed between January 2000 and December 2002. In 483/510 cases, the correlation between biopsy results and subsequent surgical excision was available.
Results The lesion diameter was < 1 cm (in 45 % of the NCBs and 72 % of the SMBs). With SMB, the average number of tissue core obtained per lesion was 12. At histology, in 67 % of NCB and in 48% of SMB diagnosis of malignancy was given. The DCIS/IDC ratio was 46/219 for NCB and 93/78 for SMB, respectively. Grade and type of the invasive and in situ carcinoma were determined in both biopsy and definitive excision, and the results compared. The agreement for overall grade was in 82 % with SMB and in 66 % with NCB. At surgical excision, 26/139 DCIS showed foci of IDC. In 15 % of SMB cases no residual neoplastic tissue was found.
Conclusions The accuracy of both methods is very high. SMB shows highest reliability and accuracy, because of the increased quantity of tissue obtained. The possibility to assess tumour size is limited. The preoperative assessment of the other prognostic factors (type and grade, tumour markers) are key determinants in planning the optimal breast lesions management.

P-026

Expression of MCP-1 mRNA and macrophage infiltrates in invasive breast carcinoma

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Introduction Monocyte Chemotactic Protein-1 (MCP-1) has been detected in many human tumors; however, its chemoattractant role for macrophages in breast carcinomas is still questionable. The aim of this study was to determine the possible correlation between tumor-associated macrophages (TAM) and the number of MCP-1 mRNA in the invasive breast carcinoma.
Material and methods Tissues obtained from 27 surgical specimens of invasive breast carcinomas and 8 cases of normal breast tissues were frozen in liquid nitrogen and kept at – 80 ^{o} C until they were used for quantification of MCP-1 mRNA by RT-PCR. The expression was calculated as a number of MCP-1 cDNA copies per 100 copies of G-6-PHD mRNA. Paraffin embedded sections from breast carcinomas were immunohistochemically processed for CD68 staining. Positively stained tumor associated macrophages (TAM) were counted on three “hot spots” (x 400) and mean value was calculated.
Results The result of RT-PCR showed that in all cases of breast carcinomas (27/27) and the majority of normal breast tissues (7/8) the number of detected MCP-1 cDNA copies was above the detection limit. Furthermore, carcinomas showed higher levels of MCP-1 mRNA (229.5 ± 275.6) then normal breast tissue (48.06 ± 69.5). The value of TAM within a single carcinoma was very inconsistent (48.3 ± 23.8). Statistical analysis did not find a significant correlation between MCP-1 expression and macrophage infiltrations (r= -0,19592).
Conclusion MCP-1 is an important factor in the recruitment of macrophages. However, the results indicate that MCP-1 is probably not the only and/or crucial factor involved in macrophage attraction to tumor locus in breast carcinomas.

P-027

Comparison of mitotic index, growth fraction (evaluated by Ki-67), ER, PR, cathepsin D, p53, and Bcl-2 status between primary breast cancer and corresponding axillary metastatic sites

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Introduction It has been postulated that the equilibrium of different clones within a tumor is eventually overcome by a biologically dominant one with enhanced metastatic potential. Therefore, the metastatic tumor should be composed of more aggressive tumor cell clones than the primary one. We analyzed mitotic index, proliferation antigen Ki-67, estrogen receptors (ER), progesterone receptors (PR), cathepsin D activity, protein p53, and Bcl-2 oncoprotein status in order to explore the assumed differences in the clonal composition between the primary breast cancer and axillary metastasis.
Materials and methods ER, PR, p53, Bcl-2, Ki-67, and cathepsin D statuses in 60 patients with the primary ductal invasive carcinoma of the breast and at least one axillary metastatic lesion were analyzed by immunohistochemistry (IHC – DAKO LSAB2 System). Mitotic index was assessed by counting the number of mitoses in primary and metastatic lesions. Statistical analysis included t-test for dependent samples, linear correlation analysis, and ROC-curve based contingency table analysis.
Results We found higher mitotic index (p=0.018, r=0.51), higher growth fraction (Ki-67, p=0.045, r=0.71), and higher level of overexpression of Bcl-2 (p=0.014, r=0.86) in axillary metastatic lesion than in primary breast tumor. There were no significant differences in ER (p=0.16, r=0.98), PR (p=0.08, r=0.87), cathepsin D (p=0.92, r=-0.06), and p53 (p=0.36, r=0.87) status. Higher mitotic and Ki-67 rates were associated with lower ER and PR expression, whereas higher cathepsin D level was associated with lower Bcl-2 expression, both in primary tumor and metastasis.

Conclusion Between the paired primary tumor and axillary metastatic lesions, samples had concordant ER, PR, p53, and cathepsin D status when analyzed by IHC. Tumor clone with enhanced metastatic potential might be characterized with higher mitotic index and overexpression of both Ki-67 antigen and Bcl-2 oncoprotein.

P-028

Basaloid breast carcinoma with pleomorphic lipoblast-like giant cells and neuroendocrine differentiation

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Liposarcomatous differentiation in breast neoplasms is a rather infrequent phenomenon and so far restricted to rare cases of malignant phyllodes tumour and true liposarcomas of the breast. The terms lipid-rich and lipid-secreting breast carcinomas encompass breast tumours in which neoplastic cells accumulate or secret lipid; however, lipoblasts are not usually seen. Recently, we have observed a distinctive type of breast carcinoma in which pleomorphic lipoblast-like cells were seen admixed with the carcinomatous component. An 82-year-old female presented with a 5cm hard lump in the right breast with palpable nodes in the right axilla. Mammography and ultrasound disclosed two irregular dense central masses in the right breast, measuring 5cm in aggregate, and an enlarged axillary node measuring approximately 3cm. A diagnosis of breast carcinoma was made and the patient received neoadjuvant tamoxifen despite the lack of estrogen receptor (ER) in the primary tumour. Four months later, her disease had progressed and she underwent right mastectomy with axillary dissection.. Histologically, both the primary tumour and the 3/24 lymph node metastases were characterised by a high-grade carcinomatous component which imperceptibly merged with pleomorphic lipoblast-like cells. Immunohistochemistry demonstrated epithelial differentiation in the carcinomatous and liposarcomatous-like components (epithelial membrane antigen, cytokeratins 5/6, 8/18, 14, 19 and MNF116). Both components were also positive for vimentin, S-100 protein, and focally for chromogranin, GCDFP-15, and E-cadherin. Hormone receptors (estrogen, progesterone and androgen receptors), Her2/neu, p63, smooth muscle actin, desmin, synaptophysin were negative. Based on the histological and immunohistochemical profile, we believe that this case represents a breast carcinoma with basaloid phenotype and showing divergent differentiation (liposarcomatous and neuroendocrine), features that have to our knowledge not been documented to date.

P-029

Collagenous spherulosis arising in an adenomyoepithelioma of the breast: a hitherto unrecognised association

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Collagenous spherulosis (CS) is a benign epithelial and myoepithelial breast lesion characterised by the accumulation of basement membrane material in the form of eosinophilic or rarely basophilic spherules that exhibit concentric and radiating fibrillar patterns. CS is an incidental finding that usually occurs in association with benign proliferative and pre-invasive lesions. Adenomyoepithelioma is a biphasic neoplasm of the breast, composed of both luminal epithelial and myoepithelial cells. Despite the shared histogenesis, CS has never been described in association with adenomyoepithelioma. We report a case of a 48-year-old female who presented with CS of the breast arising in an adenomyoepithelioma. The differential diagnosis included adenoid cystic carcinoma. Immunohistochemistry with antibodies for cytokeratins (CKs) 19, 14 and 5/6, smooth muscle actin (SMA), calponin, smooth muscle myosin heavy chain (SMMHC), p63, collagen IV, oestrogen receptor (ER), progesterone receptor (PgR), Ki67, and c-kit was performed. Myoepithelial cells of the adenomyoepithelioma and the cells surrounding the collagen spherules of CS showed a similar profile (CKs 14 and 5/6, SMA, calponin, SMMHC, p63, and S100 reactivity), luminal cells of both lesions also showed overlapping immunophenotypes (Ck19, ER and PgR positivity). Collagen IV highlighted the spherules in CS. Ki67 labelling indices were similar in both lesions. C-kit expression was restricted to the adenomyoepithelioma and no expression was observed in the CS. The association is important to recognise as it could potentially lead the unwary to a misdiagnosis of adenoid cystic carcinoma, cribriform carcinoma or cylindroma of the breast.

P-030

Lymphoepithelioma-like carcinoma of the breast

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Introduction Lymphoepithelioma of the nasopharynx is an undifferentiated carcinoma with a prominent lymphoid infiltrate. Tumors, with histologic features similar to nasopharyngeal lymphoepithelioma in other locations are referred as lymphoepithelioma-like carcinoma.(LEC). In differential diagnosis of this lesions, medullary carcinoma and lymphoma must be considered. In this study, we report a case of LEC of the breast which is very rare tumor..

Material and method A 59-year-old female was noted to have a lesion of the right breast on mammography. An excisional biopsy was performed and sections revealed a well-demarcated elastic mass of 3 cm in diameter. Routine histologic examinations and immunohistochemical analysis were performed and compared with reported cases in literature.

Results Clinical features, macroscopic and microscopic findings, immunohistochemical analysis were consistent with LEC of the

breast. On immunostaining, the tumor cells showed strong staining for epithelial membrane antigen (EMA) and low weight cytokeratin (LCW).Lymphoid cells stained positively for leucocyte common antigen (LCA).The tumor cells showed no staining for CD30. Staining for UCHL -1 and L26 demonstrated that there were more T cells than B cells. The tumor cells were immunohistochemically negative for Epstein-Barr virus (EBV).

P-031

Comparative evaluation of DNA-ploidy and survival rate of patients with breast cancer

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Introduction This retrospective study was performed to investigate the role of DNA- ploidy and S-phase fraction in determining prognosis in advanced breast cancer.
Methods The study included 68 female patients with breast cancer, investigated for the period 1996-2002. The nuclear DNA-content has been measured by CAS-200 (Beckton Dickinson, Image analysis) on intraoperative cytologic samples of the tumour (imprints). The tumours have been divided into diploid, tetraploid, aneuploid and hyperdiploid.
Results 37 of all patients had aneuploid and multiploid histogram, whereas 75% of them had developed distant metastases. Lymph metastases have been registered in 15% of the cases with diploid histogram. S-phase has been considerably higher in patients with aneuploid than those with diploid histogram. The survival of 57 of all 68 patients has been analyzed as to the type of their histogram and as to the presence of metastases in lymph nodes and distant organs. The results showed that 9 breast cancer patients (15.7%) with aneuploid histogram and metastases in lymph nodes had a survival of less than 5 years, compared to the better survival rate of those patients with diploid histogram and no metastases in lymph nodes. The higher S-stage has correlated to the metastases in lymph nodes and distant locations.
Conclusion The analysis of all results proved that the DNA-morphometry could successfully be used as an independent criterion for evaluation of the biologic behaviour of breast cancer tumours and their prognosis.

P-032

Hypercholesterolemia impairs angiogenesis in patients with breast carcinoma and therefore lowers the risk of metastasis

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Introduction Angiogenesis plays a key role in tumor progression and it is also necessary for the development of distant metastasis. Previous experimental studies indicate that both diet-induced and genetic hypercholesterolemia (HCH) impairs angiogenesis. Our aim was to investigate the effect of HCH on angiogenesis induced by breast carcinoma and to elucidate the possible mechanisms by which it inhibits the progression of the cancer.
Material and method Fifty-one patients with invasive ductal carcinoma were included in the study. Of 51 cases 28 had HCH and 23 had no HCH. All patients were followed for a mean of 48 months after radical mastectomy. The intratumoral microvessel density (MVD) was evaluated immunohistochemically using anti-CD31 antibody and correlated with clinicopathologic parameters. Also the expression of bFGF and VEGF were examined immunohistochemically and graded semiquantitatively.
Results MVD was significantly correlated with tumor grade, VEGF and bFGF expression, recurrence, lymph node metastases (LNM), distant metastases (DM) and survival ($p<0.01$). Patients without HCH had significantly higher MVD (77.4 8.2) than those with HCH (54 5) ($p<0.05$). In addition the risk of LNM and DM were found higher in cases without HCH when compared to cases with HCH ($p<0.05$). Both bFGF and VEGF expression showed significant negative correlation with HCH ($p<0.05$).
Conclusion We suggest that, HCH impairs angiogenesis by suppressing bFGF and VEGF expression and therefore lowers the risk of metastases in cases with invasive breast carcinoma.

P-033

Use of CD10 antibody as a marker of myoepithelial cells in breast lesions

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Introduction The CD10 antibody (CALLA) is usually expressed in lymphoblastic acute leukemia and in follicular center cells of lymph nodes. It is also used in breast cancer cell culture to differentiate epithelial cells from myoepithelial cells. Usually the Smooth Muscle Actin (SMA) is used to identify myoepithelial cells in breast lesions, but this antibody is also positive in blood vessels. This study compares the expression of CD10 and SMA as a marker of myopepithelial cells in breast lesions.
Materials and methods We have compared 61 breast lesions, both benign and malignant (7 adenosis, 8 fibroadenomas, 5 intracystic papillomas, 1 hamartoma, 1 gynecomastia, 9 intraductal carcinomas, 11 invasive ductal carcinomas, 5 tubular carcinomas, 2 mucinous carcinomas, 6 invasive lobular carcinomas, 2 medullary carcinomas, 3 phyllodes tumors). The antibodies used have been CD10 (56C, 1:10, DAKO) and SMA (1A4, 1:200, BioGenex).
Results We observed CD10 expression in the cell membrane of myoepithelial cells and also in some stromal myofibroblastic cells. SMA expression was observed in the cytoplasm of myoepithelial cells, in some stromal myofibroblastic cells and also in smooth muscle cells. Proliferative breast lesions and benign tumors contain myoepithelial cells expressing CD10 and SMA. In malignant lesions the myoepithelial cell component is lost.
Conclusions The CD10 is a myoepithelial cell marker and yields a good, reproducible staining in the studied cases. We think that the use of CD10 combined with SMA is helpful in the differential diagnosis between benign and malignant lesions (epitheliosis vs intraductal carcinoma, tubular carcinoma vs atrophy, adenosis vs invasive ductal carcinoma, papilloma vs intracystic papillary carcinoma).

P-034

p63 expression in benign and malignant breast lesions

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Introduction The identification of myoepithelial cells in breast lesions remains of diagnostic value for the discrimination between benign and malignant lesions. P63 is a p53 homologue, which is expressed in epithelial basal cells of different organs. The aim of the present study has been to investigate the immunohistochemical expression of p63 in breast lesions.
Materials and methods We examined immunohistochemically 266 formalin-fixed, paraffin-embedded, archival breast samples, which consisted of 140 benign lesions and 126 malignancies (30 in situ carcinomas, 62 invasive ductal carcinomas, NST and 34 invasive carcinomas special type). The monoclonal antibodies anti-p63 (Biocare Medical/Menarini), anti-a-smooth muscle actin (Biogenex) and anti-S-100 protein (Biogenex) were used.
Results In all cases, p63 expression was nuclear. In all benign lesions, there was an almost continuous basal rim surrounding the epithelial structures, whereas it was present but discontinuous in the in situ carcinomas. All invasive ductal carcinomas, NST, were devoided of p63 staining. P63 immunoreactivity was noted in a minor fraction of neoplastic cells in 15/24 (62.5%) in situ papillary carcinomas and in 9/27 (33.3%) invasive papillary carcinomas. The stromal cells were negative to p63. There were S-100 positive luminal epithelial cells in almost all cases, whereas many fibroblasts of the stroma were positive to smooth muscle actin.
Conclusion It appears that p63 is a more sensitive and specific myoepithelial marker than those currently used, with no staining of stromal myofibroblasts. The observed staining in scattered cells, only in papillary neoplasms, might be a clue to the speculated different histogenesis of this specific type of breast tumors.

P-035

Prediction of aggressive outcome in T1N0M0 breast cancer

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Introduction Small tumour size at the time of diagnosis is one of the most significant prognostic factor of favourable outcome in invasive breast cancer. However, unpredictable breast cancer recurrences and deaths also occur among the group of patients with T1N0M0 breast cancers. Aims: We set out to identify the clinically useful prognostic features predicting unfavourable outcome in patients with T1N0M0 invasive breast cancer.
Material and methods The study comprises 72 cases of invasive ductal or lobular carcinomas diagnosed and treated in Turku University Hospital and Jyväskylä Central Hospital during the years 1987-1997. Twenty-seven patients had an aggressive disease with a recurrence or death of breast cancer. The prognostic factors of each of the patients with aggressive disease were compared with control patients matched by tumour size, age at diagnosis and histological type of tumour. The follow up time was 4-14 years. The clinicopathological prognostic factors of the patients were surveyed.
Results The prognostic features indicating unfavourable outcome were immunohistochemically detected high Ki-67 expression, high p53 expression, low estrogen receptor expression and positive Her2/neu chromogen in-situ hybridization. The number of mitotic figures (Mitotic Activity Index, MAI), histological type of the tumour or number of studied axillary lymph nodes did not provide prognostic information.
Conclusion Our study summarizes the immunohistochemical features and criteria predicting unfavourable outcome in T1N0M0 invasive breast cancer.

P-036

Detection of overexpression of C-erbB2 through immunohistochemical and amplification of her2/neu established by fish in breast cancer. A comparative study

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Introduction The prognostic and predictive implications of oncogene HER2/neu activation are very controversial, due to the extensive variation of results, mainly due to the different methods applied. Activation of HER2 in node positive patients as an independent prognostic factor has been established and correlated with tumour sensitivity to different therapeutic agents. The overexpression of this membrane receptor has converted it into a new therapeutic aim, requiring the application of a more suitable methodology for each case.
Objectives To evaluate the most suitable method (immunohistochemical vs. FISH) to determine HER2 activation and its correlation with other prognostic factors.
Material and methods A study of 42 cases of invasive breast carcinoma. Histological type: 90% infiltrating ductal carcinoma; age: 30-77, hormonal receptors: 61.9% (RH+), 23.8% (RH-); histological grade (Bloom-Richardson mod): 14.2% (G1), 28.5% (G2), and 47.6% (G3). Immunohistochemistry was performed in 20 patients with (Herceptest Dako), and 22 with anti C-erbB2 (Dako 1:200). 4 scores were used for evaluation: absence of membrane staining (0), faint and incomplete in > 10% of tumour cells (1+), weak to moderate in > 10% (2+), strong complete in > 10% (3+). FISH study was performed on paraffin sections through Path Vision system with evaluation of 60 nuclei.
Results A good correlation between immunohistochemical overexpression and amplification by FISH was observed, especially in cases evaluated as (3+) 7/7 (100%), and as (0) 6/6 (100%). No amplification by FISH occurred in 94.4% of (1+) 17/18 and in 81.8% of (2+) 9/11. 50% of negative hormonal receptor tumours showed amplification, while only 19.2% of positive receptor tumours amplified. None of the tumours classified as (G1) showed amplification.
Conclusions We emphasise the necessity to study gene amplification through FISH in cases with weak immunohistochemical over-expression (2+).

P-037

Expression of p53, p21waf1/cip1, p27kip1 and p34cdc2 in human breast cancers and its prognostic implications

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Introduction Cell cycle progression is governed by cell cycle regulators and inhibitors such as cyclin dependent kinases (cdks), p53, p21/WAF1 and p27/Kip1. The purpose of this study was to correlate the immunohistochemical expressions of p53, p21/WAF1, p27/Cip1 and p34cdc2, with its clinicopathologic prognostic parameters in human breast cancers.
Materials and methods Paraffin-embedded tissue sections from 102 patients with human breast carcinomas were examined for immunohistochemical staining. Primary antibodies used in immunohistochemical staining were mouse monoclonal antibody to human p53, p21/WAF1, p27/Cip1 and p34cdc2.
Results The expression rates of p53, p21/WAF1 and p34cdc2 were 49.1%, 40.2% and 29.3% in breast carcinomas, respectively. In normal breast tissues, p53, p21/WAF1 and p34cdc2 were not expressed. The expression rate of p27/Cip1 was 29.3% in breast carcinomas and 100% in normal breast tissues. The correlation between the expression of p53 and p21/WAF1 was noted($p=0.030$). The expression of p27/Cip1 was correlated with that of progesteron receptor(PR) ($p=0.035$). The expression of p21/WAF1 was correlated with that of estrogen receptor (ER) ($p=0.042$) and PR ($p=0.047$). No correlation was demonstrated between survival in patients and expression of p53, p21/WAF1, p34cdc2 and p27/Cip1.
Conclusion The loss of normal cell growth cycle by abnormal cyclin dependent kinases, inhibitors and steroid hormones may play an important role in human breast carcinogenesis. The p53 dependent p21 pathway, p27 gene loss and cdc2 overexpression were important in development and progression of human breast cancer.

P-038

Withdrawn

P-039

Interobserver variability in determination of HER2-protein overexpression by immunohistochemistry using Herceptest assay - 100 cases study

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Background Since January 2002 until December 2002 in 5088 cases of breast cancers evaluation of HER2 protein was performed. Evaluation of immunohistochemical stains was accomplished in sixteen oncological centers in Poland. Comparison of results achieved by researchers from all participating oncologial centers was the aim of our study.
Material and method Among 5088 cases evaluated in population screening project total 100 cases from all centers were selected and presented to 16 representatives of those centers. The immunohistochemically stained slides came from all 16 laboratories. All IHCs were done using FDA-approved DAKO Herceptest. Evaluation was performed without knowledge of the original
Results The results from each researcher were coded. The study lasted 6 hours. The average concordance was nearly 70%, while the lowest concordance was 51 and the highest - 93%. This wide range of the results might be due to several things. Firstly, the experience in IHC evaluation varied among the participant of the study. Secondly, the quality of the stains differed significantly. Thirdly, the researchers worked under time pressure and the material was selected to include more cases considered 'difficult' (2+ according to DAKO HercepTest or technically inadequate).
Conclusions To achieve higher concordance among the obtained results, evaluation should be performed by the experienced pathologists. * - participants from: Bialystok, Bydgoszcz, Gdansk, Gliwice, Kielce, Krakow, Lublin, Lodz, Olsztyn, Poznan, Szczecin, Warsaw and Wroclaw. The population screening project study was supported by Roche-Polska.

P-040

A morphometric analysis of early invasive breast carcinoma using serial sectioning and 3D reconstruction technique

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Introduction Mechanism of early invasion of breast ductal carcinoma has not been completely investigated not only from the genetic point of view but also morphologically. One of the reason is that starting sites of invasion from ductal carcinoma in situ (DCIS) might be extremely diminutive that routine histopathological examination should hardly reveal them usually. Thus, we examined a large number of complete serial sections from breast carcinomas so as to find out destroyed sites of duct wall from which carcinoma cells have started on invasion. Also we reconstructed 3D images from the sections so as to visualize the sites and to measure the size of those.
Materials and methods Used were three surgical specimens of breast cancer having extensive intraductal components with multiple invasive foci. After formalin fixed and paraffin embedded, six blocks from three cases were selected and sectioned serially in 3μm thickness. Total amount of the serial sections reached 724 sheets (mean 121/1block) indicating 2.172μm in actual thickness. After HE stained, invasive lesions connected with DCIS were searched and the lesions were reconstructed as 3D images with a computer-assisted-3D-reconstruction system.
Results Although numerous sections containing multiple invasive foci were examined, areas which connected with DCIS to invasive lesion through the destroyed site of duct wall were only three. The site were approximately 100μm, 150um and 50μm in diameter respectively, should be called as 'hole' of the duct wall. Invasive lesions were extended along the ducts with the holes.
Conclusion The holes, starting sites of invasion, were apparently smaller than surrounded invasive lesion.

P-041

Histological and immunohistochemical profile of invasive pleomorphic lobular carcinoma

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Introduction Invasive pleomorphic lobular carcinoma (IPLC), a distinct subtype of invasive lobular carcinoma (ILC), shares the typical histologic patterns of classical ILC, but the nuclei are more pleomorphic and increased mitotic activity is a prominent feature.
Methods and results We reviewed 20 cases of IPLC and compared them with 40 cases of clssical ILC. Immunohistochemical analysis was performed in all cases for estrogen (ER) and progesterone receptors (PgR), p53, c-erbB-2, and Ki67. In this series, the average patient age was 54.9 years (age range, 37-76 years) for IPLC and 56.2 years (age range 33-82 years) for ILC. Tumor size ranged from 1 to 9.5 cm (mean size 3.09 cm) for IPLC and 0.9 to 6 cm (mean size 2.8 cm) for ILC. Axillary lymph node metastasis was detected in 75% of IPLC, and 35% of ILC. Fifty percent of IPLC and 15% of ILC cases had extranodal extensions ($p<0.01$). Tumor cells were large with eccentric pleomorphic nuclei and eosinophilic granular cytoplasms. High mitotic activity was observed. Immunohistochemically ER and PgR positivity in IPLC was 75% and 65%, and in ILC 75% and 70%, respectively. IPLC cases showed 50%, and ILC cases 15% p53 positivity ($p<0.01$). C-erbB-2 was detected in 55% of IPLC, and 45% of ILC. Ki67 mean rates were 26% and 12% for IPLC and ILC.
Conclusion IPLC exhibits distinct histologic fetures, higher expression of p53 protein and Ki67 rate in comparison with classical ILC. The aggressive clinical course of patients with IPLC is supported by higher rates of axillary lymph node metastasis and extranodal extensions.

P-042

Does breast phyllodes tumour harbour c-kit mutations?

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Introduction c-kit is a proto-oncogene that codes for a transmembrane tyrosine kinase receptor: CD117. Once activated, c-kit propagates signalling events throughout the cell via multiple signal transduction pathways. It was described an activating mutation in exon 11 of c-kit gene in gastrointestinal stromal tumours (GIST), which can be blocked by using a tyrosine kinase inhibitor (STI571). Phyllodes tumours (PT) are rare biphasic breast tumours that similarly to GISTs show a monoclonal stromal proliferation and high recurrence rates. Aims: To study the immunohistochemistry expression and prevalence of activating c-kit mutations in PT in order to explore whether this disease could be a potential target for treatment with STI571.
Materials and results We analysed 18 PT (12 benign and 6 malignant) by immunohistochemistry for c-kit expression and direct sequencing of exons 9, 11, 13 and 17 of c-kit gene for evaluate the mutational status. c-kit expression was detected in 10 out 18 cases (5/12 benign and 5/6 malignant). No mutations were found for exons 9, 11 and 13. In exon 17 we found the same silent alteration in 2 out of 18 cases.
Conclusions Although c-kit expression is a frequent finding in PT of the breast, we did not find activate mutations similar to those described in GISTs.

P-043

Expression of cyclin D1 in ductal breast carcinoma: relationship with histological parameters and estrogen receptors

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Introduction Recently, the role of cyclin D1 expression in breast cancer had received attention. It causes the activation of cyclin-dependent kinases in G1 phase and phosphorilation of the Retinoblastoma gene, required for entry of cell into S phase of the cell cycle. Recent investigations confer the role of cyclin D1 once again in G2 phase. The aim of this study was to analyse the expression of cyclin D1 in human breast cancer cells and to compare its value with the estrogen receptors and histological parameters.
Material and methods Immunohistochemical analysis for cyclin D1 and estrogen/progesteron receptors (ER/PR) was performed on 40 formalin-fixed infiltrating ductal breast carcinomas. Scoring was done using semiquantitative system. The stainining score was ranged from 0-3 (called cyclin D1-negative) and score larger or equal to 4 (called cyclin D1-positive).
Results According to the staining score 20% of the breast carcinomas were cyclin D1 positive, and those carcinomas were associated with higher nuclear and histological grade, while only 33% were ER-positive.
Conclusion These reslults suggest that higher expression of the cyclin D1 is related with poor prognostic parameters.

P-044

C-erb B2 overexpression in breast cancer with negative lymph nodes

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Background It is known that c-erb B2 overexpression in advanced breast cancer represents a predictive factor of tumoral agressivity. The aim of this study was to assess the involvement of c-erb B2 and other prognostic factors expression (hormonal receptors, EGFR, PCNA) in breast cancer with negative lymph nodes.
Methods We investigated histopathologically and immunohistochemically 30 selected cases with G2 ductal invasive carcinoma and no lymph nodes involvement, from 200 breast cancers. We

used a monoclonal antibody panel (estrogen and progesteron receptors, EGFR, c-erb B2, PCNA) in formalin fixed paraffin embedded tissues.
Results We found a significant increased c-erbB2 overexpression associated with a low ER and PgR expression and minimal micro-angiogenesis as expressed by EGFR. The average of proliferative rate assessed by PCNA was 35.3%.
Conclusion Immunohistochemical c-erbB2 overexpression in negative lymph nodes breast cancer, may suggest an early amplification of its encoding gene, which can predict an aggressive biological behaviour (invasion and metastasis), so that this type of patients would benefit of humanized anti-her2/neu monoclonal antibody treatment.

P-045

Aspiration cytology of the breast with or without ultrasound guidance. Quality control

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Introduction Aspiration cytology of the breast is one of the three components of the triple test for breast cancer diagnosis. Ultrasound (US) guidance increases the complete sensitivity of this technique. The aim of this study was to compare the results between a group of mammary lesions aspirated with or without US using the European Quality Assurance Guidance.
Material and method We analyzed 206 mammary lesions by aspiration cytology with or without US guidance. One group was formed by 103 breast aspirates from women older than fifty years old, included in an screening programme, with palpable and non palpable lesions, using US guidance. Another group was formed by 103 breast aspirates from outpatients origin of all ages only with palpable lesions using no US guidance. We analysed and compare the results between both groups using using the European Quality Assurance Guidance for Breast Screening Pathology.
Results In both groups the positive predictive value of the C5 results were 100% (false positive rate=0%). The complete sensitivity of US guided aspirates were 91.1% and for the non guided aspirates were 83%. We compare the rest of values and analyze our deviations from the standards.
Conclusion The quality of our breast aspiration practice is between the margins of the standards in both techniques, with an increment in the sensitivity with US guidance and a light loose of specificity in the screening group (probably because of patients age characteristics).

P-046

Ultrastructural study of intercellular junctions in metastatic and non-metastatic breast cancer

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Introduction Functional and morphologic characteristics of intercellular junctions are involved in tumor dissemination. The purpose of this study was to investigate whether the number of intercellular junctions in breast cancer is predictive for the presence of lymph node metastases.
Materials and methods 76 infiltrating duct carcinomas were the subject of this study, 50 of them without (G1) and 26 with metastatic disease (G2) in axillary lymph nodes. The number of intercellular junctions was counted, and the length of the cell-to-cell contact surfaces was measured in randomly taken electron micrographs. The number of desmosomes/100µm of cell membrane apposition has been statistically compared between both groups.
Results Cell junctions in tumors with lymph node metastases were usually smaller and poorly developed. There were no statistically significant differences in the number of intercellular junctions between both groups [G1=14%, (10,75%; 25%). G2=12,5% (7,75%; 29%). p=0,546].
Conclusions Although intercellular junctions are more rudimentary in tumors that metastasize, their number is not significantly reduced. Therefore, it will be necessary to combine number, size and development of cell junctions in a single parameter to attempt prediction of tumor dissemination.
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P-047

Analysis of the relation between presence of myoepithelial cells in tumour stroma and expression of prognostic indices in tumour cells of ductal breast cancer

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Introduction Numerous publications have been devoted to effects of myoepithelial cells on biology of breast cancer. The cells are thought to be able to inhibit invasion of neoplastic cells by production of inhibitors for serine proteases. Some authors suggest that, acting in a paracrine way, myoepithelium can affect breast cancer cells. Present study aimed at analysis of the relation between presence of myoepithelial cells (smooth muscle actin, SMA) in the structure of a ductal breast cancer and expression of the recognized prognostic factors (ER, PR, HER-2, TGF alpha, Ki67 and p53) in the cancer cells.
Materials and methods The studies were performed on samples of primary ductal breast cancers originating from 60 females, including 20 cases at G1, 20 cases at G2 and 20 cases at G3 grade. Immunocytochemical reactions were performed using antibodies directed to SMA, ER, PR, HER-2, TGF alpha, Ki67 and p53.
Results The study showed that the highest content of myoepithelial cells was noted in tumours of G1 grade while the lowest content of the cells was observed in tumours of G2 grade (Kruskal-Wallis ANOVA rank test, p=0.0018). In the entire group (G1-G3) a positive correlation was detected between the presence of myoepithelial cells and intensity of HER-2 expression (gamma correlation, p=0.0017) and a negative correlation with proliferation intensity measured by expression of Ki67 antigen (gamma correlation, p=0.00067). No relation was observed between manifestation of myoepithelium and expression of the other studied variables.

Conclusion Our studies confirmed the potential for paracrine communication between myoepithelial cells and cells of ductal breast cancer.

P-048

Immunohistochemical VEGF expression in breast carcinomas. Correlation with prognostic factors

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Introduction VEGF (vascular endothelial growth factor) is considered as one of the most important angiogenic factors in breast cancer and its expression in tumor cells correlate with microvessel density. Several studies have proposed that VEGF may be a relevant prognostic factor. Aim: To study the immunohistochemical expression of VEGF in different histological types of breast cancer and its relation to established prognostic and predictive factors.
Material and methods We have analyzed 84 carcinomas (55 of invasive ductal type, 15 lobular, 5 mucinous, 2 papillary and 7 ductal in situ). Immunohistochemical staining was performed on formalin fixed, paraffin sections, using an avidin-biotin immunoperoxidase technique. A rabbit polyclonal antibody for human VEGF was used at a 1:100 dilution. Immunoreactivity was scored from 0 to 3+.
Results 44 (61,3%) of all invasive carcinomas showed VEGF expression (ductal: 61%, mucinous: 100%). All but 2 lobular carcinomas were negative or weakly positive. Both papillary carcinomas showed strong positivity. Staining in DCIS was generally absent or weak. VEGF positivity correlated inversely with nodal involvement (p=0.001) and RE status (p=0.05). There were no correlation of VEGF staining with tumor size, histological grade, p53 and c-erbB-2.
Conclusion Expression of VEGF is characteristically weak or absent in lobular carcinomas and them no relevant as a prognostic factor in this type of carcinomas. As VEGF staining correlate with absence of nodal involvement, its prognostic value may be mainly due to facilitate invasion of blood vessels, more than to lymphatic spread.

P-049

Clinical and histopathological parameters as predictors of clinical outcome for patients with vulvar carcinoma

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Introduction Squamous carcinoma accounts for approximately 90% of all primary vulvar carcinomas. Although the anatomic localization of this tumor allows early diagnosis and considerably easy surgical accessibility, the prognosis for these patients remains relatively grim. The explanation for this finding should be sought in the fact that most of the patients are diagnosed in the advanced clinical stage of the disease. Since radical vulvectomy (a complex and debilitating procedure) is often a therapy of choice, the recent studies have been focused on determining reliable prognostic parameters that would allow individualized, and hopefully simpler therapeutic modalities to be applied in a certain group of patients with the same effectiveness. The aim of this study was to determine the most important clinical and histopathological parameters that influence the prognosis and outcome of patients with invasive squamous carcinoma of the vulva in order to offer them the most suitable form of therapy. Moreover, it was designed to assess the clinical significance of DNA content analysis by flow cytometry.
Patients and methods 43 cases of squamous cell carcinoma of the vulva, diagnosed between 1978. and 1996. were selected. In order to assess the influence of the various prognostic factors on survival, the study included clinical and pathohistological parameters as well as the results of flow cytometric DNA analysis of paraffin-embedded tumor samples from all cases. Clinicopathological parameters focused on the age of patients, clinical stage of the disease, menstrual status, diameter and localization of the tumor, histological grade, nuclear grade, combined histological and nuclear grade, depth of tumor invasion, tumor growth pattern, presence of giant cells in the tumor and the form of therapy, while the flow cytometric DNA analysis comprised DNA ploidy, proliferative activity and DNA index.
Results Five-year survival of our selected population of patients was 62,3+/-7,8%. The results of univariate statistical analysis confirmed that statistically significant prognostic parameters included the age of patients (p=0,033), clinical stage of the disease (p=0,014), histological grade (p=0,047), nuclear grade (p<0,001) and the depth of invasion (p<0,001). On the other hand, the results of multivariate statistical analysis showed that only combined histological and nuclear grade (p=0,015) and the depth of tumor invasion (p<0,001) can be considered independent, statistically significant prognostic parameters.
Conclusion The age of patients at the time of diagnosis, clinical stage of the disease, histological grade and nuclear grade are considered to be the parameters of crucial relevance for the prognosis of patients with squamous cell carcinoma of the vulva. However, only combined histological and nuclear grade, as well as the depth of tumor invasion were proven to be independent statistically significant parameters relevant for the outcome of patients with this disease.

P-050

Can a pleomorphic vulvar leiomyoma be a tumor with local aggressive behavior? A case report

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Introduction Vulvar leiomyoma is uncommon and its pleomorphic or bizarre form is especially rare. Bizarre vaginal and vulvar (Bartholin's gland area) leiomyomas were reported in only few cases. This tumor is hard to differentiate from leiomyosarcoma. The main criterion to make a distinction between them is the mitotic activity, which is less than 3 per 10 HPF in the former.
Case report A 26-year-old female was referred to the clinic for the evaluation of paravaginal tumor in distal vaginal third and right vulvar area with a history of 6 month duration. She had no history of any earlier disease, and other examinations revealed normal findings. Macropathology: The tumor was a large solid mass measuring 10x7.5 cm in diameter. The tissue was yellow and seemed partly necrotic.

Micropathology Tumor consisted mostly of spindle cells arranged in bundles with round or oval nuclei and large prominent nucleoli or showed hyperchromatism in places where there were cells with giant nuclei. Cytoplasm was eosinophilic, elongated with small vacuole. There were areas where cells had two or more nuclei with the earlier mentioned polymorphism. Areas of hyaline or myxoid degeneration of the stroma were found. Mitoses were not found although the tumor was examined by three independent pathologists from different countries. The tumor margin looked infiltrative. The tumor was immunohistochemically positive for vimentin, smooth actin and desmin. Cytokeratin MNF 116, EMA, S-100, CD-34, and CD-117 were negative.
Conclusion We describe a vulvar smooth muscle tumor with marked polymorphism, without mitoses, with myxoid changes in the stroma and with infiltrative margins. Because of all these characteristics the tumor was considered biologically aggressive and additional surgery with resection of the tumor site and regional lymphadenectomy was performed. Three years after the last surgery the patient is alive and well.

P-051

Trophoblast apoptosis and proliferation in preeclamptic placentas

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Introduction Preeclampsia is a specific disorder of pregnancy with an increased risk for both neonatal and maternal mortality rates. It is associated with shallow invasion of the placental cytotrophoblast within uterus developing decidual vasculopathy and results in dereased blood flow. The aim of the present report is to determine whether hypoxia influences the rates of apoptosis and proliferation and to compare them with those of normal controls.
Materials and methods Samples were taken from 20 liveborn, singleton preeclamptic pregnancies and 20 normal controls. We used routine light microscopy, immunohistochemical and quantitative studies for Bcl-2, and Bax (to assess apoptosis) and PCNA (to assess cellular proliferation). Bcl-2- and PCNA-positive trophoblast cells were counted in a minimum of 10 high-powered fields, of 3 consecutive sections free from any infarction of at least 3 paraffin blocks.
Results While increased apoptosis has been estimated in study groups, the rates of proliferation seems to be unaffected.

P-052

Placental pathologic features of preeclampsia

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Introduction The clinical and placental features of preeclampsia are subject of an extensive study. Clinical evidence suggests that this pregnancy specific disorder may be demographically heterogenous. Our purpose is to record the range of the most common pathologic features in placental specimens from a gypsy cohort and to compare them with those in nonethnic Bulgarian population.
Materials and methods Review of 20 preeclamptic placentas from liveborn singleton deliveries of gypsy mothers and 20 matching controls from Bulgarian population revealed histologic lesions that were considered as belonging to one of the following groups: (1) vascular lesions; (2) acute or chronic inflammation and (3) coagulation-related lesions. For each separate case, additional demographic and reproductive history was collected from the patients' charts. All placentas were studied by previously published standard protocols. Nonparametric statistical analysis was applied.
Results In gypsy preeclamptic group, lesions related to coagulation (52% vs 39%) and vascular lesions (26% vs 29%) were more frequent. Inflammatory features were equally distributed among the two groups (22%).

P-053

Versican in epithelial ovarian cancer; relation to hyaluronan, clinicopathological factors and prognosis

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Introduction Versican is a chondroitin sulfate proteoglycan, which belongs to the aggrecan gene family. It has not been studied in epithelial ovarian cancer before. The aim of this study was to clarify the relation between versican and hyaluronan in epithelial ovarian cancer and to find out if versican expression affects prognosis in these patients.
Materials and methods Localization of versican was studied immunohistochemically in 299 primary epithelial ovarian cancers and their 43 metastases, as well as in 6 normal ovary specimens. The staining was scored according to the percentage area of strong versican signal of total peri- and intratumoural stroma as low or high. Epithelial staining of the tumours was scored as positive or negative.
Results Low and high levels of strong stromal versican staining were observed in 133 and 166 carcinomas, respectively. A low level of strong stromal versican was found to be a significant marker for early FIGO stage and mucinous histology. Stromal versican staining correlated significantly with stromal hyaluronan staining. Cancer cell-associated versican positivity was seen in 151 tumours and correlated with clear cell histology and early FIGO stage. The 5-year prognosis of patients decreased with increasing stromal versican levels for overall survival. The 5-year prognosis for recurrence-free survival was slightly better when cancer cells were positive for versican. In Cox's multivariate analysis neither stromal nor cancer cell-associated versican had any prognostic value.
Conclusion Enhanced stromal versican expression correlates with hyaluronan accumulation and is associated with unfavourable disease outcome, but it is not an independent prognostic marker in epithelial ovarian cancer.

P-054

Cytobrush-collected malignant cervical epithelial cells display a cancer-specific gene expression pattern in cDNA array analysis

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Introduction Although it is well known that persistent high-risk HPV infections are the principal causative agents of cervical cancer, the discrepancy between high infection rates and the relatively low rates of high-risk HPV positive women who actually develop carcinoma of the cervix suggest that development of cervical cancer is a multistep process involving additional factors and mutational events. Therefore, differentially expressed genes in normal and malignant cervical epithelial cells and the feasibility of gene expression profiling on cervical cells obtained by regular cervical swabs were investigated.
Material and methods Cervical epithelial cells obtained by regular cytobrushes from 3 women with high-risk HPV positive invasive cervical cancer and from 3 HPV negative women without suspicious PAP smears were pooled and subjected to cDNA array analysis. Dot blotting was then performed to detect the proteins which corresponded to the most highly up-regulated genes in the cDNA array analysis.
Results mRNA expression of c-erbB-2, C-kit, VEGFR-1, N-myc, K-ras, cyclin-dependent kinase inhibitor 4 (p16INK4A), cyclin D1, NM23-H1, NM23-H2, c-MET, KGF, KGFR, and STAT 1 was increased in malignant samples. Several genes associated with anti-apoptosis, such as bcl-2 like protein, cell structuring and/or cell attachment were also upregulated in tumoral cells. Conversely, decreased gene expression was observed for members of the transforming growth factor receptor superfamily (TGF-beta), interleukin 1 (IL-1), insulin-like growth factor binding proteins (IGFBPs) and for members of the integrin family.
Conclusions We identified a mRNA and protein expression pattern that might have an important role in the pathogenesis of invasive cervical carcinoma and appears to be specific for malignant cervical epithelium. We hypothesize that the presence of a cancer-specific gene-expression signature assessed by cDNA array analysis or immunohistochemical techniques could eventually turn out to become an additional tool to evaluate the dignity of routinely obtained cervical swabs.

P-055

Prognostic features and molecular markers in ovarian borderline tumors

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Introduction Ovarian cancer represent the fourth most common cancer in women. Borderline ovarian serous tumors (BSOT) affect women of childbearig age and their prognosis is outstanding. The aims of this study were to characterize the group of patients with BSOT and evaluate the significance of various molecular markers expression versus serous papillary ovarian carcinomas (SPOC)
Material and methods We analyzed a total of 102 cases including: 64 cystadenoma, 10 borderline and 28 cystadenocarcinoma. For the BSOT the median age was 40 years. Eight women with BSOT underwent radical surgery and two fertility sparing surgery. The recurrence of disease appeared in one case with fertility sparing surgery. All the cases were analyzed on haematoxilin-eosin stained and immunohistochemistry (the indirect tristadial Streptavidin-Biotin method) for estrogen receptor (ER), progesteron receptor (PR), p53 protein (p53), proliferative activity (Ki67) and carcinoembrionic antigen (CEA).
Results The immunostaining demonstrated a significant increase in the expression of ER (90%) and a decrease for p53 (10%) and Ki67 (6%) in BSOT vs SPOC (ER-60%, p53-50% and Ki67-40%).
Conclusions The main differences between BSOT and SPOC was regarding the expression of p53 and Ki67. There was no significant correlation between Ki67 and the known histologic parameters of prognostic significance. Transvaginal ultrasound and CA 125 serum levels are the most effective diagnostic techniques for the follow up of patients treated conservatively for BSOT.

P-056

Gonadoblastoma and germ cell tumours of the ovary in childhood and adolescence

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Introduction Gonadoblastoma with dysgerminoma or other germ cell tumor occurs almost exclusively in girls and young women with underlying gonadal disorder. The aim of this study was to review biopsy examinations of the tumours, stage, treatment strategy, follow up and outcome of five girls with gonadoblastoma and germ cell tumours.
Material and methods Retrospective review of 5 patients with gonadoblastoma and germ cell tumours was performed. The patients were 13,14, 14, 15 and 17 years old. The first 14-year-old girl (karyotype, 46 XX, normal ovary bilateral) with gonado-blastoma, dysgerminoma and choriocarcinoma had IIIc stage disease. She was treated by chemotherapy with cisplatinum and radiotherapy. Four patients had gonadoblastoma and dysger-minoma. The second 14-year-old girl (mixed gonadal dysgenesis, karyotype, 46, XY) had stage Ib and was treated by chemotherapy with cisplatinum. Two patients were treated with surgery only: 17-year-old girl (streak gonads, karyotype, 46, XY) - stage Ib and 15-year-old girl (karyotype 46, XX, der 22/ 45, XX, - 22) - stage Ia. The 13-year-old patient (karyotype, 46, XX, normal ovary bilateral) - stage Ia disease, had adnexotomy only, but because of recurrence (stage IIIc) 13 months after the original diagnosis, she was treated by chemotherapy.
Results Our review confirmed the original diagnosis of gonado-blastoma and dysgerminoma and in one case gonadoblastoma, dysgerminoma and choriocarcinoma. All patients were followed up for 42 to 170 months. Only one patient, who was initially treated with surgery only (stage Ia), had disease recurrence, but achieved

durable remission with chemotherapy and further surgery. The latest examinations revealed that all patients were in good health.
Conclusions Because the gonads are often replaced by a tumour, a diagnosis of the underlying abnormality may be impossible. Gonadoblastoma is regarded as in situ malignancy, and there is a high possibility for development of invasive germ cell tumour such as dysgerminoma. Young girls with unilateral encapsulated gonadoblastoma and dysgerminoma can be treated only by unilateral oophorectomy or salpingo-oophorectomy and subsequent careful follow up to prevent damage of contralateral ovary and preserve the fertility. The patients with gonadoblastoma and mixed germ cell tumor are treated in accordance with other tumour structures. The paper is sponsored by Ministry of Health of the Czech Republic (International Grant Agency) No 6511 – 3, CZ

P-057

Cadherin/catenin expression in squamous lesions of cervix

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Introduction Cadherins and their associated cytoplasmic protein catenins are cell adhesion glycoproteins that are altered in tumor progression The aim of this study is to reveal the altered expression of E-cadherin, P-cadherin, α-catenin and β-catenin in cervical squamous cell carcinoma progression from dysplastic lesions (low grade squamous intraepithelial lesion-L-SIL, high grade squamous intraepithelial lesion-H-SIL) to invasive squamous cell carcinoma.
Material and method Immunohistochemical method was used in formalin-fixed, paraffin embedded representative tissue sections of 25 L-SIL (CIN I), 25 H-SIL (CIN II-III) and 30 invasive squamous cell carcinomas. The presence of immunostaining and the pattern of distribution of E-cadherin, P-cadherin, α-catenin and β-catenin were analyzed.
Results E-cadherin was detected in 91.7 %, 41.7 %, 6.9 % of L-SIL, H-SIL and invasive squamous cell carcinomas respectively. The difference was statistically significant (p=0.0001). The expression of P-cadherin and α-catenin was reduced significantly from L-SIL through invasive carcinoma (p=0.009 for P-cadherin, p= 0.0001 for α-catenin). β-catenin expression was revealed in all lesions with nearly the same frequency, 75.9 % L-SIL, 83.3 % H-SIL and 79.2 % invasive carcinomas.
Conclusion Loss of adhesion proteins, E-cadherin, P-cadherin and α-catenin is observed in cervical squamous cell carcinoma progression. The presence of β-catenin in H-SIL and invasive carcinomas might suggest its role in tumor progression by its effect in Wnt signaling pathway in cervical carcinomas.

P-058

Cadherin/Catenin alterations in squamous cell carcinoma of cervix. Correlation with clinicopathologic variables and Human papilloma virus (HPV) status

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Introduction P, E- cadherin and their cytoplasmic domains alpha and beta-catenins are epithelial cell adhesion complexes. Their alteration is important in tumor progression. Beta catenin is also involved in Wnt signaling cascade which regulates cell proliferation. We investigated the altered pattern of cadherin/catenin complex in cervical squamous cell carcinomas. We also tried to find out if there was a correlation between adhesion proteins and clinico-pathologic variables (stage, depth of invasion, lymph node met-astases) and HPV status.
Material and methods E-cadherin, P-cadherin, alpha catenin and beta catenin expression in 15 squamous cell carcinomas were investigated by immunohistochemical method. The presence of HPV 16/18 infection was detected by in situ hybridization.
Results There were 11 large cell keratinizing, 3 nonkeratinizing and 1 small cell subtypes of squamous cell carcinoma. Lymph node metastases were found in 6 carcinomas. HPV 16/18 infection was detected in 8 of 15 carcinomas, all were large cell keratinizing except one. No correlation was found between the HPV status and cadherin and catenin expression of the tumors. E- cadherin was negative in 12 carcinomas, P- cadherin was positive in only 4 carcinomas. Strong cytoplasmic beta catenin positivity was revealed in 11 carcinomas. Alpha catenin reactivity was seen in 5 of 15 carcinomas. Neither cadherins nor catenins expressions were correlated with other clinicopathologic variables.
Conclusion Our results reveal that E-cadherin and P-cadherin expressions are lost in squamous cell carcinomas. However alpha and beta catenin immunopositivity in cervical squamous cell carcinomas might suggest catenins' role in tumor progression in a different way other than their activity in cell adhesion.

P-059

Prognostic significance of karyotype analysis in ovarian carcinomas

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Introduction Traditional prognostic factors of ovarian carcinoma (OC) include disease stage, age, histologic grade and residual disease after surgery. At present it is quite difficult to predict the survival benefit of platinum analogues based chemotherapy. The aim of this study was to evaluate the clinicopathological / cytogenetic profile in a series of OCs and the reliability of karyotype in predicting benefit of chemotherapy and prognosis.
Materials and methods A series of 44 OCs (28 serous, 10 undifferentiated, 3 endometrioid, 2 mucinous and one mixed carcinomas) was investigated for grade (1/2:29% vs3/4: 71%), stage (I/II: 43% vs III/IV: 57%), residual disease after surgery (59%), chemotherapy (81%), remission (75%) vs progression (25%) after chemotherapy and follow-up (mean:56, range: 15-96 months). Cytogenetics were performed on direct preparations using the QFQ banding technique and describing abnormalities according to ISCN. Karyotypes were classified as diploid/near-diploid (41%)

and complex (hyperdiploid, multiple cell lines, fragmented chromosomes) (59%). Statistical analysis using chi-square and Fisher test was done.
Results Statistical analysis demonstrated that low stage (p=0.00005) and low grade (p=0.0004) were predictors of good prognosis, while residual disease correlated with a poor outcome (p=0.00001). Diploid/near-diploid karyotype correlated with a favourable prognosis (chi-square: 3.84). Complex karyotypes appeared much more frequently associated with chemoresistance (57% vs 7%).
Conclusion Our results confirm the correlation between grade, stage, residual disease and prognosis of OCs. In addition, these data suggest that a diploid/near-diploid karyotype might predict benefit of chemotherapy and favourable prognosis.

P-060

Behaviour of microinvasive (MIC) squamous- and adenocarcinoma of the uterine cervix in the light of world literature and experience from our clinic

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Introduction The aim of this study was to critically assess the progression- and recurrence rates of microinvasive (MIC) Stage IA cervical carcinoma, based on Medline search, and to compare these data with our own experience on these tumours in Ljubljana Medical Centre.
Materials and methods In the 25 published studies covering the years 1976-1999, a total of 4.311 squamous cell MICs were reported, including 2.967 stage IA1 lesions, 1.269 in stage IA2, and 75 cases of occult stage IB, classified according to FIGO or SGO classifications. Our experience in Ljubljana is based on the management of 400 stage IA squamous cell carcinomas (SCC), accumulated in the past 30 years and 33 early invasive adenocarcinomas (AC) collected in 5 years.
Results In the reported 4.311 SCC MIC cases, lymphatic space involvement was recorded in 342 (8%) cases, and in 53 of those, the disease recurred, and progressed to death in 15 patients. In 14 series, MICs were associated with 72 (1.7%) recurrences. In seven series, 20 (0.5%) cases of death due to cancer were reported. Lymph node metastases were found in 30 (0.7%) cases. Even when stage IA1 and IA2 carcinomas were treated with conservative surgical approach, very low risk of recurrence (1.7%), lymph node disease (0.7%), or death caused by cancer (0.5%) was reported by these studies. Most authors conclude that MIC with the depth of invasion <3 mm, without confluent growth or lymphatic invasion can be safely treated with conization. During 1966-1972 lymphadenectomy was included in 290 operations of MIC in Ljubljana. The pelvic LNs were invariably free of cancer. The 5-year survival was 97%, while 6 patients were lost to follow-up. In Medline search 1.170 cases of stage IA ACs were analysed in a 35-year period. Lymph node metastasis were found in 15 (1.3%) of 531 cases with lymphadenectomy, complicated with recurrent disease in 11 (2.1%) cases Compared to the SCC MIC, the risk of recurrence in AC was considerably higher, 3.4% and progression to death 1.4% (436 cases analysed by Östör, 2000). In our 33 AC patients diagnosed in stage IA there was no recurrence or death of disease.
Conclusions According to the world literature and our own experience, the prognosis of MIC of both squamous and glandular type is very favourable. This suggests that conservative management of stage IA cervical cancer is indicated and safe when strict evaluation of tumour extension and surgical margins of the cone are assured.

P-061

AgNOR counts, histological and immunohistochemical characteristics of placenta in preterm delivery with live and dead newborns

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Introduction The pathology of placenta constitutes one of the most poorly understood domains of general pathology. The significance of histological characteristics of preterm delivery is disputable. The aim of this study was to investigate histological and immunohistochemical characteristics and AgNOR counts of placenta in preterm delivery with alive and dead newborns.
Materials and methods We studied placental tissues in preterm deliveries of 19 live (first group) and 12 dead (second group) newborns (N1 Obstetrics Clinic of TSMU, 1999-2002). Paraffin sections were stained using H&E, AgNOR (Bio-Optica) and immunohistochemistry by Ki-67, CD68, CD31, bcl-2 (DAKO).
Results In the first group the number and area of chorionic villi was 2 times lower, the number and area of villous vessels 10.5 times higher, the number of villous lymphocytes is 4.1 times higher, neutrophils 1.27 times higher, Hofbauer cells 1.6 times lower, syncytiotrophoblasts 2.5 times lower (their area was the same), syncytial knots 2 times lower (their area is 2 times higher). The width of Nitabuch layer was 2 times greater, decidual tissue 2.2 times greater, the number of decidual cells was 3.3 times higher, decidual lymphocytes 1.2 times higher, neutrophils 1.4 times higher, macrophages 1.2 times lower, AgNOR(+) syncytiotrophoblastic cells 1.8 times lower, AgNOR granules did not differ, AgNOR(+) decidual cells 3 times higher, AgNOR granules 1.2 times higher, Ki-67(+) syncytiotrophoblasts and cytotrophoblasts 2 times higher.
Conclusion Preterm delivery shows incoordination between proliferation and involution of placental tissue. In death of newborns, morphological changes reflect non-efficacy of adaptative-compensative reactions of chorion-decidual system.

P-062

Tumor suppressive function of gelsolin in ovarian carcinoma

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Introduction The ubiquitously expressed actin-binding protein gelsolin is known to play a role in modulation of the actin network and regulation of cell motility and cell growth. Decreased gelsolin expression occurs in transformed cells and in carcinomas of the colon, bladder, breast, lung and prostate.
Methods and results In the present study we analyzed expression of gelsolin in 241 matched human normal and tumor tissues using a Cancer Profiling Array.

Results We found a decreased expression of gelsolin in cancer tissue of female reproductive organs including ovary. Further we examined the expression of gelsolin in human benign and malignant ovarian tissues by immunohistochemistry, observing a diminished expression of gelsolin in borderline tumors and ovarian serous carcinomas compared to epithelium of normal ovaries and benign adenomas. In addition, low levels of gelsolin protein were observed in four of six ovarian carcinoma cell lines in contrast to immortalized ovarian surface epithelial cells. Re-expression of gelsolin in the ovarian carcinoma cell lines OAW42 and ES-2 resulted in a suppression of tumor cell survival in vitro. Finally, we showed an up-regulation of gelsolin in ovarian cancer cell lines after inhibition of both DNA methylation and histone deacetylation using 5-aza-2'deoxycytidine and Trichostatin A, respectively.
Conclusion Our results suggest that the gelsolin gene has a potential function as a class II tumor suppressor gene in human ovarian epithelium.

P-063

Steroid cell tumor of the ovary: presentation of two cases

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Two cases of steroid tumor of the ovary, diagnosed at our department during the last five years among a total of 254 ovarian tumors, are presented. Steroid cell tumors, previously classified as lipid or lipoid cell tumors are rare neoplasms of sex-cord-stromal origin derived in almost all cases from ovarian stromal lutein cells or from Leydig cells and rarely from adrenal rest cells. They have been subclassified as steroid cell tumors not otherwise specified (NOS), stromal luteomas and Leydig cell tumors. In our report we present two cases of the NOS subtype: one occurred in a 24-year-old woman and the other in a 72-year-old postmenopausal woman. Both patients presented with a pelvic mass and signs of virilization (resulting effect of elevated serum testosterone). Both tumors were composed of characteristic eosinophilic-granular or vacuolated (lipid rich) neoplastic cells lacking crystals of Reinke and expressing the appropriate immunohistochemical phenotype. Based on thorough pathological examination and evaluation of several findings such as tumor size, necrosis, haemorrhage, cellular atypia, mitotic activity, we classified the first case as a malignant and the second as a benign one. Various aspects of the clinical and pathological presentation, differential diagnosis and treatment of these tumors are discussed.

P-064

Choriocarcinoma following term pregnancy

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Introduction Choriocarcinoma is the most malignant form of gestational trophoblastic disease. Most cases develop after an abnormal gestation mainly complete hydatidiform mole. Uncommonly choriocarcinoma follows a normal pregnancy. The aim of this study was to stress the importance of a correct and rapid diagnosis which will allow a high rate of success in therapy for this neoplasm.
Material and methods Histopathologic and immunohistochemical studies were performed to an endometrial curettage and a hysterectomy specimen.
Case report A 33–year-old female presented with uterine bleeding three months after a normal delivery from a term gestation in which placenta was not submitted for study. Endometrial curettage was performed, and it showed a dimorphic population of atypical cytotrophoblast and syncytiotrophoblast cells. Cytotrophoblast cells had pale cytoplasm with single nucleus, eosinophilic nucleoli and prominent mitotic figures. Syncytiotrophoblast appeared as large multinucleated cells with polygonal basophilic cytoplasm. Extensive necrotic and hemorrhagic areas were found. There was no presence of placental villi. Neoplastic cells expressed strongly ß-hCG and cytokeratin. With a diagnosis of choriocarcinoma, hysterectomy was performed and showed areas of normal proliferative endometrium, and hemorrhagic areas with nests of trophoblastic neoplastic cells infiltrating among myometrial muscle. There was no involvement of cervix or adnexa. Treatment was completed with chemotherapy, and one year after diagnosis the patient has not evidence of disease.
Conclusions The modern treatment with chemotherapeutic drugs is highly effective in complete cure of choriocarcinoma. Therefore a quick histological diagnosis has a very important role to reduce the risk of metastasis disease, even avoiding surgery to preserve reproductive function.

P-065

Role of angiogenesis in uterine leiomyomas treated with GnRH agonists

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Introduction The mechanism of action and the histological features of uterine leiomyomas (UL) treated with GnRH are still a matter of debate. Recent reports suggest that neovascularization induced by specific angiogenic molecules may play a role in the response to GnRH. Also a reduced proliferative activity was observed and postulated as a possible histopathological feature of UL treated with GnRH. The aim of this study was to assess the possible role of angiogenesis and to quantify the percentage of proliferative cells in uterine leiomyomas in response to treatment with GnRH from premenopausic patients.
Material and methods A total of 60 patients were studied, with two groups comprising 30 samples each, including patients treated with GnRH and age-matched control patients. Clinical (age, length of treatment, assessment of response to treatment by ultrasonography) and histopathological dates (microvessel density (vessels/mm2) and cellular proliferation assesed by Ki-67 stain in 200 cells were studied.
Results The study group showed CD31= 59,28 vessels/mm2 (mean) and Ki-67= 4,38 (mean). The control group showed CD31= 38,95 vessels/mm2 (mean) and Ki-67= 9,3.
Conclusion The leiomyomas treated with GnRH showed a) high number of microvessel density, b) low proliferative activity and c) significant reduction in size compared with patients without treatment.

P-066

Potential prognostic significance of apoptosis related oncogenes: p53, bcl-2 and mdm-2 in early stage cervical carcinoma

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Introduction Evaluations of expression of apoptosis related oncogenes are being increasingly called upon in an attempt to better understand the carcinogenesis of cervical carcinoma and to provide possible prognostic information. The aim of this study was to analyze the expression of bcl-2, p53 and mdm-2 oncoproteins and cellular proliferative marker Ki-67 in early stage cervical carcinoma, with an emphasis on their association with human papillomavirus (HPV) infection, recurrence rate and lymph node status.
Material and methods Using immunohistochemistry, 69 radical hysterectomy specimens with cervical carcinoma (pT1b1/pT1b2) were studied. Evaluation of expression of p53, bcl-2, mdm-2 and Ki-67 was performed in surface area, center and invasion front of the neoplasms. The HPV presence was determined by CARD in situ hybridization.
Results and conclusion In the invasion front bcl-2 was expressed in 31 (45%), p53 in 37 (53%) and mdm-2 in 33 (47%) cases. HPV infection was detected in 40 (58%) cases. Carcinomas with a higher Ki-67 labeling index were more frequently HPV positive than HPV negative (82.5% vs 17.5%, $p<0.01$). No association was found between p53, mdm-2 or Ki-67 and either lymph node status or recurrence rate. Negative staining for bcl-2 was associated only with the presence of lymph node metastasis (74% vs 26%, $p=0.05$), and not with the recurrence rate. Significant correlation among expression of bcl-2, p53, mdm-2 oncoprotein and Ki-67 values was also observed. These results suggest that further study of a larger series is needed to confirm whether bcl-2, either alone or in combined evaluation with other markers, could be a useful marker to identify more aggressive behavior in early stage cervical carcinoma.

P-067

Malignant potential of dysplastic endocervical epithelium assessed by ploidy status, S-phase fraction and c-myc expression

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Introduction During the past three decades, the incidence of cervical invasive primary adenocarcinoma (ACA) has increased. At the same time, incidence of squamous cell carcinomas of the cervix decreased, due to early detection of their well documented precursors lesions. The aim of this study was to determine if dysplastic endocervical cells (EC) have neoplastic potential as a precursor lesions of adenocarcinoma in situ (AIS) of the cervix. The malignant potential was determined by assessing the ploidy status, proliferative activity and c-myc expression in normal, dysplastic and malignant EC separately.
Patients and methods Serial sections from 49 patients with diagnosed AIS or primary invasive ACA were analyzed. One representative slide of cervix which showed normal, dysplastic and malignant endocervical glands simultaneously was chosen from each patient. Determination of ploidy status and S-phase by flow cytometry and expression of c-myc oncoprotein by immunohistochemistry in normal, dysplastic and malignant EC was done.
Results The morphologicaly normal EC were diploid, with normal proliferative activity and no expression of c-myc oncoprotein. The dysplastic and malignant EC had a significant proportion of cells with aneuploidy, higher proliferative activity and c-myc oncogene overexpression. The χ^2 test showed significant association of malignant EC with aneuploidy ($p=0.008$) and high proliferative activity ($p=0.042$). As one third of dysplastic EC are aneuploid, with high mitotic activity, they probably have malignant potential. Dysplastic endocervical cells had statistically significant association with c-myc oncogene expression ($p=0.028$).
Conclusion Our results support the existence of pre-malignant glandular lesions while the immunohistochemical detection of c-myc oncoproteine could be helpful in detection of EC with malignant potential even without dysplastic morphologic changes.

P-068

Immunohistochemical expression of c-erbB-2 in early stage cervical carcinoma: correlation with human papillomavirus infection and prognosis

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Introduction The attempts to determine the prognostic significance of c-erbB-2 oncoprotein expression (OPE) and its relation to human papillomavirus (HPV) infection in cervical cancer have yielded controversial**.** The aim of this study was to evaluate the patterns of c-erbB-2 OPE in early stage cervical carcinoma and to assess its prognostic value by exploring its relationships to various clinicopathological characteristics, HPV status and recurrence rate.
Materials and methods Radical hysterectomy specimens from 71 cervical carcinoma patients (pT1b1/1b2) were investigated immunohistochemically for c-erbB-2 presence. The c-erbB-2 score (range: 0-400) was determined in the surface area, center and invasion front of each carcinoma. CARD in situ hybridization was used for HPV detection.
Results and conclusion Strong c-erbB-2 OPE was detected in 21, 20, and 32 cases in the invasion front, center and surface area of the tumor, respectively. There was a significant difference in positive staining rate of c-erbB-2 between squamous cell, mixed carcinomas and adenocarcinomas (23%, 50% vs 83%, $p=0.005$). C-erbB-2 OPE was significantly higher in carcinomas with abundant than in tumors with less abundant peri-tumoral lymphocytic infiltration (36.5% vs 10.5%, $p=0.032$). In HPV positive carcinomas (41), c-erbB-2 was detected more frequently in type 31/33 versus type 16/18 lesions (75% vs 18.8%, $p=0.002$). No association was found between c-erbB-2 expression and recurrence rate, lymph node metastasis or any other clinicopathological variable investigated (age, tumor diameter, depth of invasion, grade, vascular invasion). Therefore, immunostaining for c-erbB-2 is unlikely to be of use as a prognostic indicator in early stage cervical carcinomas, while further study is warranted to examine relationships between HPV infection and c-erbB-2 OPE.

P-069

Determination of estrogen, progesterone receptor and Ki-67 immunoreactivity in early stage cervical carcinoma: association with human papillomavirus infection and prognosis

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Introduction The predictive values of estrogen (ER) and progesterone receptor (PgR) status and cell proliferation kinetics in cervical carcinomas are still unsettled. The purpose of this study was to clarify the associations among ER/PgR status and Ki-67 expression and to determine their relationship to human papillomavirus (HPV) infection, recurrence rate and other clinicopathologic parameters (age, tumor diameter, depth of invasion, histotype, grade, vascular involvement, inflammatory infiltrate, lymph node status) in early stage cervical carcinomas.
Materials and methods ER, PgR and Ki-67 immunostaining was performed in 72 cervical carcinoma radical hysterectomy specimens (pT1b1/pT1b2). ER/PgR staining was scored in a semiquantitative fashion, while to evaluate the cell proliferation, the Ki-67 labelling index (LI) was assessed in the surface area, center and invasion front of each tumor. HPV status was determined by CARD in situ hybridization.
Results and conclusion ER positivity was detected in 11 (15%), while PgR positivity in 14 (20%) carcinomas. ER/PgR values were in correlation with Ki-67 LI in all three tumors' compartments ($p<0.01$). In contrast to ER/PgR status, Ki-67 LI was strongly associated with HPV infection ($p<0.01$). No relationship was found between PgR or Ki-67 immunoreactivity and either recurrence rate or any other clinicopathological variable investigated. Nevertheless, reduced ER expression was significantly associated with larger tumor diameter ($p=0.04$) and poor differentiation ($p=0.03$), as well as lymphovascular involvement ($p=0.04$) and lymph node metastases ($p=0.02$). These results suggest that ER, PgR and Ki-67 expression are closely related to neoplastic cell proliferation, probably induced by HPV infection. Their determination may provide additional prognostic information in early stage cervical carcinomas.

P-070

Immunohistochemical assessment of heat shock protein 70 expression in epithelial ovarian carcinomas: Relationship with apoptotic markers as over-expression of bcl-2 and p53

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Purpose Heat shock proteins (hsps) occupy a central role in the regulation of intracellular homeostasis and especially hsp70 and hsp27 are potent antiapoptotic proteins. Overexpression of hsp70 protects cells from stress-induced apoptosis. The aim of this study was to determine the distrubition pattern of hsp70-expression in ovarian carcinomas by immunohistochemistry and to evaluate its relationship with the prognostic parameters and over-expression of bcl-2 and p53 proteins.
Materials and methods Thirty-five patients with ovarian carcinoma were included in the present study. Expression of hsp70 was assessed by immunohistochemistry. The relationship between expression of these proteins and other prognostic markers such as stage was evaluated.
Results Hsp70 was expressed in all of ovarian cancer cells with different intensity. The staining was localized in the cytoplasm and/or nuclei. There was a negative correlation between stage and both intensity of hsp70 positivity and bcl-2 positivity (respectively: $r= -0,478$; $p<0.01$; $r= -0.386$, $p<0.05$). Intensity of hsp70 positivity was statistically associated with bcl-2 positivity ($r=0.435$, $p<0.01$). There were no significant associations between p53 positivity and bcl-2 positivity, intensity of hsp70 or stage of ovarian carcinoma.
Conclusions These data show that the intensity of hsp70 positivity was significantly stronger in early stage than in advanced stage ovarian carcinomas. Hsp70 expression detected by immunohistochemistry was correlated with bcl-2 expression but not with p53 protein expression.

P-071

Endometrial avb3 integrin expression and pinopode formation in women receiving hormone replacement therapy

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Background avb3 integrin and pinopodes have been proposed as new markers of endometrial receptivity. There is also a very limited experience on the responses of these markers to hormonal therapies. The aim of this study is to evaluate the effect different hormone replacement treatment on these markers.
Design We studied 12 fertile controls and 16 women with ovarian failure receiving hormone replacement therapy (estradiol valerate (EV) and vaginal progesterone (VP), group E+EP). Those women were divided randomly into two subgroups of 8 patients. Group E+P received EV for 14 days and VP alone for 14 days. Group E+P+EP was given E+EP therapy but including episodic VP on days 8 and 11. All women underwent two endometrial biopsies during a single menstrual cycle (days +7 to +8 and four days later). avb3 integrin was detected in frozen tissue using the EnVision system. Pinopode formation was evaluated in tissue fixed in glutaraldehide using a Zeiss DSM940A scanning electron microscopy. Both avb3 integrin and pinopode formation were semiquantitatively evaluated.
Results Patients in group E+P+EP showed markedly decidualized endometria in the midluteal biopsy and a significant increase of avb3 integrin expression ($p=0.01$ for both parameters). No significant changes in pinopode formation were observed in midluteal biopsies. No differences were observed in late luteal biopsies.
Conclusions avb3 integrin expression and pinopode formation are signs of endometrial maturation related to progesterone action but their significance in terms of endometrial receptivity in the clinical setting remains to be shown. Supported by grant PI020036 (Fondo de Investigaciones Sanitarias).

P-072

Gestational trophoblastic disease associated with ectopic pregnancy: a report of three cases

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Introduction Gestational trophoblastic diseases (GTD) are extremely rare conditions, especially the ones associated with ectopic pregnancies (EP).
Case reports Only three cases (0.26%, 3/1133) of GTD associated with EP were diagnosed at the Department of Histopathology and Clinical Cytology in the last 14 years. The first patient, age 29 underwent salpingectomy for clinical suspicion of tubal pregnancy, whereas in the second, age 49 hysterectomy with bilateral salpingo-oophprectomy (HSOP) was performed for a clinical diagnosis of ovarian endometriosis. The third patient, age 42 underwent HSOP for malignant ovarian tumour which metastasised to lungs. Pathological findings: Chorionic villi were found in the tubal lumen of the first patient, showing marked hydropic degeneration, reduced/absent vascularisation and excessive trophoblastic cell growth. Diagnosis of partial hydatiform mole was established, and the patient remained under surveillance for the HCG level. Atypical trophoblastic cells, which penetrated the full thickness of the tubal wall and invaded the ovarian blood vessels of the second patient, led to the diagnosis of an invasive mole. The third patient was diagnosed with choriocarcinoma due to the presence of a tumour consisting of malignant trophoblastic cells that infiltrated the right ovary, tube and uterine cornu. The last two patients received chemotherapy and have been well for 15 and 118 months, respectively.
Conclusion Due to extremely low incidence of GTD associated with EP, these entities are rarely clinically recognised. Therefore, a careful histopathologic examination of the adnexal masses is essential for the establishment of the correct diagnosis and further treatment of the patients.

P-073

Advantages of liquid-based cytology in the diagnosis of atypia (ASCUS) - a follow-up study

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Introduction In an organized screening programme for cervix cancer the diagnosis atypia (ASCUS) has always been a problem. The subsequent histological examination shows different diagnoses, such as negative, inflammation, CIN I-III (SIL) and carcinoma. In the County of Funen, a well-organized screening programme has existed since 1989.Using the cytological diagnosis of atypia, we distinguish between atypia 1 (ASC-US) probably inflammatory and atypia 2 (ASC-H) probably dysplasia. In previous studies on PAP-smears we have shown histological follow-up diagnoses for atypia 1(ASC-US): negative 84.3% and CIN I-III 15.7%, and for atypia 2 (ASC-H): negative 41.6%, CIN I-III 57% and carcinoma 1,4%. Since June 2001 we have implementated liquid-based cytology (LBC) ThinPrep, Cytyc. The aim of this investigation is to show the histological follow-up diagnoses of cytological atypia when using LBC-technique.
Materials and methods The total number of LBC-samples in 2002 were examined. The recommandations for atypia 1 are a repeated cervical sample, and for persistent atypia 1 or atypia 2 colposcopy, cervical biopsies and abrasion within 3 months.
Results In 2002 a total of 34,585 smears, including 30,060 LBC-samples, were examined. The diagnoses of the LBC-samples were: negative 92,9%, inadequate 2,8% (a reduction of 50% compared with PAP-smears), atypia 2,8 % and CIN I 4%. Preliminary histological follow-up diagnoses for atypia 1 and 2 were: negative 59% and CIN I-III 41%.
Conclusion ThinPrep shows two significant advantages: a markedly reduced number of cytological atypia diagnoses (40%) compared with PAP-smears and a higher diagnostic quality with fewer false positive diagnoses.

P-074

Ovarian Sertoli-Leydig cell tumor with heterologous elements of rhabdomyosarcoma: A case report

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Introduction Ovarian Sertoli-Leydig cell tumors (SLCT) are very rare sex cord-stromal tumors and among them, tumors with heterologous sarcomatous elements are exceptional. Aims: A unilateral ovarian stage Ic SLCT with heterologous mesenchymal elements is presented.
Materials and methods A 29-year-old woman, para 1, was admitted to hospital with severe abdominal pain. At laparotomy, resection of a large right ovarian cyst was carried out. Histological examination revealed a SLCT, and subsequently surgical staging was performed. No adjuvant therapy was given. At 2 years follow-up she was well and without signs of recurrence. Routine staining of slides from 34 paraffin blocks and immunohistochemistry (IHC) were available.
Results Gross examination revealed a 18 x 14 x 11 cm large multilocular ovarian cyst with smooth interlocular septa and outer surface. Microscopy disclosed a SLCT of intermediate diff-erentiation with a very few Leydig cells. Major areas consisted of a hypocellular stroma with edema, hemorrhage and necrosis. In a few sections small foci of cartilage were seen. In a number of sections groups of small cells with indistinct cytoplasm and hyperchromatic nuclei were present. Mitotic and apoptotic figures were common. IHC revealed a positive reaction for desmin, myf4 and myogenin, indicating rhabdomyosarcoma of embryonal type.
Conclusion The most important prognostic factors of SLCT are stage and grade of differentiation. Variants of SLCT with immature skeletal muscle are often fatal. IHC with myf4 or myogenin with a high sensitivity and specificity for rhabdomyosarcoma, combined with inhibin is very important in differing immature Sertoli cells from rhabdomyoblasts, in order to diagnose the rhabdomyosarcoma and to grade the SLCT correctly.

P-075

Expression of the nerve growth factor (NGF) receptors TrkA and p75 shows biological diversity in ovarian, breast and mesothelial malignancy

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Neurotrophins and their receptors are known to have a central role in biologic processes in the nervous system. However, recent studies have additionally highlighted the role of NGF and its receptors TrkA (inducer of proliferation) and p75 (inducer of apoptosis) in the development and progression of malignant tumors, including those of non-neural origin. We studied protein expression of p75 and activated phosphorylated TrkA (p-TrkA) in 145 serous ovarian carcinomas (80 effusions, 65 solid tumors), 107 breast carcinomas (42 effusions, 65 solid tumors) and 81 malignant mesotheliomas (MM) (24 effusions, 57 biopsies) using immuno-histochemistry and immunoblotting. p-TrkA was expressed in ovarian carcinoma cells in effusions in only 15/80 (19%) specimens, compared to 52/65 (80%) solid tumors, while the opposite was true for p75. In contrast, p-TrkA membrane expression was higher in effusions ($p=0.058$) of MM patients, while the opposite was true for p75 ($p=0.008$). In addition, p-TrkA (but not p75) expression was significantly higher in peritoneal MM compared to their pleural counterparts (20/20 vs. 46/61 positive tumors; $p=0.014$). In breast cancer, p-TrkA expression in effusions was significantly higher than in primary tumors ($p<0.001$) and lymph node metastases ($p=0.001$), while p75 expression was lower in both effusions ($p=0.019$) and primary tumors ($p=0.039$) compared to lymph node metastases. Our results provide the first evidence regarding the role of NFG receptors in tumor progression through site-specific changes in expression. Specifically, they may provide biologic evidence for the uniformly and rapidly fatal outcome of breast cancer patients with effusions and of MM patients.

P-076

The significance of mast cells in the differential diagnosis of uterine smooth muscle tumors

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Aim To assess the role of the mean mast cell count in the differential diagnosis of uterine smooth muscle tumors.
Material and method Twenty-cases of ordinary leiomyoma, 6 cases of atypical leiomyoma, 6 cases of leiomyosarcoma, 8 cases of mitotically active leiomyoma and 38 cases of cellular leiomyoma were included in the study. The morphological features and clinical presentation was evaluated retrospectively. Toludine Blue stain was used to highlight the mast cells in the endometrium, myometrium and smooth muscle tumors. At least 40 high power fields were counted.
Results Considerable variation in mast cell numbers was found among smooth muscle tumors ($p<0.01$). The intratumoral mean mast cell count was found significantly higher in ordinary leiomyomas (82.7 14), when compared with other tumors, including atypical leiomyoma (68.4 18.4), mitotically active leiomyoma (45 10.3), cellular leiomyoma (34.2 5.9) and leiomyosarcoma (23.8 8.3) ($p<0.01$). No significant differences were found between these tumor groups in regards of the mean mast cell count of endometrium and myometrium adjacent to the tumor. In the endometrium, a drop in the number of mast cells has been demonstrated with advancing age, particularly after menopause ($p<0.05$). In addition 2 cases showed eosinophilia, 4 cases showed basophilia and 5 cases showed allergic reactions to different causes. A significant positive relationship was found between the intratumoral, myometrial mast cell number and basophilia.
Conclusion These data suggest that intratumoral mast cell quantitation may allow the differential diagnosis in uterine smooth muscle tumors.

P-077

Mutation analysis of proliferation genes in ovarian tumours by DHPLC

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Ovarian carcinoma is the most frequent cause of death in the world among gynaecologic malignancies. Diagnosis is usually late, and the biological behaviour is highly variable, depending on several factors such as the histopathological grading, the local and/or peritoneal spread and the proliferation rate. We choose a list of genes involved in cell growth and proliferation (IGF2, IGFBP3, CTNNBETA1, CDK-1C, FGFR4). Among these, so far, we analyzed IGFBP3 gene and studied loss of heterozygosity (LOH) in tumour tissue in order to identify potential relevant genes in ovarian cancer homeostasis. Twenty cases of formalin fixed, paraffin embedded ovarian carcinomas from the archives of the Department of Pathology of Padova University and five effusions (from peritoneal or pleural recurrences) of ovarian carcinomas, were selected for this study. Genomic DNA and tumour DNA for each case were extracted from blood cell, tumour or not-cancer surgery biopsy. LOH was searched using 2 published single nucleotide polymorphism (SNPs) in IGFBP3 gene. PCR product covering the SNPs from genomic and tumour DNA were DHPLC separated. Three of the twenty patients whose tumour DNA was obtained from paraffin material, were heterozygous at nucleotide 323 C/A (dbSNP ref. n. 2132570) and 1704 A/C (dbSNP ref. n. 2854744) of the IGFBP3 gene in genomic DNA. Analysis of tumour DNA failed to reveal LOH (preserved heterozygosity). In effusion cases, one of five patients showed heterozygosity at the same nucleotides. In these patients, LOH was also not identified. Our preliminary results suggest that DHPLC analysis for LOH is feasible, rapid and maybe of potential interest in the identification of genes involved in ovarian cancerogenesis.

P-078

Squamous intraepithelial lesions and Ki67

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Introduction The progression of low grade squamous intraepithelial lesion (L-SIL) to high grade squamous intraepithelial lesion (H-SIL) occurs in around 20% of cases. There are no morphologic changes to predict this progression. Aims: To study whether the Ki 67 immunostaining pattern and labeling present upon diagnosis of L-SIL is able to predict the regression or persistence/progression of the lesion to H-SIL of the cervix over a two year period following diagnosis.
Materials and methods 113 cervical biopsies from 113 patients diagnosed cytologically as cervical squamous intraepithelial lesion (SIL) were used. Biopsies were performed immediately after the cytological diagnosis resulting in 89 L-SIL and 24 H-SIL. Ki 67 immunostaining was performed on all cases and was classified as suprabasal immunostaining pattern (SBIP) for cases without Ki-67 immunoreactive cells in the upper two thirds of the epithelium thickness, or dispersed immunostaining pattern (DIP) for cases with Ki-67 immunoreactive cells in the upper two thirds of the epithelium thickness. 200 nuclei in 25 HPF including the total thickness of the epithelial layer of each sample were counted to determine the mean number of cells which reacted against Ki-67 antibody. In situ hybridization was used to demonstrate the presence of HPV and its typing. Chi Square Test, Fisher's Exact Test and Student T Test were used for statistical analysis with significance set at $p<0.05$.
Results Of the 113 biopsies performed 89 cases resulted in L-SIL (54 S-BIP and 34 DIP patterns) and 24 in H-SIL. 14% of the L-SIL cases that resulted in a S-BIP pattern showed persistence/progression of the lesions while 65% of the cases that resulted in a DIP pattern showed persistence/progression of the lesions. The difference between these values where statistically significative ($p<0.001$). Regarding Ki-67 labeling index (LI) in L-SIL cases, we found that 25% of the cases (15 out of 58) with a Ki-67 LI less than 28% persisted/progressed, while 51% of cases (16 out of 31) with Ki-67 LI over 28% persisted/progressed. In this case there was also a statistically significative difference ($p=0.015$). We found no statistical differences ($p=0.64$) regarding HPV "in situ" hybridization. 48% of the cases (14 out of 29) where HPV was detected by in situ hybridization persisted/progressed while 28% of the cases did not show HPV by hybridization.
Conclusions The L-SIL cases which showed DIP and high Ki-67 LI were significantly related with persistence or progression to H-SIL of these lesions, while L-SIL cases which showed SBIP and low Ki-67 were significantly related to regression of the lesions. Therefore, we conclude that Ki-67 immunostaining pattern may be of prognostic value to predict regression or persistence/progression of L-SIL.

P-079

Sclerosing stromal tumor of the ovary

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Introduction Sclerosing stromal tumor (SST) of the ovary is a rare tumor. It was first described by Chalvardjan and Scully in 1973 as a distinct subtype in the thecoma-fibroma group. Most SSTs occur in young patients below the age of 30 years. The most common clinical symptom in patients is menstrual irregularity. In differential diagnosis, thecoma, fibroma, and hemangiopericytoma must be considered. Aim: In this report, 2 cases of SST of the ovary were described and compared with other ovarian tumors.
Material and methods Two cases of ovarian SST were examined. The first case occurred in a 28-year-old female. At operation, the patient was found to have the ovary replaced by a tumor mass measuring 5x4x2 cm. The second case occurred in a 24-year-old woman and the tumor mass measured 9x5x5 cm. The surgical material was fixed in 10% formaldehyde solution and prepared for paraffin sections. In addition to H&E staining, immunohistochemical analyses were performed.
Results The clinical features, gross features, light microscopic findings and immunohistochemical results were described and compared with literature. The findings were consistent with SST, which is a very rare tumor of the ovary. The tumor cells showed positive staining for vimentin and desmin. We discuss the results and differential diagnosis.

P-080

Unususal chorangioma of the placenta - A case report

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Chorangiomas (CA) are common lesions, occurring in 1 of 100 placentas. It is not clear whether CA are hyperplasias, hamartomas, or true neoplasms. Depending on their size and location, they may be devoid of symptoms or may cause difficulties for the infant and the mother. We describe a case of manual removal of the placenta in association with CA. On gross examination the tumor had a nodule-in-nodule appearance and was histologically composed of two well defined nodules. The larger, outer one had the appearance of an usual chorangioma; the inner nodule showed highly cellular vascular proliferation. This architecture might represent a morphological variant of the same disease. In the present case, the placenta had to be removed manually. It remains unclear if this was due to impaired contractility of the uterine wall or due to a lack of decidualisation, following a worse vascular support in the surrounding area of the CA. It can´t be excluded that placenta accreta was caused by strictures after Cesarian section which had been performed at the first delivery two years ago. On the other hand a placenta with a nodule form of CA could be less elastic than a placenta with regular architecture. However manual removal of the placenta may be regarded as a further complication of CA's larger than 4 cm in diameter.

P-081

Are MUC 2 and MUC 5AC reliable markers in the differential diagnosis of primary and metastatic ovarian carcinomas?

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Introduction Aberrant expression of mucin glycoproteins has been observed during malignant transformation of human ovarian epithelium. However, there is limited information regarding the expression of these mucin proteins in primary and metastatic ovarian carcinomas. The aim of this study was to examine immuno-histochemical expression of MUC 2 and MUC 5AC mucin proteins in primary and metastatic carcinomas of the ovary in order to assess their value in the differential diagnosis.
Materials and methods Forty eight ovarian tumours comprising 37 surface epithelial carcinomas (8 endometrioid, 5 clear cell, 11 serous papillary and 13 mucinous type) and 11 metastatic carcinomas of gastrointestinal primaries were included in the study. The age of the patients, the histological type and grade of the tumours were evaluated in relation to the expression (percentage distribution and staining intensity) of MUC 2 and MUC 5AC studied immunohistochemically.
Results MUC 2 was expressed in 28 (75.7%) primary ovarian tumours while MUC 5AC was expressed in 27 (73%) primary tumours in comparison to 100% expression of MUC 2 and 82.3% expression of MUC 5AC in metastatic ovarian tumours. The differences in MUC 2 expression between non-mucinous (46.04 ± 38.11) and metastatic tumours (56.36 ± 41.72) and metastatic and mucinous tumours (28.46 ± 39.97) were statistically significant ($p< 0.01$). The difference in MUC 5AC expression was statistically significant ($p< 0.05$) between non-mucinous (27.92 ± 33.49) and mucinous tumours (51.54 ± 38.91).
Conclusion MUC 2 proves to be highly reliable in the differential diagnosis of primary and metastatic mucinous tumours of the ovary.

P-082

Deregulation of sFRP1 and sFRP4 RNA-expression in endometrial stromal sarcoma

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Introduction Endometrial stromal sarcomas (ESS) are very rare uterine tumours. Whereas histology and immunohistochemistry of this tumour are well documented, little is known about molecular mechanisms involved. To characterize ESS-altered genes, we generated a genome-wide library by SSH-hybridisation and used it to produce cDNA arrays. Particular emphasis was also placed on data obtained from QRT-PCR analyses.
Materials and methods Using SSH-library a gene-bank with app. 4000 different clones was established. Total RNA was extracted from frozen tumour tissue and used for cDNA hybridisation. QRT-PCR analyses were performed both on frozen and paraffin-embedded tissue samples.
Results After cDNA-array analysis more than 300 genes were selected, sequenced and rearranged to different functional groups. Among the most strongly deregulated genes were secreted frizzled-related protein-1 (sFRP1) and sFRP4. These are putative genes that regulate wnt-signalling pathway and can influence different cell events including cell proliferation. Compared to post-menopausal endometrium the expression of sFRP1 and sFRP4 was increased in low-grade ESS, but decreased in aggressive ESS form. These results were additionally verified on paraffin embedded tissue probes by QRT-PCR analysis.
Conclusion Deregulation of sFRP1 and sFRP4 expression appears to be one of the crucial features of ESS. Whereas in low-grade ESS these two genes are up-regulated, their down-regulation could be associated with high-grade form of ESS. Therefore a sFRP1 and sFRP4 may not only represent useful markers for early ESS diagnosis but may also have prognostic impact and help to distinguish between low- and high-grade ESS. (This work was supported by LORE-SALDOW – Research Fund)

P-083

C-kit immunoreactivity in female genital tract carcinosarcoma (FGT-CS)

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Introduction FGT-CS is a highly malignant neoplasm with poor response to current chemotherapy. The proto-oncogen c-kit encodes a transmembrane-tyrosine-kinase (t-k) receptor involved in cell diferentiation, that has been found to be expressed in certain solid tumors and may play a role in their tumorigenesis. A t-k inhibitor specific for c-kit (STI571) has been reported to have therapeutic effects in tumors expressing the aberrant forms or high quantities of target protein. Contradictory results are reported in the literature about the expression of c-kit in CS. The aim of this study was to investigate the immunoreactivity for c-kit in CS.
Materials and methods Immunostaining with anti-c-kit (CD117) polyclonal antibody (Dako) was evaluated in 34 CS (23 endometrial, 9 ovarian, 1 cervical and 1 tubal). Only cases with membrane staining were considered to be positive.
Results C-kit was expressed in 5 cases: in 3 cases only in the carcinomatous component (2 endometrial, 1 tubal), in 1 case only in the sarcomatous component (endometrial) and in 1 case in both components (ovarian). Positivity was focal and less than 5% of cells in all cases.
Conclusion CS of the female genital tract rarely express c-kit. It is unlikely that patients with these tumors will benefit from treatment with the currently avalaible t-k inhibitors.

P-084

Uterine heterologous sarcoma with the component of osteosarcoma on the base of lipoleiomyoma - a case report

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Uterine homologous and heterologous sarcomas are rare neoplasms that are usually misdiagnosed as a component of malignant mixed Mullerian tumor (MMMT). We present a case of a 53-year-old woman who had a heterologous sarcoma with a component of osteosarcoma on the base of lipoleiomyoma, that can be differentiated from MMMT by the absence of epithelial component. In heterologous sarcomas, the other components of neoplasm are rhabdomyosarcoma, chondrosarcoma, osteosarcoma, liposarcoma and combined forms. These neoplasms are misdiagnosed as endometrial stromal sarcomas or as a component of MMMT. They are more agressive than MMMT.

P-085

Immunohistochemical detection of ER-β in ovarian sex cord-stromal tumors

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Introduction Since the discovery of a second subtype of estrogen receptor, ER-beta (ER-β), in 1996, approximately 10 years after the cloning of ER-α, several reports have been published regarding the tissue distribution of this receptor. In mammals so far studied ER-β predominates in the ovary. High expression of ER-β was observed by in situ hybridization or immunohistochemistry in granulosa cells of the ovarian follicles. The aim of the present study was to examine immunohistochemically the expression of ER-β in ovarian sex cord-stromal tumors.
Materials and methods Ten cases of ovarian granulosa-stromal cell tumors (five adult granulosa cell tumors, two juvenile granulosa cell tumors, and 3 sclerosing stromal tumors) and two cases of Sertoli-Leydig cell tumor were immunostained for ER-β (polyclonal ERb88, Biogenex). Representative sections from ten cases of ovarian carcinomas were immunostained for comparison.
Results Positivity for ER-β was observed in 50% to 80% of the nuclei in adult granulosa cell tumors and 40% to 75% of the nuclei in sclerosing stromal tumors. Only 10-15% of the nuclei from juvenile granulosa cell tumors were positive for ER-β. In Sertoli-Leydig cell tumors positivity was more extensive in areas of diffuse growth. Four cases of ovarian carcinomas exhibited positivity.
Conclusion Positivity for ER-β is a common finding in ovarian sex cord-stromal tumors. These findings might have therapeutic implications.

P-086

Adult fibrosarcoma of the vulva

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Introduction Sarcomas are rare and unusual lesions of the vulva. The aim of this study was to present a case with analysis of clinical, morphologic and immunohistochemical findings.
Materials and methods A 59-year-old female patient presented a rapidly growing of a mass in the pubis and left labium major of vulva of 17x16x10 cm associated with a second satellite lesion (2 months later) in the right inguinal area of 7x6x5 cm.
Results Histologic examination of both lesions disclosed a malignant neoplasm composed of uniform and orderly spindle cells with occasional epithelioid cells, oriented in curving or interlacing fascicles, forming a herringbone pattern with mitotic figures (8/10 HPF) and areas of necrosis and hemorrhage. The immunohistochemical profile showed strong reactivity for CD 34 and vimentin, and focal and weak reactivity for smooth muscle actin. Immunoexpression for S100, NSE, BCL-2, HMB-45, MelanA, AE1AE3 and desmin was lacking.
Discussion and conclusions We present a case of a malignant mesenchymal tumor of vulva. Due to histological features and behavior, pathologists must discard others spindle cell tumors, such as malignant melanoma, malignant peripheral nerve sheath tumor, monophasic synovial sarcoma or leiomyosarcoma. Fibrosarcoma is a diagnosis of exclusion and a wide immunohistochemical panel is useful to make differential diagnosis.

P-087

Serous tumor of borderline malignancy of the fallopian tube - a case report

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Introduction Serous tumors of borderline malignancy (STBM) of the fallopian tube are exceedingly rare. We report a case of STBM of fallopian tube in a 34-year woman.
Material and methods A 34-year old woman was found to have right adnexal mass on her routine gynaecologic examination. Serum CA-125 was not elevated. At laparoscopy a swollen fallopian tube was removed. During the operation a fine needle aspiration was performed. The cytological findings of the adequate Giemsa-stained smears were recorded. Haematoxylin and eosin-stained sections from the routinely fixed and paraffin embedded surgical material were histologically analysed.
Results A papillary tumor, with maximal diameter of 3 cm in the dilated part of the fallopian tube toward the fimbrial end, was found. Histologic features of the tumor were similar to the atypical proliferative serous tumor of the ovary. Two years after the operation the patient is well, without evidence of the disease.
Conclusion To the best of our knowledge only four cases of STBM of fallopian tube have been reported. Our case is unusual due to lack of any symptoms.

P-088

CDX2 immunoreactivity in primary and metastatic ovarian mucinous tumours

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Introduction The caudal related homeobox transcription factor

CDX-2 regulates intestinal differentiation of the epithelial cells. Recent studies have suggested that in the setting of an unknown primary, CDX-2 immunostaining may be used in identifying a metastatic carcinoma as colonic in origin. Aim: We sought to evaluate the immunoreactivity of CDX2 in a series of primary and metastatic ovarian mucinous neoplasms.
Materials and methods 59 cases of mucinous tumour of the ovary, including 28 benign cystadenomas, 15 borderline mucinous tumours and 16 mucinous adenocarcinomas, were tested for CDX2 immunoreactivity. 25 cases of serous and endometrioid lesions as well as 5 cases of ovarian metastases from colonic adenocarcinoma were also evaluated.
Results CDX2 immunoreactivity was detected in 45/59 (76%) cases of primary ovarian mucinous tumour. Particularly, 20/28 (71,5%) cases of cystadenomas, 10/15 (66,5%) cases of borderline tumours and 15/16 (93,5%) cases of adenocarcinomas were immunostained. Immunoreactivity was usually related to an intestinal morphology. No immunoreactivity was observed in serous or endometrioid lesions whereas all five cases of ovarian metastases from colonic adenocarcinoma were immunoreactive for CDX2.
Conclusions CDX2 immunoreactivity may be detected in the majority of benign, borderline and malignant ovarian mucinous tumour whereas serous and endometrioid lesions are unstained for this marker. CDX2 immunoreactivity is not useful in distinguishing between primary and metastatic ovarian mucinous carcinomas whereas it may be useful in the distinction between endocervical and intestinal type of mucinous tumour of the ovary, which may have clinical relevance. An ovarian primary should be added to the list of CDX2 positive metastatic carcinoma in the setting of an unknown primary.

P-089

Expression of p16INK4A in serous lesions of the female genital tract

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Introduction A spectrum of serous lesions including benign, borderline and malignant tumours and endosalpingiosis commonly arise in tissues of the female genital tract. p16INK4A is a tumour suppressor protein which functions as a cyclin dependent kinase inhibitor and plays a vital role in the cell cycle control at the G1/S phase transition. Although well recognized as a discriminator of benign and potentially malignant squamous and glandular cervical lesions, the expression of p16 in ovarian lesions and their mimics is less well established. The aim of this study was to evaluate p16INK4A expression in serous lesions of the female genital tract and to investigate its potential diagnostic utility.
Methods Immunohistochemical analysis of p16INK4A (mouse monoclonal anti-p16 antibody) was performed on cases of serous epithelial lesions of the female genital tract (n=26) comprising endosalpingiosis, borderline serous cystadenomas of ovary and peritoneum, benign serous cystadenoma of ovary, serous adeno-carcinoma of ovary and peritoneum and normal fallopian tube epithelium.
Results All cases of endosalpingiosis were positive (definite cytoplasmic staining with occasional nuclear positivity) for p16INK4A expression, as were all normal fallopian tube epithelium samples. Varying degrees of p16INK4A expression were found in the serous neoplasms examined, with no clear pattern associated with any histological subtype.
Conclusion Diagnostic difficulties may arise in the distinction between benign serous glandular lesions and more sinister potentially malignant serous neoplasms. In this study the pattern and degree of expression of p16, although showing some differences, does not allow definitive classification of these lesions.

P-090

Epithelioid trophoblastic tumor of the lower uterine segment. An immunohistologic study

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Introduction Epithelioid trophoblastic tumor [ETT] is an unfrequent gestational trophoblastic disease [GTD], whose epithelial-like cells derive from chorionic-type intermediate trophoblast. ETT mostly shows a benign behaviour, but metastases have also been reported, and pathologic prognosticators are not as yet recognized. The kinase-inhibitor p57KIP2 has been seen to be lacking in malignant GTDs, while different growth factors, such as the C-kit receptor, have been related to growing trophoblasts of p57-null embryos. Since C-kit is said to promote trophoblast growth and invasiveness, this study was aimed at immunolocalizing p57 and C-kit in ETT, as compared to a proliferation antigen.
Materials and methods - After a preterm birth of a living infant, a 37-year-old woman underwent hysterectomy for a postpartum metratonia. The patient had an antecedent miscarriage. A cervical ETT was found, and immunohistology was performed on paraffin sections, using C-kit, p57, and Ki67 antibodies, following a microwave antigen-unmasking. Positive reactions were recorded either as a focal or diffuse immunostaining.
Results and conclusions An escavated, hemorrhagic, endocervical lesion was found, which focally infiltrated the myometrium. Light microscopy showed poorly limited nodules of epithelial-like cells, arranged in cords and nests, with interposed hyaline material, extensive hemorrhages, and necrotic areas. Atypical mononucleate and syncytiotrophoblastic cells occasionally occurred. Immunohistology revealed a diffuse presence of C-kit-expressing tumor cells. The p57 nuclear staining was focally observed to be negative or weak, and Ki67 was totally unlabeled. In conclusion, this slowly-growing ETT is lacking in p57, but the C-kit over-expression substantiates trophoblastic survival and invasiveness.

P-091

Carcinosarcomas of the corpus uteri

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The aim of our study was to analyze the clinical and morphological characteristics of carcinosarcomas of the corpus uteri. 3 carcinosarcomas of the corpus uteri were studied in postmenopausal women. Paraffin-embedded material was stained with hematoxylin and eosin, trichromic, reticulin and PAS staining. Antibodies against EMA, cytokeratins, vimentin, desmin, and PCNA were also used. The size of tumor, depth of myometrial invasion, presence of vascular invasion, necroses, cellular atypia and differentiation, and mitotic activity were analyzed. All tumors were characterized by large size, foci of hemorrhage and necrosis.

Morphological and immunohistochemical analysis proved biphasic structure of the tumors. Epithelial component was represented by adenocarcinomas with high (2 cases) and moderate (1 case) degree of differentiation. All presented homologous pattern – leiomyosarcomas. No metastasis was encountered at the time of diagnosis. As EMA was intensely positive, both in malignant epithelium and in the majority of stromal component, we conclude that carcinosarcomas of the corpus uteri represent combination tumors with both elements originating from a single common stem cell. This conclusion was emphasized by the presence of PAS positive residues in both components.

P-092

Knowledge evaluation among Albanian women on risk factors of cervix cancer

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Aim This study evaluates the rate of awareness of women on risk factors and the preventive ways for the cervix cancer.
Methods The data were obtained through the questionnaire in a sample of 1660 women aged 20-60 years randomly selected throughout Albania (random cluster sample). The questionnaire included questions on: social-demography, sexual behavior, women health, and sources of the health information.
Results Only 20% of interviewed women were informed on PAP-test. In 60% of cases it was the doctor notified the women of the cluster for this screening as an examination of cervix cancer prevention. The PAP-test examination had been performed by only 12% of the women who had prior knowledge on PAP-test. In about 90% of cases the examination was performed for diagnostic reasons and not for prophylactic control reasons. In proportion to total only 2.5% had performed the test, which presents a very problematic situation. Among the women who performed PAP-test, 40% were aware of the need of periodical check-up every 1-2 year, but 50% of them never performed it. The reason for not doing periodical screening seemed to be the daily routine and fear of the unknown events. 80% of women who performed PAP-test were from the cities. Only 10% of women use contraceptive methods of the family planning. About 35% of women had undergone at least one abortion.
Conclusions The study supports the concept that information on health services as well as health education of women at risk plays an important role in early detection and prevention of cervix cancer.

P-093

Expression of checkpoint proteins p53, Hus-1 and Rad-9 and its relation with apoptosis in ovarian epithelial tumours

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Introduction Eukaryotic cells show surveillance mechanisms (checkpoints) of genomic integrity. An impaired checkpoint has been linked to genomic instability, and, in higher eukaryots has been associated to risk of cancer. The genes to be studied are involved in the mitotic checkpoint and DNA damage checkpoint (like p53, the tumour supressor gene frecuently mutated in human cancers). These proteins are also involved in the checkpoint responding to incomplete DNA replication (replication checkpoint) termed the Rad/Hus group of proteins. Failure of this checkpoint progress can result in genomic instability, and, in higher eukaryotes may lead to tumorigenesis or certain degenerative disease states.
Material and methods We have obtained antisera against recombinant hHus1 and hRad9. For this we will clone and overexpress in bacteria and immunize animals to obtain antisera. We will carry out extensive analysis of the expression levels of several proteins by Western blot analysis. We looked for possibles associations among Hus-1 and Rad-9 immunohistochemical expression and prognosis in ovarian carcinomas prior to chemotherapy. We studied, in addition to the levels of expression of the mentioned proteins, the localization and modifications of these proteins in 130 epithelial ovarian tumours (benign, borderline and carcinomas) in order to establish the probable relationship between these proteins and ovarian carcinogenesis.
Results Increased levels of checkpoint hus-1 protein was related to malignant transformation across all types of ovarian carcinomas ($p<0,05$). Similar results were observed with the expression of rad-9 protein, however was not statistically significant ($p>0,05$). In univariate survival analysis, the expression of hus-1 and rad-9 protein were not significantly correlated with shorter survival and shorter free period of illness ($p>0,05$). There were positive correlation between the apoptotic index, p53 and bax and the expression of checkpoint protein hus-1 ($p<0,05$), but none relation was detected with rad-9 expression ($p>0,05$).
Conclusions 1- The expression of hus-1 is higher in ovarian carcinomas and low in benign and borderline tumours. 2- The expression of hus-1 shows a positive correlation with the apoptotic index and with the pro-apoptotic factors (p53 and bax). 3- There was not statistical significant differences in the expression of Hus-1 and Rad-9 among the different histological types of ovarian tumours. 4- There was a positive correlation between the expression of Hus-1 protein with other prognostic factors such as the FIGO stages, and the histological grade.

P-094

Unilateral gonadoblastoma associated with 46, xy pure gonadal dysgenesis (Swyer's syndrome): a case report

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Gonadoblastoma develops almost exclusively in gonadal dysgenesis and is often associated with dysgerminoma. We report a 29-year-old female patient with Swyer's syndrome and gonadoblastoma in the right ovary. The patient was a phenotypic female with 46, XY karyotype. The external genitalia were female, the body structure was androgenic with virilization and primary amenorrhaea, with normal serum level of the testosterone. The dimensions of the gonadoblastoma were 2 cm x 2 cm x 1 cm, with proliferation of primitive germ cells, sex cord stromal cells and Sertoli cells organized into nests, separated by dense fibrous stroma

with numerous microcalcifications. The patient underwent bilateral salpingoophorectomy and total hysterectomy. The authors underline the importance of the correct diagnosis and the need of bilateral salpingoophorectomy for preventing the possible progression of the gonadoblastoma into malignant germinal cell tumor.

P-095

Histological and electron microscopic identification and analysis of uncommon particles in tissue after use of orthopaedic implants

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Introduction Deposits of solid products occur widely in tissue either from endogenous sources due to pathological changes, or from exogenous sources. After use of orthopaedic implants, particles produced by wear are commonly recognizable and identifiable in tissue by the surgical pathologist. Foreign body related tissue re actions may contribute to aseptic loosening of implants, which is regarded as one of the most significant complications in orthopaedic surgery. In the present study we report on very uncommon particles in periprosthetic tissue after aseptic loosening of metallic implants. Our study was performed to identify particles in periprosthetic tissue and to clarify whether they may contribute to the loss of implants.
Materials and methods Periprosthetic tissue including joint capsule, acetabular and femoral interface membranes of 62 cases of aseptic loosened cementless total hip replacements was histologically investigated. Four cases with present uncommon particles were selected for electron microscope investigation and dispersive x-ray analysis.
Results In four cases the histological investigation revealed uncommon green to yellow coloured foreign bodies. By the use of electron scanning microscopy and energy dispersive x-ray analysis, these particles were identified as deposits rich in chromium, phosphorus and oxygen. This detection indicates the presence of solid corrosion products derived from implant surfaces.
Conclusion Particles produced by wear and/or corrosion are associated with an alteration of the environment and the development of periprosthetic osteolysis which may result into the loss of the implant. The surgical pathologist should be aware that corrosion products may occur. Their identification is helpful to determine implant failures.

P-096

Paratesticular lipoleiomyosarcoma: a case report

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Introduction Leiomyomatous differentiation in liposarcoma is a rare phenomenon and may occur as a well differentiated liposarcoma with an intrinsic malignant smooth muscle component, so called " lipoleiomyosarcoma" or in the context of "dedifferentiated" liposarcoma.
Material and methods We have studied a paratesticular lipoleiomyosarcoma in a man 63 years old. Gross examination revealed 4 tumor nodules dispersed between identifiable testis and epididymis, the largest of which measured 13x7x4 cm. The specimen cut surface revealed smooth yellow tissue with distinct areas of gray myxomatous change. Microscopically the specimen consisted of a well differentiated lipoma-like, sclerosing liposarcoma with mature adipocytes and fibrous septa containing spindled and floret-like cells. The smooth muscle accounted for about 40% of the total tumor mass and consisted of fascicles and bundles of spindle cells with very little atypia or pleomorphism. Mitotic activity was moderate at most and focal in distribution. The cells colored well with immunohistochemical stain against SMA.
Results and conclusions An extraordinary rare combination-type tumor is presented. Only two previous examples of paratesticular lipoleiomyosarcoma have been published. The nature of combination-type tumors and dedifferentiated liposarcoma is addressed. The existence of two lines of tissue differentiation in the same tumor suggests the possibility of two separate tumors arising together or two lines of differentiation appearing from one pluripotent precursor cell. The differential diagnosis includes angiomyolipoma, which should be HMB45 positive.

P-097

Expression of estrogen receptor (ER) and progesterone receptors (PR) in renal angiomyolipoma

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Introduction Angoimyolipomas are the most common mesenchymal tumours of kidney. They are more common in female. Possibly estrogen and progesterone influence on tumours growth. We investigated the expression of estrogen receptors (ER) and progesterone receptors (PR) in renal angiomyolipomas.
Materials and methods 28 renal angiomyolipomas from 24 patients were tested. The expression of ER and PR was studied immunohistochemically. The expression of HMB-45, alpha-smooth-muscle actin and vimentin was also studied in all cases.
Results Most angiomyolipomas were of classic morphology with one exception. In that case, classical angiomyolipoma was combined with cystic hamartomatous lesions and involvement of regional lymph nodes. The vast majority of cells in the cases with typical morphology expressed alpha-smooth-muscle actin. Expression of HMB-45 was diffuse or focal. In the case with cystic hamartomatous lesions, an inverse correlation of expression of HMB-45 and alpha-smooth-muscle actin was observed in both renal and extrarenal components. Of the 28 tumours, two tumours were only ER positive and three tumours were ER and PR positive. In four cases, focal immunoreactivity for ER was mainly observed in the spindle shaped cells. In two cases, the immunoreactivity for PR was diffuse, but less than 5 percent of cells were positive. ER and PR positivity was diffuse, strong and widespread only in the case with cystic hamartomatous lesions.
Conclusion To our knowledge, estrogen and progesterone are not critical players in all angiomyolipoma growth, but only in the specific cases.

P-098

Proto-oncogene c-kit and c-myc expression in Kaposi sarcomas

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Introduction c-kit proto-oncogene encodes a transmembrane receptor tyrosine kinase that has been shown to be structurally related to the platelet-derived growth factor receptor. c-kit gene mutation has been shown as a mechanism of c-kit oncogene activation in human mast cell disease and gastrointestinal stromal tumors. c-myc oncogene is believed to function as a sequence specific transcription factor regulating genes which control cell growth, differentiation and cell death. c-myc point mutation has been identified in Burkitt's lymphoma. The aim of our study is to identify the expression of c-kit and c-myc proteins in Kaposi sarcoma.
Materials and methods Tissue sections from 77 Kaposi sarcomas were stained immunohistochemically with anti-c-kit and anti-c-myc proto-oncogene protein product.
Results Only 31% of Kaposi sarcomas were positive for c-kit and the staining in these lesions was usually focal. The percentage of positive cells was 5% - 10%. c-myc expression was noticed in 36/77 (~47%) of Kaposi sarcomas. Staining was also observed in early Kaposi lesions.
Conclusions 1) c-kit and c-myc protein expression in Kaposi sarcomas suggest that these genes may play a role for tumor growth. 2) c-myc protein expression in early Kaposi lesions suggests that this gene may be implicated in the initiation of these tumors.

P-099

CD10 expression in malignant myofibroblastic and smooth muscle tumors

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Introduction Myofibroblastic tumors are sometimes difficult to be distinguished from smooth muscle tumors. Recently, CD10, a cell surface metalloprotease, was shown to be present in myofibroblasts within the stroma of infiltrating cancers. To clarify the specificity of CD10 expression in myofibroblasts, we performed immunohistochemical studies on myofibroblastic and smooth muscle tumors.
Methods and materials consisted of 25 myofibroblastic and 22 smooth muscle lesions, ranging from reactive to benign and malignant tumors. All myofibroblastic lesions arose in soft tissue and smooth muscle tumors in soft tissue and organs. Using paraffin sections, immunohistochemical staining for CD10 (clone: 56C6, Serotec) was carried out by an automated stainer (BENCHMARK, Ventana).
Results Significant CD10-expression was demonstrated only in malignant or borderline lesions, including malignant fibrous histiocytoma (3 of 3 cases), low grade myofibroblastic sarcoma (4 of 7), inflammatory myofibroblastic tumor (3 of 3), and leiomyosarcoma (3 of 6). However, no positive reaction was seen in reactive lesions and benign tumors, such as scar (1 case), nodular fasciitis (4), superficial fibromatosis (3), desmoid (4), leiomyoma (7), vascular leiomyoma (3), angiomyolipoma (2), and glomus tumor (4). Furthermore, the intensity of staining was almost parallel to the microscopic anaplasia.
Conclusion The present study demonstrated that CD10 expression is not specific to myofibroblasts, but seems to be related to the biologic aggressiveness of myofibroblastic and smooth muscle tumors. Therefore, CD10 expression may be a potential predictor of malignancy in these tumors.

P-100

Correlation of metallothionein (MT) and antigen Ki-67 expressions with grade of malignancy (G) in some soft tissue sarcomas

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Introduction In soft tissue sarcomas the most significant prognostic criteria include grade of malignancy (G) and size of the tumour. Recently, metallothionein (MT) expression in cells of some malignant tumours has been shown to carry prognostic potential. Present study aimed at comparing expressions of MT and of Ki-67 antigen with G in selected sarcomas of soft tissues.
Materials and methods Immunocytochemical studies were performed on paraffin sections in 54 cases of malignant fibrous histiocytoma (MFH) and 18 cases of liposarcoma. Intensity of MT and Ki-67 expression was evaluated and the obtained results were compared to G grades.
Results Expression of MT was noted both in the cytoplasm and in cell nuclei in all studied sarcomas. Its intensity was high in 33% tumours, moderate in 35% tumours and in 32% it was low. In MFH and in liposarcoma a correlation was demonstrated between MT expression and G ($r = 0.7$; $r = 0.8$; $p < 0.05$), between MT expression and expression of Ki-67 ($r = 0.7$; $r = 0.75$; $p < 0.05$) as well as between Ki-67 expression and G ($r = 0.53$; $r = 0.74$; $p < 0.05$).
Conclusion Expression of MT in soft tissue sarcomas of MFH type and liposarcoma type strongly correlates with intensity of proliferation and with the grade of the tumour. Both markers may be helpful in determining the extent of malignancy of these tumours.

P-101

Occurrence of tumor-like lesions in pediatric orthopedics - 9 years of experience

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Introduction Tumor like lesions: solitary and aneurysmal bone cyst, non-ossifying fibroma, eosinophilic granuloma and fibrous

dysplasia may be found in the bones during the childhood and adolescence. They can cause pathologic fractures and deformities of involved bone. Rarely, some of them (fibrous dysplasia), undergo malignant transformation. The aim of this study is to analyse all the cases of tumor-like lesions of bones in children and adolescents, during the period time1993-2001.

Materials and methods The study was performed at the Institute for Pathologic Anatomy, School of Medicine Belgrade. The data source was The Bone and Joint Tumor Registry. The study was a retrospective analysis of 216 patients who had tumor-like lesion before the age of 18.

Results Solitary bone cyst was the most common lesion (64 patients). The median age was 9 years for fibrous dysplasia and solitary bone cyst, 16 years for aneurysmal bone cyst and eosinophilic granuloma, and 14 years for non-ossifying fibroma. Male to female ratio was 1:1.2 for fibrous dysplasia, 1:1.25 for aneurysmal bone cyst, 2.7:1 for solitary bone cyst, 2.3:1 for eosinophilic granuloma and 1:1 for non-ossifying fibroma. The most common sites were femur for fibrous dysplasia and aneurysmal bone cyst, humerus for solitary bone cyst, tibia for non-ossifying fibroma and bones of scull and vertebrae for eosinophilic granuloma. The most frequent symptoms and signs were pain, pathologic fractures and swellings.

Conclusion The results of our study mostly correspond to those of the current literature.

P-102

Extraspinal myxopapillary ependymoma. Case report and literature review

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Introduction Ependymomas are glial tumors that on rare occasions occur in subcutaneous locations dorsal to the sacrum and coccyx.

Case report A 35-year-old woman presented with a soft tissues tumor in the intergluteal region. MRI revealed a 7cm large mass, with solid and cystic areas, located below the coccyx, without bone alteration. Surgical excision was performed.

Pathological features Grossly, the tumor was encapsulated and showed solid, spongy and myxoid areas with a peripheral hemorrhagic focus. Histologically it consisted of medium-sized cuboidal-columnar cells, with round-oval nuclei, granular cromatin and eosinophilic cytoplasm. Tumor cells showed myxopapillary and cystic-papillary growth pattern or were arranged on fibrovascular stalks, with a central hyalinized wall vessel, surrounded by a single layer of cuboidal cells. Tumoral necrosis and hemorrhage were observed. The peritumoral stroma was highly hyalinized. The immunohistochemical stains showed vimentin(+), S-100(+), GFAP(+) and keratins(-).

Discussion and conclusion Extraspinal ependymoma (EE) is a rare tumor usually localized subcutaneously in sacrococcygeal region of young adults; most are of myxopapillary type. EE may arise from normal remnants of the neural tube or from abnormal remnants resulting from embryologic malformations. EE has a greater propensity to metastasize than its intraspinal counterpart, because of easier accessibility to lymph channels. In a study of 32 cases, Elson found a metastases incidence of 20% and no clinical or histologic data was found to predict the biological behavior of the tumor. The differential diagnosis must be done with choroid plexus papilloma, myxoid chondrosarcoma, chordoma, meningioma, adenoid cystic carcinoma and paraganglioma.

P-103

Madura foot caused by Pseudoallescheria Boydii - a case report

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Introduction Fungal infections of bone represent about 0,1-0,2% of all osteomyelitis cases. The disease most often affecting the feet - mycetoma pedis, is also known as Madura foot. In our geographic regions mycetoma is extremely rare. It is endemic in tropical and subtropical countries.

Case report We present a case of mycetoma pedis - Madura foot. The patient was a 50 years old woman. The clinical signs were pain, indurations and local redness with a long history of 10 years. The surgical material was routinely stained with hematoxylin-eosin (HE). Pathologically, a granulomatous inflammation of the bone was confirmed. All pathological characteristic pointed to fungal infection in the form of mycetona pedis. Special stains (PAS, Grocott's hexamine-silver and Giemsa) for fungi were performed: and the diagnosis of a mycetoma was confirmed. For the definitive microbiological analysis tissue inoculation on the Sabouraud dextrose agar laboratory media was done. Pseudoallesheria boydii - a sexual staging of Monosporium apiospermum was isolated. After microbiological verification of fungal infection the patient was treated surgically. Seven months after first operation the patient developed similar clinical signs again. The diagnostic procedure was repeated and mycetoma was confirmed again. The surgical therapy was performed as a therapy of choice because Pseudoallesheria boydii does not react to treatment with antimycotic drugs.

P-104

Pleomorphic variant of a spindle cell lipoma

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Spindle cell lipoma is a rare entity with an uncertain histogenesis; although the current classification includes this tumor in the lipomatous neoplasms, the immunohistochemical and electron microscopic data suggest a much closer link to the fibrous solitary tumor. We report the case of a 35 years woman with a 4/2/2/ cm nodule in the scapular area; the histopathologic examination revealed a tumor proliferation consisting mainly of giant cells, most of them multinucleated with floret-like appearance. The stroma consisted of an abundant collagen deposition with rope-like fascicles and prominent myxoid changes, most striking in the periphery of the tumor. The tumor proliferation was contiguous with nearby adipose tissue with no capsule in-between. Immuno-

histochemical tests revealed diffuse positivity for CD 34; PCNA was positive in 3 % of the cells. Smooth muscle actin, desmin and S100-protein were negative in tumor cells. The patient is well with no recurrence of the tumor one year after the resection. Despite the highly pleomorphic histopathologic appearance the tumor is benign with an uneventful clinical behaviuor.

P-105

Expression of p53 protein in GCT of bone with lung metastases

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Introduction The giant cell tumor of bone (GCT) is a local osteolytic tumor with variable degrees of aggressiveness. In rare cases pulmonary metastases can be observed. The stromal cells are the neoplastic components of the GCT, and are probably responsible for biological behavior of the tumor.
Method and results In the last 10 years 2 cases of GCT with lung metastases were diagnosed at the Institute of pathology, School of medicine, Belgrade. Both tumors displayed characteristic histological appearance of GCT: a high number of osteoclast-like multinucleated giant cells and two mononuclear cell types. The first one resembles a monocyte, the second are fibroblast-like stromal cell. Mononuclear cells had benign cytological characteristics without polymorphism and mitosis. To elucidate the mechanism of metastasing, we searched for the molecular abnormalities of p53. We examined the expression of p53 in stromal cells immunohistochemically. Unexpectedly, both tumors were p53 negative and we could not show correlation between p53 expression and metastasing of GCT to the lung.
Conclusion Although p53 positivity in other tumors is closely associated with aggressive biological behavior we could not confirm this association for GCT.

P-106

Trichoblastic fibroma or primary soft tissue adamantinoma?

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Introduction Benign extragnathic tumors that may mimic the histological features of odontogenic ameloblastoma (and ameloblastic fibroma) include trichoblastic fibroma and primary osseous and soft tissue adamantinoma. Trichoblastic fibroma is a rare ectomesenchymal tumor of hair germ involving most often the dermis and subcutis of the head and neck. The mean age at presentation is 63,8 years.The youngest case reported was that of a shoulder tumor in a 49 year old female. Its occurrence in the soft tissue of the upper arm has not been documented. Primary soft tissue adamantinomas are extremely rare with isolated cases reported in the region of the jaw and the leg ie. close to sites where odontogenic and primary osseous adamantinomas occur, respectively. No cases involving the upper arm have been reported. We report a case of a benign appearing adamantinoid tumor involving the deep soft tissue of the upper arm.
Methods A 33 year old Black male presented with a slow growing mass involving the deep soft tissue of the left upper arm without associated cutaneous or osseous abnormalities.
Results The adamantinoid features in this benign appearing biphasic tumor included nests and acini of palisading columnar epithelium exhibiting reversed nuclear polarity, areas reminiscent of stellate reticulum and an intimately associated spindle cell proliferation.
Conclusion Our favored diagnosis was a trichoblastic fibroma but we discuss the complete differential diagnosis of all tumors with adamantinoid features with respect to clinical, morphological and immunohistochemical characteristics.

P-107

Intraosseous lipoma - a case report

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Intraosseous lipoma is a rare benign tumor of mature adipocytes arising in the medullary canal, most often diagnosed incidentally. Most of these tumors occur in the long bones, with propensity for the proximal femur, fibula, and tibia. Ribs, calvarium, sacrum, metatarsus and calcaneus are other frequent sites. We report a case of a 49-yearold female with a histologically verified solitary intraosseous lipoma involving the distal part of the right fibula. Radiographically the tumor was a lytic benign-appearing lesion with sharply demarcated borders surrounded by a zone of sclerosis. Computed tomographic scan showed the lesion to have features of adipose tissue. The examination was made on surgically removed distal part of the right fibula. For histological verification of intraosseous lipoma, resected bone was decalcified, embedded in paraffin and stained with standard staining with hematoxilin-eosin. Histopathologic examination revealed mature adipose tissue with widely scattered trabeculae of bone and central calcifications. This case emphasizes the characteristics of intraosseous lipoma, and highlights the need for clinicopathologic and roentgenographic correlations.

P-108

FasL, C-ErbB2, Cyclin D1, p53 and TGFB3 expression in osteosarcomas: Evaluation of relationship with clinicopathologic parameters

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Introduction FasL, c-erbB2, cyclin D1, p53 and TGFB3 are molecules which were shown to be related to prognosis of tumors. In this study expression of these molecules were evaluated in 36 osteosarcoma (Os) cases. The correlation between the expressions and age, gender of the patients, location, grade, and histologic subtype of tumors were investigated.
Material and method FasL, c-erbB2, cyclin D1, p53 and TGFB3 expressions were detected immunohistochemically.
Results Thirty-six Os cases (23 osteoblastic, 8 fibroblastic, five chondroblastic) were included in the study. Twenty-two were males

and 14 were females. Os were located in the extremities (25), jaw (6), pelvis (3), vertebra (2). Twenty-five of 36 were high grade and 11 were low grade tumors. FasL and p53 were detected in 41.7% of the cases, TGFB3 in 80.6%, c-erbB2 in 16.7%, and cyclin D1 in 8.3%. There were statistically significant correlations between FasL and tumor grade (p: 0.001), FasL and TGFB3 (p= 0.027), FasL and c-erbB2 (p= 0.003), p53 and TGFB3 (p= 0.027). No correlation was found among other parameters.

Conclusion Expression of these molecules and detection of correlation between some molecules show that pathogenesis of Os is a complex mechanism regulated by several molecular factors. Better insight into tumorigenesis may help to provide new therapeutic approaches and idntification of patients with better prognosis.

P-109

Does hemosiderin deposition have important role in accumulation of macrophages in total hip prostheses loosening?

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Introduction Granulation tissue with macrophages is usually found around loose total hip prosthesis (THP), usually explained with deposition of wear debris from THP. However, we often found a lot of hemosiderin (an insoluble form of storage iron) in macrophages. The aim of this study was to evaluate the amount of hemosiderin in granulation tissue around THP removed after relatively short period of duration, and compare it with an interface tissue around THP of very long duration.

Material and methods We assessed the interface tissue around the loosened THP of the longest duration described ever (Judet's THP was removed after 51 years) and compared it with the granulation tissue around THP of short duration (less than six years). At histology H&E, Giemsa, and Weigert-Van Gieson staining were used. For the demonstration of hemosiderin Perls' reaction and for macrophages CD68 were used. We compared the results of histologic evaluations performed according to modified Mirra's classification: semi quantitative assessment of neutrophils, mononuclear histiocytes, chronic inflammatory cells, giant-cells, metal particles, polymethacrylate globules, necrobiosis, bone chips, necrosis and necrotic debris. But, we also included the assessment of the intensity of hemosiderin deposition.

Results Comparison of granulation tissues of short duration THP to that of 51-year duration revealed a surprising difference in the amount of hemosiderin particles with intensive intracellular hemosiderin deposition in mononuclear macrophages and giant cells in the first group. In the 51-year-old THP there was predominantly hypocellular tissue with very little amount of mononuclear macrophages containing slight amount of intracellular hemosiderin deposition and no multinucleated giant cells at all.

Conclusions We don't know why hemosiderin deposition is more evident in early loosening of THP. Hemosiderin deposition may give rise to accumulation of macrophages, perhaps increasing the process of osteolysis and granulation tissue formation. But more 'aggressive' forms of loosening may also cause recurrent bleeding. Whether the higher level of hemosiderin is the cause or the result of the loosening of THP is to be determined.

P-110

The types of tumor and peritumor stroma and biological behavior of fibrohistiocytic tumors

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Introduction Tumor-host interaction induces different types of tumor and peritumor stroma which may reveal some aspects of tumor biology. The aim of this study was to establish a correlation between different types of tumor and peritumor stroma of fibrohistiocytic tumors (FH tumors) and their biological behavior.

Materials and methods The presence of colagenous-reticular (CR), myxoid (MYX) and mixed (MX) tumor and peritumor stroma was determineted on selected paraffin sections stained by Masson-trichrome, silver impregnation, alcian blue with and without hialuronidase pretretment and PAS reaction of 121 FH tumors: 69 benign (BFH), 14 dermatofibrosarcoma protuberans (DFSP) and 38 malignant (MFH), all graded in three degrees of malignancy. The collected data were statistically evaluated by means of Student T proportion test.

Results The difference in presence of MYX and MX tumor and peritumor stroma in relation to the CR type was statistically significant between MFH and BFH ($p<0,001$) or DFSP ($p<0,05$). With an increasing malignancy grade of MFH percentage of tumors with CR tumor stroma was decreasing and percentage of tumors with MYX and MX tumor stroma was increasing. However, MFH of G1 and G2 malignancy grade did not differ in distribution of peritumor stroma types, but 73,7% of MFHG3 had MYX and MX peritumor stroma.

Conclusion While MYX and MX types of tumor and peritumor stroma are associated with agressive, CR type of tumor and peritumor stroma is associated with benign and less agressive FH tumors.

P-111

Dynamic angiogenic models of human soft tissue tumors transplanted into immunodeficient nude mice

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Introduction Animal models were used to study the vascular neogenesis of tumors. We undertook experiments using human sarcomas transplanted into the subcutaneous pad of immunodeficient nude mice. The aim of this study was analysis of: the microvasculature present within the tumor before the transplant; the early stages of the neovascularization; and the types of vascular connections established between the host and the tumor graft.

Material and methods Implants of 0.3-0.4cm in size of a Ewing's sarcoma (ES), an osteosarcoma (OS) and a fibrosarcoma (FS) were analyzed. The study was successively followed for 24, 48, 72 hours, one, two, three and four weeks. HE, PAS and immunohistochemistry for angiogenic factors were complemented with electron microscopy.

Results After 24-48 hours, the implants over-expressed several angiogenic factors. At this stage, the tumors initiated an active angiogenic remodeling in the surrounding stroma where capillary congestion and microhemorragies occurred. Endothelial sprouting of the host capillaries was seen. Vascular co-option between the endothelial cells, newly formed endothelial cells and the tumor cells was observed. No clear basal lamina was seen. Tumor-endothelial cell mosaics and vascular mimicry were detected only in the ES and OS.
Conclusions Vascular expression and neogenesis varies considerably from the fast ES to the FS while the OS reproduces patterns that are common in all three. Based upon present experience it seems evident that each tumor shows a different type of neovascularization. These models provide excellent examples of all the systems that are involved in the recapillarization of a sarcoma. This work has been supported by grants FIS 01/ 0673 and FIS 01/702 from the Ministry of Health, Madrid, Spain.

P-112

Prognostic significance of intratumoral neoangiogenesis in childhood osteosarcoma and Ewing sarcoma

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Introduction Intratumoral neoangiogenesis quantified by microvessel density (MVD) has been shown to be a strong prognostic indicator in a number of malignant tumors, primarily carcinomas. Its association with prognosis in bone sarcomas has been subject to less extensive research. The aim of our study was to investigate clinical significance of neoangiogenesis in osteosarcoma and Ewing sarcoma.
Patients and methods A retrospective immunohistochemical study was performed. 39 patients with osteosarcoma and 27 patients with Ewing sarcoma were included. Sections from diagnostic biopsies were immunostained using factor VIII and CD 34 (DAKO) antibody and microvessels were counted at 400x magnification on three microscopic fields showing highest MVD per patient. MVD was correlated with overall and event-free survival by Kaplan-Meier and log-rank analysis. The difference between the mean microvessel counts in patients with or without metastasis and alive or dead was assessed by Mann-Whitney test.
Results In patients with osteosarcoma significant statistical difference was found as regards the death risk and risk of development of metastasis between patients with high (>31 vessels/field) and low (<31 vessels/field) microvessel counts (log-rank test p=0.0027 and p=0.0209, respectively). In patients with Ewing sarcoma a trend was noted for the patients with lower (<31.5 vessels/field) microvessel counts to have better outcomes than patients with higher vessel counts, however, the difference was not statistically significant.
Conclusion These findings strongly suggest that neoangiogenesis quantified by microvessel density is predictive of metastasis and poor prognosis in osteosarcoma. Valorization of its role in patients with Ewing sarcoma awaits further studies.

P-113

Epithelioid sarcoma - clinicopathological findings in 6 cases

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Epithelioid sarcoma is a soft tissue sarcoma of unknown histogenesis with a predilection of young adults and hand/forearm location.The tumor is often multinodular showing central necrosis. Epithelioid sarcoma has a high potential of local recurrence and metastasis. Six cases were presented. Five patients were male with a mean age of 30. Tumor locations were finger, hand and forearm. Cytokeratin and vimentin were positive in all cases. Four cases showed positivity for CD34. Desmin and CD31 were negative. Ki-67(MIB-1)proliferation index was 12-15% in 4 cases. Initial surgery performed in all of the patients has been local excision with negative margins.Three patients received radiotherapy or chemotherapy after the primary excision.Except for one patient who was lost to follow-up, local recurrence was detected in all the patients in a period of 2 months to 2 years.Three patients died with distant metastasis. Epithelioid sarcoma can be easily identified with its typical features, but it can be confused with non-tumor lesions in patients having a long clinical history and a small tumor. Radical surgery and regional lymph node dissection may bring benefit; the incidence of the lymph node metastasis is high. Radiotherapy and chemotherapy can be added to therapy in the recurrent or metastatic state of the disease.

P-114

Intramuscular hemangioma of mixed type

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Introduction Intramuscular hemangiomas are relatively uncommon but significant lesions, and their unorthodox location and infiltrative pattern often raise the question of malignancy to clinician and pathologist. We present the histomorphological and immunohistochemical data of a hemangioma in a 20-year-old man who presented with an enlarging painful soft tissue mass of his right upper leg.
Methods The complete local excision of tumor arising in the quadriceps muscle was done. Sections of formalin-fixed, paraffin-embedded tissue material were stained with HE, Van Gieson, PAS, PTAH, Gomori's reticulin and trichrome, and immunohistochemically for detection of factor VIII-related antigen.
Results Grossly, the lesion (1x2,5 cm) was poorly circumscribed, reddish brown and partially haemorrhagic. Microscopically, it was composed both of small, capillary-sized and large, cavernous vessels lined by endothelial cells and surrounded by connective and mature adipose tissue. The adipocytic component wasn't prominent. Many cavernous spaces were separated by dense fibrous tissue and some of them were thrombosed. Foci of hemosiderin deposits and moderate lymphocytic infiltration were

also noted. The small vessels in some areas hadn't discernible lumina so that areas appeared solid. Mitotic figures and intraluminal papillary tufting were not seen, but infiltration of skeletal muscle bundles and rarely perineural spaces were present. The muscle fibres showed atrophy, degenerative changes and signs of regeneration. The cells lining both cavernous and capillary-sized vessels showed expression of factor VIII-related antigen.
Conclusions Presented case of tumor arising in the quadriceps muscle show histomorphological and immunohistochemical features of mixed type hemangioma with pronounced infiltrative pattern.

P-115

Alveolar soft part sarcoma: a review of 8 cases at National cancer institute from Colombia

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Aims To review the histology and immunohistochemistry of alveolar soft part sarcoma with comparison of clinical features.
Material and methods Histology slides, paraffin blocks and files of patients were reviewed.
Results and discussion There were 8 patients retrospectively analysed, 5 female and 3 male, between 1 year and 39 years old. The initial symptom was mass. The most frequent location was thigh (25%), followed by the leg, orbit, chest wall, trunk, arm and forearm. Six patients had haematogenous metastases at the time of diagnosis (lung 4, brain 1, lung and bone 1). Histologically, all tumours were composed of nests of medium sized cells arranged in pseudo-alveolar or lobular patterns, separated by fibrovascular stroma. PAS & PAS diastase were positive in all cases, with rhomboid or rod-shaped crystals. Immunohistochemical staining for Myo D1 showed cytoplasmic positive in 8 cases (100%), desmin in 4 and smooth muscle actin in 2 cases.
Conclusion Alveolar soft part sarcoma is an uncommon neoplasm. The microscopic picture is a constant and typical feature of the lesion, and immunohistochemistry is a useful complementary method for diagnosis.

P-116

Malignant peripheral nerve sheath tumor with a t(X;18)

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Introduction Malignant epithelioid schwannoma is a rare variant of malignant nerve sheath tumor (MPNST) that often causes differential diagnostic problems with other neoplasm.
Material and methods A 56-year-old woman presented with a palpable tumor located in the supraclavicular region. The lesion was partially resected. Grossly, the tumor was firm, gray to whitish and slighty nodular. Standard histological procedure and inmunohistochemical study was performed.
Results Microscopic examination revealed a solid proliferation of moderately large, rounded epithelioid cells, with pleomorphic nuclei, prominent nucleoli and atypical mitoses. In peripheral areas the tumor showed smaller cells with nuclei arranged in palisades and central necrosis. Immunohistochemical studies showed vimentine, S-100 protein, and cytokeratine positivity and HMB-45 negativity. The histopathology and the immunohistochemical results were consistent with MPNST, but a SSX2/SYT gene fusion was detected by molecular analysis, a characteristic chromosomal change of synovial sarcoma (SS).
Conclusions Spindle cell tumors with epithelioid areas can be difficult to diferentiate by histologic pattern alone. Cytogenetic analysis and molecular studies may reveal the diagnosis by demonstrating a characteristic chromosomal abnormality or molecular rearrangement. SSX/SYT gene fusion results from the t(X;18)(p11.2;q11.2). The translocation fuses the SYT gene from the chromosome 18 long arm to either of 2 highly homologous genes on the chromosome X short arm, SSX1 or SSX2. The translocation t(X;18) is currently regarded as a specific molecular marker of SS. Our case shows, that we might consider that SS may have a larger histologic spectrum than previously thought.

P-117

A case of idiopathic retroperitoneal fibrosis (Ormond's disease) and renal supracapsular liposarcoma

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Background Idiopathic retroperitoneal fibrosis is an uncommon inflammatory disease characterized by proliferation and fibrosis of retroperitoneal tissue complicated by obstruction and encasement of retroperitoneal structures.
Case report We describe a 46-year-old female patient who underwent a gynecologic operation for left ovarian cyst in October 2001. A periaortic mass alongside with peritoneal nodules were biopsied which revealed endometriosis, necrotic xantomatous nodules and Ormond's disease. The last diagnosis was rejected and tumor misinterpreted as malignant fibrous histiocytoma. The course of the disease progressed and on computed tomography a left renal supracapsular tumor measuring 20/10/8cm together with a diffuse retroperitoneal fibrosis was demonstrated. In November 2002, a tumor localized in the adipose capsule of the left kidney was removed together with the kidney weighing 7,5kg. The kidney was markedly atrophic. A parallel excision of ten retroperitoneal tissue masses with a size up to 10/6/4cm was performed. Our final histological diagnosis was highly differentiated liposarcoma. Four months later the abdominal CT and the tumor markers were normal.
Conclusion The symptoms in our case were not specific for renoureteral obstruction and included abdominal pain and mass, weight loss and ascites during the year prior to second operation and finally urinary symptoms. We interpret our case as a slowly growing perirenal lipomatous tumor gradually compressing the kidney and causing slowly progressing retroperitoneal fibrosis.

P-118

A rare case of thoracic angiomatosis in an adult and review of the literature

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Introduction Angiomatosis is a rare condition in which large segments of the body are involved by proliferating vessels. The

majority of angiomatoses present during childhood or infancy. A diffuse angiomatosis affecting thoracic organs is a rare, frequently fatal disorder. The origin of the lesion is unknown. The lesion consists of thin-walled sometimes bloodless capillary blood vessels. The definite diagnosis is usually made by open lung biopsy or autopsy. In our paper we are presenting the rare case of angiomatosis of pleura and lung in 37-years old woman and the review of the literature.

Materials and methods The patient was presented to the hospital with complaints to the pain in the right chest, dyspnea, weakness. The lesion manifested on chest radiographs and CT scans with unilateral pleural fluid and nodular pleural thickening. Thoracocenthesis was performed and 600 light brown fluid was drained. Cytologically the effusion contained erythrocytes and lymphocytes. Further 3000 ml more fluid was obtained. The open-lung biopsy revealed the extensive diffuse angiomatosis affecting visceral and parietal pleura and subpleural lung tissue. The lesion consisted of bloodless, thin walled, endothelium channels. Factor VIII-related antigen and CD31 were the most reliable immunocytochemical markers in highlighting the endothelia.

Results The palliative methods of treatment were not effective, and the patient was treated by total pleurectomy.

Conclusion Diagnosis is based exclusively on histologic finding as the only possibility of diagnosis. Open pleurectomy is the most succesful treatment in preventing reaccumulation of the effusion.

P-119

Immunolocalisation of cysteine proteinases and their inhibitors in asthma

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Background Chronic inflammation with remodelling of the airway wall structures is an essential characteristic in asthma patients. Lysosomal proteinases are involved in extracellular proteolysis, which leads to matrix changes of subepithelial connective tissue in the airway wall. The aim of the study was to localise the expression of different cysteine proteinases and their endogenous inhibitors in lung tissue of asthma patients.

Material and methods We included ten specimens of bronchial biopsies of asthma patients and lung tissue specimens of fatal asthma case. Tissue specimens were fixed in formalin and embedded in paraffin. H&E slides were used for morphological examination. Immunohistochemistry was performed on an automated stainer (Benchmark, Ventana, Tuscon, AZ, USA). We used monoclonal antibodies to visualize cysteine proteinases (cathepsin B, H, L and S) and their inhibitors (stefin A and B, cystatin C) (Krka, Ljubljana, Slovenia).

Results Cathepsin B showed the most intense and wide distributed positive reaction. It was localized in the respiratory epithelium, macrophages, lymphocytes, mucous glands and smooth muscle cells. Cathepsin H showed intense reaction localized in the respiratory epithelium, macrophages, lymphocytes, mucous glands, but not in the smooth muscle cells. Cathepsins L and S showed positive reaction only in macrophages. The positive reaction of all three inhibitors was weaker compared to cysteine proteinases; it was observed in the respiratory epithelium, macrophages and mucous glands.

Conclusion These results suggest involvement of cysteine proteinases and their endogenous inhibitors in asthma, showing distinct immunoreactivity and different distributions in the lung tissue of asthma patients. Since the inhibitor expression is less intense we can speculate that remodelling of the extracellular matrix may be also a consequence of imbalance between cysteine proteinases and their inhibitors.

P-120

The correlation between aberrant connexin 43 mRNA expression induced by promoter methylation and nodal micrometastasis in non-small cell lung cancer

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Introduction Reduced connexin (Cx) 43 gene expression has been shown in most of lung tumors and lung cancer cell lines. Although aberrant Cx43 gene expression was linked with lung tumorigenesis, our understanding to the mechanism was still limited. We hypothesized that the evidence of aberrant Cx43 gene expression was gradually intensified from adjacent normal lung tissues surrounding tumors towards to tumor tissues.

Materials and methods In this study, 90 lung tumors and adjacent normal tissues were collected to examine Cx43 mRNA expression by reverse transcription-polymerase chain reaction (RT-PCR).

Results Our data showed that Cx43 mRNA expression in adjacent normal lung tissue was significantly correlated with nodal involvement ($P = 0.03$), but the similar trend was not observed in tumor tissues. To verify whether lack of Cx43 mRNA expression was resulted from promoter methylation, PCR-based methylation assay was performed for Cx43 promoter methylation analysis. High frequency of promoter methylation was observed in Cx43 mRNA negative patients (21 of 33, 63.7%) compared with Cx43 mRNA positive patients (3 of 57, 5.3%, $P < 0.0001$). To elucidate whether aberrant Cx43 gene expression is originated from adjacent normal lung tissues, 25 lung tumors and each of five adjacent normal tissues with various distances from tumor tissues were collected to examine Cx43 mRNA and protein expression by RT-PCR and western blot, respectively. As the results shown, Cx43 mRNA and protein expressions were gradually decreased from adjacent normal lung tissues to tumor tissues with a positive correlation to the distance from tumor tissues. Gel shift assay data also revealed that shifted band binding with AP1 was only observed in adjacent normal tissues which were far from the tumor tissues.

Conclusion These results indicate that promoter methylation may interfere with AP1 binding to promoter to cause aberrant Cx43 gene expression. Thus, Cx43 mRNA in adjacent normal tissue surrounding lung tumor simply detected by RT-PCR may be act as a molecular marker of nodal micrometastasis in NSCLC.

P-121

Fhit, cell adhesion molecule and matrix metalloproteinase expression, and cell proliferative activity during peripheral lung adenocarcinogenesis

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Introduction An adenoma-carcinoma sequence is recognised in peripheral lung. Atypical adenomatous hyperplasia (AAH) and bronchioloalveolar carcinoma (BAC) are the pre-invasive precursors of invasive adenocarcinoma, which frequently has a 'residual' peripheral BAC component. The biology of this neoplastic progression is poorly understood. The aim of this study was to examine the expression of the tumour suppressor gene FHIT, CD44v6, E-cadherin, beta-catenin, MMP-2, TIMP-2, and the cell cycle marker MCM2 at different stages in the proposed sequence.
Materials and methods Sections from formalin-fixed, wax-embedded tissue blocks of 118 AAH, 18 BAC and 133 invasive adenocarcinomas, stained immunohistochemically for the above molecules, were scored semiquantitatively. In invasive tumours, any BAC component was scored separately. For MCM2 a labelling index was determined as the ratio of immunopositive to total cells counted.
Results Fhit protein is abundant until the disease becomes stromally invasive ($P<0.0001$). CD44v6 levels are high in AAH and BAC, but fall in invasive disease ($P=0.007$). E-cadherin and beta-catenin levels rise between AAH and BAC ($P<0.001$), and remain well expressed thereafter. MMP-2 and TIMP-2 expression is relatively low in AAH, rises in BAC but falls when tumour becomes stromally invasive ($P<0.01$). The BAC component of invasive tumours expresses some molecules in a manner similar to pure BAC but mirrors expression in invasive tumour for others. MCM2 counts increase from 0.87%(low grade AAH) to 65.5% in invasive adenocarcinoma ($P=0.01$ or less)
Conclusion In peripheral adenocarcinogenesis, loss of Fhit is a relatively late event, while CD44v6 loss occurs earlier. Fhit loss has potential as a surrogate marker of invasion. E-cadherin and beta-catenin expression probably reflects changes in lesion architecture. MMP-2/TIMP-2 may be implicated in BAC but down-regulated as stromal invasion develops. Increasing cell cycle entry is consistent with neoplastic progression.

P-122

Immunohistochemical study of squamous and adenosquamous cancer of lungs

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Aims Expression of proliferation markers and factors of invasion in different forms of lung carcinomas.
Methods Using formalin-fixed and paraffin-embedded tissues, we examined by immunohistochemistry tenascine, collagen IV, CD44, MMP1, MMP9, TIMP1, TIMP2, CD95, Bcl2, p53, PCNA, Ki67, cyclin D1 in 30 cases of lung carcinoma - 15 squamous and 15 adenosquamous with (first group) and without (second group) metastases.
Results The positive staining with tenascine was observed in all cases of squamous and adenosquamous carcinoma, but the most strong expression was found in extracellular matrix and tumor cells of carcinomas of first group. The overexpression of MMP1 and MMP9 was found in cases with metastases in tumor cells and stromal cells. The reaction was localised on periferical zone of tumor complexes. At the same time reaction with TIMP1 was low in tumor of second group and very low – in first group, reaction with TIMP2 was moderate in metastatic tumor and low – in second group. Overexpression PCNA and Ki67 changed from 75% to 90% in 1 group and from 30% to 50% in 2 group. The increase expression of cyclin D1 was only in adenosquamous lung carcinoma. The overexpression of p53 was found only in metastatic tumors.
Conclusion There are some difference between squamous and adenosquamous lung carcinomas in expression of proliferation markers, and cyclin D1, but no difference in expression of metalloproteinases and their inhibitors. We found more prominent differences in groups of metastatic and nonmetastatic tumors.

P-123

Lack of Her-2/neu amplification or overexpression in malignant pleural mesothelioma (MPM)

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Introduction The Her-2/neu receptor, a member of the epidermal growth factor receptor (EGFR) family, represents a novel therapeutic target for the newly marketed recombinant anti-Her2 monoclonal antibody Herceptin®. MPM mortality continues to rise steeply (5% to 10% per year) with no known curative modality. Approximately 70% of MPMs have high levels of expression of EGFR. Two recent studies have reported expression of Her-2/neu in MPM but there was no analysis of overexpression or amplification. In this study, the amplification/overexpression of Her-2/neu gene has been evaluated in a series of 35 consecutive MPM biopsy specimens.
Materials and methods The tumour specimens had been formalin-fixed and paraffin-embedded. Immunohistochemistry (IHC) for Her-2/neu overexpression was performed using the Dako HercepTest kit. Only membrane staining intensity and pattern were evaluated using a 0 to 3+ scale with 2+ being the lower limit for a positive test. Amplification of the Her-2/neu gene was tested with the fluorescent in-situ hybridisation (FISH) method (Ventana INFORM Her-2/neu Probe) in 10 out of the 35 cases. Cases were considered as amplified by FISH when the mean number of fluorescent signals per nucleus was greater than four.
Results Of the 35 MPMs evaluated for the overexpression of the Her-2/neu receptor, all 35 scored 0 on the IHC test and all 10 cases additionally assayed by FISH were negative.
Conclusions Although the sample size was limited, these findings suggest that MPM do not overexpress or amplify Her-2/neu, which therefore should not be considered a prognostic and predictive marker in MPM. (This work was funded by the Alessandria branch of the Italian League against Cancer).

P-124

Morphological changes of bronchial glands at the localisation of preceding biopsy

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Introduction At the location of preceding biopsy, in sero-mucous bronchial glands, there are certain changes of regenerative type. Hyper-plastic and meta-plastic squamous epithelia partially or wholly fills the acinus and duct of secretory glands. Aims: Regenerative atypical characteristics found in repeated materials can be misinterpreted as epidermoid or muco-epidermoid carcinoma.
Material and methods The research used 44 materials made by re-biopsy of bronchia, with regeneratory changes in bronchial glands. Presence of morphological parameters was examined semi-quantitatively, and then processed with methods of descriptive statistics.
Results In 59.09% lobular architectonics of the lesion was preserved; fibrin exudates were present in 90.91% cases; granulocytes in stroma were present in 68.18% cases; granulation tissue in 97.44% cases; planocellular metaplasia of duct and acinus was present in every single case. In 43.18% cases mucin-secreting cells were found in squamous epithelia. The squamous cells were diskeratotic in 35% cases; with polymorph nuclei in 27.73% cases; hyper-chromatic in 20%; multi-nuclear in 12.5%; with visible nucleolus in 72.5%, and with mitotic activity in 32.5% cases. In 61.36% cases the squamous cells were located in the fibrous tissue. Mucus in interstitium was present in 41.86% cases; cystoid formations were present in 23.25% cases.
Conclusions The changes are built of solid material, undifferentiated and/or squamous cells located in the granular tissue or in the fibrin. Infiltrates of granulocytes are found in fibrin, stroma, and squamous epithelia. Epithelial cells in regeneration show atypical cell characteristics in small percentage and therefore are hard to differentiate from carcinoma.

P-125

Immunohistochemical expression of Her-2/neu in patients with lung carcinoma

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The HER-2 protein or p185her2 is a membrane receptor with tyrosine kinase activity encoded by HE2/neu gene. Overexpression of HER-2/neu has been observed in many human cancer, including lung cancer. There are different reports about the expression of this protein in different lung cancer. The aim of the study is to determine the expression of HER-2 protein in the spectrum of lung cancer (adenocarcinoma, squamous cell carcinoma and small cell carcinoma). The study population consisted of two groups: 19 patients that have undergone to surgical treatment and 10 patients that have undergone to fiber-optic bronchoscopy and biopsy for primary diagnosis.Tissue specimens were neutral formaline fixed and paraffin embedded. Standard histochemical and immunohistochemical staining were used for diagnosis. Expression of Her-2/neu protein was determined by immunohistochemical staining with Hercep TestTM (DAKO). The results were graded 0-1 as negative and 2-3 as positive. Overall incidence of HER-2/neu overexpression was 34,4% (10 of 29). Higher incidence was found in patients with adenocarcinoma 45,4% (5of 11); squamous cell carcinoma 30,7% (4of 13) and small cell carcinoma 20% (1 of 5). No statistical significant difference was seen with age and gender. HER-2/neu overexpression was more relevant for patients with advanced tumor: all patients with squamous cell carcinoma were in stage 3b and 4, and 80% with adenocarcinoma were in stage 3a and 3b. These results are satisfactory and encourage us to continue this work in the follow-up study to evaluate HER-2/neu role as predictive and prognostic factor for patients with lung cancer.

P-126

The expression of the EGF-receptors (EGFR, ErbB-2, ErbB-3 and ErbB-4) in neuroendocrine tumors of the lung

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Introduction EGF and TGF-alfa play a major role in development of several human solid tumors. Hereby the expression of EGF receptors has been investigated to elucidate their role in tumor growth and progression. The aim of this study was to evaluate the expression and distribution of EGF receptors, EGFR, ErbB-2, ErbB-3 and ErbB-4 in neuroendocrine tumors of the lung, in order to relay them to histology, progression and prognosis of these tumors.
Material and methods We applied immunohistochemistry to paraffin sections of neuroendocrine tumors using MoAbs specific for EGFR, ErbB-2, ErbB-3 and ErbB-4.
Results In all cases of neuroendocrine carcinoma the majority of neoplastic cells displayed a nuclear and cytoplasmic positivity for ErbB-4. On the contrary in 75% of cases EGFR, ErbB-2 and ErbB-3 receptors were focally and randomly expressed in about a third of neoplastic cells. Carcinoid tumors displayed only a focal positivity for ErbB-4 in about two third of the cases, although they were all EGFR negative. Moreover about a third of carcinoids displayed a cytoplasmic and focal membrane positivity for ErbB-2 and ErbB-3.
Conclusions Our data suggest that ErbB-4 and EGFR may play a costant role in the pathogenesis of neuroendocrine lung carcinomas; moreover the modulation of expression of EGF receptors may contribute to elucidate the pathogenic relationship between neuroendocrine carcinomas and carcinoids of the lung.

P-127

Laminin alpha2 chain positive vessels and metastatic tendency in neuroendocrine lung carcinomas

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Introduction We previously demonstrated that laminin-α2-chain positive vessels are markers of early angiogenesis in tumors such as glioblastomas and supraglottis, breast and lung non small cell carcinomas. The aim of this study was to evaluate distribution of laminin-α2-chain positive vessels in lung neuroendocrine carcinomas to relay their number to metastatic tendency of these tumors.

Material and methods We applied immunohistochemistry on six neuroendocrine lung carcinomas using MoABs specific for extracellular matrix proteins and integrins; moreover transwell test migration assays evaluated the rate of transendothelial migration of AE-2 neuroendocrine carcinoma cell line throught a monolayer of EAHY endothelial cell line expressing laminin-α2-chain to establish wheather this protein may favour in vivo vascular invasion and metastasis.
Results 80%, 75%, 90%, 65%, 80% and 70% of vessels were respectivelly distributed within the parenchyma of these tumors. Studies on contignous sections demonstrated that parenchymal vessels were positive for all ECMPs, but laminin-α2-chain, which was respectively expressed in 100%, 30%, 80%, 30%, 60% and 100% of parenchymal and in 100%, 0%, 30%, 0%, 100% and 70% of stromal vessels of these tumors. In all cases about 25% of neoplastic cells were α6 positive. Preliminary results of transwell test migration assay indicate that the pretreatment of the AE-2 cell suspension with anti laminin-α2-chain and anti α6-integrin MoAbs reduced their migration through the EAHY monolayer.
Conclusions As known α6-integrin is a receptor of laminin-α2-chain, therefore the up-regulation of this protein in a large number of vessels during tumor angiogenesis may favour metastasis increasing neoplastic cell adhesiveness to newly formed vessels.

P-128

Brain death as a cause of primary lung graft disfunction in an isolated rabbit lung model

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Introduction early lung dysfunction observed after lung transplantation has been attributed to an increase of vascular permability. Factors involved in this mechanism are unknown. Our hypothesis was that brain death (BD) is a preconditioning factor that induces primary lung graft dysfunction.
Methods twenty-four rabbits were randomized to control (CRL) or induced BD (increasing intracraneal pressure above systolic pressure for 30 minutes) groups and were ventilated in volume controlled mode (tidal volume 10 ml/kg, positive end expiratory pressure (PEEP) 0 cmH2O and respiratory rate (RR) to obtain normocapnia) for 120 minutes. Isolated lungs were perfused with 300 ml/min (constant flow) and ventilated in pressure controlled mode (peack pressure 30 cmH2O, PEEP 5 cmH2O and RR 20) for 30 minutes. Pulmonary edema and lung injury were assessed by weight gain, change in ultrafiltration coefficient before and after ventilation; and extent of hemorrhage (scored by histology: intensity of alveolar hemorrhage, percentage of alveoli with hemorrhage and number of vessels with perivascular hemorrhage) Mean values (3 samples per lung) of the 3 indices of hemorrhage showed above were scored (0 to 5) and added to obtain a score of overall lung hemorrhage (range 0 to 15) for each lunch. Data are expressed as mean and 95% confidence interval and were compared with paired t test.

Results

	CRL	BD	p
Weight gain (g)	0.43(0.20-0.65)	0.92 (0.52-1.32)	0.031
Hist score (0-15)	5.4 (3.37-7.42)	8.80 (6.15-11.45)	0.036
Ultraf coeficient	0.00199	0.00315	
	(-0.00397-0.00165)	(-0.00402-0.00170)	0.404

Conclusions under these experimental conditions BD contributes to pulmonary edema and lung hemorrhage.

P-129

Expression of cyclooxygenase-2 in nonsmall cell lung carcinomas

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Introduction Cyclooxygenase (COX) is an inducible enzyme that catalyses the conversion of arachidonic acid to prostaglandins and thromboxanes. COX-2 is induced by cytokines and growth factors and has been shown to be important in carcinogenesis. The aim of this study was to investigate the expression of COX-2 and clinicopathologic factors in nonsmall cell lung carcinomas (NSCLC) with regard to survival times.
Materials and methods Seventy-seven NSCLC specimens were evaluated for COX-2 expression by immunohistochemical method. Staining patterns were assessed semiquantitatively and correlated with tumor histologic type, TNM stage and prognosis. Chi-square and Kaplan-Meier survival analysis was performed.
Results COX-2 was not expressed in the normal bronchial epithelium. COX-2 expression was detected in 36 (46.8%) NSCLC. Expression of COX-2 was significantly higher in adenocarcinomas than in other histologic subtypes (p=0.018). There was no significant relationship found between COX-2 expression and age, gender, TNM stage, and prognosis.
Conclusion This study suggests that an increase in COX-2 expression may be associated with the development of adenocarcinomas. But, prognostic significance of COX-2 expression is controversial.

P-130

Prognostic significance of cyclin-dependent kinase inhibitor p27 and Bcl-2 protein in nonsmall cell lung cancer

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Introduction The expression of p27, which is known a cyclin-dependent kinase inhibitor, has considerable value for the prognosis of cancer patients. Bcl-2 gen product (Bcl-2 protein) is implicated in oncogenesis by its ability to prolong cell death through the inhibition of apoptosis. We studied the expresssion of p27 and bcl-2 protein to investigate their association between clinicopathologic factors and prognosis in nonsmall cell lung cancer (NSCLC)
Materials and methods Patients with NSCLC were included in our study.There were 30 patients(25 men,5 women), 15 patients

had adenocarcinoma (AC) and 15 had squamous cell carcinoma (SCC).Tumors were examined immunohistochemically with p27 and bcl-2 protein expression, using formalin-fixed, parafin-embedded tissue. For the evaluation of p27 expression, the number of nuclear staining was counted and showed as a percentage.Bcl-2 protein was primarily identified in the cytoplasm of tumor cells. Statistically, correlation between immunoreactivity and the clinicopathologic factors were evaluated.

Results P27 immunoreactivity was observed in 12(40%) of 30 cases. There were 9(60%) SCC, 3(20%) AC. Statistically, these findings were found significant (p=0,025). Percentage of p27 positive nuclei, the average being 25%. Positive bcl-2 immuno-staining was found in 8(26,6%) of 30 cases. 5 cases (33,3%) of 15 SCC and 3 (20%) of the 15 AC were positive. These findings were not found significant (p=0,68)

Conclusions There is no correlation between p27 and bcl-2 expression and clinical parameters, clinical stage, pathologic grade and prognosis. Our study suggest that p27 immunreactivity in NSCLC may provide prognostic information.

P-131

Pulmonary hamartoma – report of four cases

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Introduction Mesenchymal lesions of the lung encompass a wide variety of benign and malignant conditions. Pulmonary hamartoma (PH) is a bening mesenchymal tumor, usually described as a solitary pulmonary nodule, composed of fat, smooth muscle and respiratory epithelium. The diagnosis is based on clinical data, imaging methods (radiography, computed tomography), fine needle aspiration cytology (FNAC) and histomorphological study. Because of the tumor rarity we find just a few reports, concerning the cytologic characteristics. The aim of the present study is to describe the cytological features of PH and to compare them with the histological findings.

Materials and methods We have examined four cases of PH, who have undergone surgery, and we have used routine histological stains on paraffin sections for the diagnosis. FNAC is performed prior surgery in one patient, and in the other three offprints of the resected tumor are prepared.

Results The cytomorphological study show predominance of spindle cells, admixed with many stellate cells, and fibromyxoid material on a serosanquinous background as well. Postoperative histopathological study reveals spindle shaped smooth muscle cells, goblet cells, cystic spaces, covered with bronchiolar epithelium, cartilaginous or myxomatous tissue, thus confirming the diagnosis pulmonary hamartoma. In

Conclusion These rare tumors may be exactly diagnosed by correlating clinical and radiological data with cytological and histological findings.

P-132

A case of neuroblastoma in subpleural localisation in a 56-year-old man

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Introduction Neuroblastoma is a malignant tumor of early childhood and is very rare in adults, thus we report this case.

Case report We describe a case of a 56-year-old man, never before notably ill. Basic laboratory values were not significantly anomalous except of mild anaemia and high erythrocyte sedimentation rate. An X-ray scan showed a spherical opacity 4 cm in diameter in the top right pulmonary field paramediastinally. CT displayed a low-density tumor 5x3, 8x4,5 cm in the apex of the right hemithorax. The tumor was well circumscribed and seemed to be in a subpleural localisation. It was subsequently punctioned with Chiba and Core needle under CT control. Cytological and histological material did not contain any malignant cells. On MR scan there was a well encapsulated extrapulmonary tumor without destruction of ribs, probably of benign origin. Tumor extirpation was indicated. The lesion was growing out of spinal chord. It was completely removed. Grossly, the tumor looked like an old hematoma. Histologically, it had the typical appearance of neuroblastoma - malignant tumor which is found in more than 90% of cases in patients under 6 years of age. A scintigrafical examination and trephine biopsy showed massive bone marrow infiltration by neuroblastoma.

Conclusion A 56-year-old man was confirmed to have a typical early childhood tumor - neuroblastoma in a subpleural localisation. Despite the poor prognosis of this tumor in adults and a wide bone marrow infiltration, the patient is well and without signs of disease after surgical resection and two cycles of chemotherapy over 22 months after the initial diagnosis.

P-133

Measurements of DNA amount and nuclear size by image cytometry: are these two features sufficient for diagnosis of early lung cancer?

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Background Lung cancer represents 15% of all cancers, but is responsible for nearly 30% of cancer related deaths due to the fact that it is most often detected in a very late stage. It has been proposed that selected nuclear features could be used to assist lung cancer detection in an early, operable stage. It was shown that both DNA amount (DNA ploidy) and nuclear size are key features for positive diagnosis in oral, laryngeal, bronchial, and gastric epithelial tumors. We used image cytometry to assess the potential of this approach using nuclei of epithelial lung cells from sputum samples.

Experimental design Sputum samples were obtained by induction method, using hyperosmolar salinic solution, from 139 lung cancer patients and 148 high risk individuals. The cells were fixed and after breaking mucosa with mechanical and chemical treatment, they were cyto-centrifuged onto the microscopy slides. Cell nuclei were stained by a DNA specific and stoichiometric (Feulgen-Thionin) stain and DNA amount (ploidy) and nuclear size of 6000

(on average) cell nuclei from each sample were measured by a high resolution image cytometer.
Results The results showed that there were no statistical differences between the DNA ploidy values between control and cancer cases, as very few cancerous cells were found in the sputa of lung cancer patients. Interestingly, there was a measurable increase in the mean nuclear area of diploid cells from cancer patients in comparison to control cases.
Conclusions Despite the slight difference in the size distribution the nuclear size feature alone does not provide effective discrimination between the negative and positive populations.

P-134

Pulmonary blastoma: report of 10 cases

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Introduction Pulmonary blastomas (PB) are rare lung tumors having unique histomorphological features. Although they were once regarded in the same group of lung neoplasms with well differentiated fetal adenocarcinomas, recently the two tumors were seperated from each other. We aim to discuss the morphological features and the changes of morphology on recurrences of pulmonary blastomas.
Materials and methods We present ten cases of PB, six of which were biphasic, two were pleuropulmonary, and two were cystic. Biphasic PB patients were five males and one female, with an age range of 29-70. Two female patients with pleuropulmonary blastoma were 6 and 22 years, and cystic PB patients were 3 and 4 years old female and male children, respectively.
Results One of our most striking histomorphological findings was that four of the biphasic PBs accommodate mature epithelium, not fetal epithelium. One of the other two cases presented with a fetal adenocarcinoma and the tumor recurred as a biphasic PB. One of the cystic PB, which arised in a background of congenital bullous emphysema, recurred with pleomorphic cells forming solid groups. The other cystic PB was diagnosed as congenital cystic adenomatoid malformation.
Conclusion Because of the changing morphological features and composition of epithelial component, classification of the pulmonary blastomas should be reviewed.

P-135

5-Lipoxygenase (5-LO) detection in pleural mesothelioma: an immunohistochemical study

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Introduction Evidence indicates that the 5-LO pathway is critical for malignant tumor cell growth. We have previously shown that human malignant pleural mesothelial (MM) cells, but not normal mesothelial cells, express a catalytically active 5-LO, which regulates MM cell proliferation and survival via a VEGF-related circuit (Romano et al., FASEB J. 15:20326,2001). Aim of our study was to investigate in vivo expression of 5-LO in human mesothelioma.
Materials and methods Immunohistochemical analyses were conducted in five micron sections from human pleural mesothelioma (9 epithelial, 2 biphasic and one fibrous type). 5-LO expression was evaluated using a 5-LO polyclonal antibody.
Results Immunohistochemical staining was observed in all cases. The staining pattern was cytoplasmic in 4 cases (2 epithelial, 1 biphasic and 1 fibrous type), both nuclear and membranous in one case of the epithelial type, both nuclear and cytoplasmic in 5 cases (4 epithelial and 1 biphasic type), nuclear, cytoplasmic and membranous in two cases of the epithelial type.
Conclusion These results are consistent with our in vitro data showing 5-LO expression in MM cells and strongly suggest that 5-LO may play a relevant role in the pathobiology of malignant pleural mesothelioma.

P-136

Expression of vascular endothelial growth factor (VEGF) and E-cadherin in human non-small cell lung cancer

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Introduction The present study is scheduled to analyze the intratumoral expression of vascular endothelial growth factor (VEGF) in human lung cancer and to determine the relation with microvessel density (MVD), E-cadherin expression and clinicopathological parameters.
Materials and methods Using immunohistochemical technique, the expression of VEGF (Neomarkers) and E-cadherin (Santa Cruz, Bioanalytica) was studied in 88 non-small cell lung cancer (NSCLC) specimens (48 squamous cell carcinomas, 30 adenocarcinomas and 10 large cell carcinomas). MVD was assessed by visual quantification of microvessels stained with anti-CD34 antibody.
Results A strong expression of VEGF (>25% of neoplastic cells) was observed in 77.27% of carcinomas (36/48 squamous cell carcinomas, 22/30 adenocarcinomas and 10/10 large cell carcinomas). VEGF expression was correlated with MVD and tumor cell differentiation ($p<0.001$). Absent or reduced E-cadherin expression (<50% of neoplastic cells) was noted in 61.36% of tumors. A higher percentage of undifferentiated carcinomas was observed in the reduced E-cadherin group than in the E-cadherin positive group ($p<0.0001$). The simultaneous strong expression of VEGF and reduced expression of E-cadherin was correlated with tumor dedifferentiation ($p<0.001$).
Conclusions The intratumoral VEGF expression correlates with tumor angiogenesis and histologic differentiation. E-cadherin expression is associated with tumor cell differentiation. Combined evaluation of VEGF and E-cadherin may provide evidence for a better understanding of the biological characteristics of NSCLC tumors, and it may appear useful in defining groups of patients with a poor prognosis.

P-137

A rare case of mucoepidermoid carcinoma of the thymus

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A 53-years old male presented with chest discomfort, dyspnea, weakness. Heart function tests were normal. CT examination revealed a well demarcated mass in the anterior-superior mediastinum as well as pleural and pericardial effusions. There was no clinical/radiographic evidence of tumor elsewhere. A surgical excision of the mass was performed. Grossly, the mass was round, well demarcated, measured 10x8.5x3 cm. The cut surface showed solid whitish or reddish with yellowish foci areas and cystic areas filled with mucinous fluid. Histologically, the tumor consisted of sheets and nests of atypical squamoid cells intermingled with gland-like lumens. Areas of clear cells were also present. In the tumor stroma, abundant amounts of mucin, foci of necrosis, lymphatic invasion, intense inflammation and cholesterol granulomas were observed. In some areas, the tumor cells were seen in contact to the lining of the cystic cavities. The latter were lined by a single layer of cuboidal or flattened or stratified epithelium with morphological features of squamous cells. At the periphery of the tumor, rare lymphoid follicles, residual thymic elements or degenerative Hassall's corpuscles were seen. These histologic features were more consistent with a mucoepidermoid carcinoma arising in association with a thymic cyst. The patient died two months after chemotherapy administration. Mucoepidermoid carcinoma of thymus is a rare entity and, to our knowledge, very few cases have been described in the International Literature. However, in case of absence of metastatic disease or other common primary tumors of thymus, the diagnosis of a mucoepidermoid carcinoma should be taken into consideration.

P-138

Tissue array for immunohistochemical profiling of intrathoracic localized fibrous tumors

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Introduction Localized fibrous tumor (LFT) is a rare neoplasm generally presenting as a pleural-based mass. The aim of this study was to investigate expression of oncoproteins, prognostic markers, steroid receptors, antimesothelial markers, extracellular matrix proteins and a variety of other markers in LFTs by tissue array using immunohistochemistry.
Materials and methods Two core tissue biopsies (4 mm in diameter) were taken from the paraffin blocks of eight CD34 positive pleural tumors to represent both hypercellular and hypocellular areas. A tissue array block of 16 tissues were prepared and examined for 21 markers.
Results There were five female and three male patients (age range: 30-75). Six intrathoracic extrapulmonary pedinculated, two intrapulmonary non-pedinculated lesions measured 3 to19 cm. The tumor cells showed variable immunoreactivity with SMA, desmin, ER, PR, CD99, CD57, CD10, CD117, bcl-1, Ki-67 and laminin. Expression of bcl-2 and collagen type III was detected in all cases. None of the LFTs showed immunoreactivity for calretinin, mesothelin, S-100, CD5, CD31, c-erbB2 and p53. The diagnosis was changed as desmoid tumor in one case.
Conclusion No difference was detected between hypercellular and hypocellular areas of LFTs with respect to immunohistochemical profile. Collagen type III and bcl-2 could be used together with CD34 in the diagnosis of LFTs. Extracellular matrix proteins, CD10, CD57 and bcl-1 could be detected in LFTs. Overlapping immunoprofile of LFTs and gastrointestinal stromal tumors for CD117, CD34, bcl-2 and CD99 deserves further study. In ER, PR, SMA and desmin positive case, desmoid tumor should be considered in the differential diagnosis.

P-139

The value of intraoperative imprint cytology in mediastinal staging in non small cell lung cancer

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Introduction Evaluation of lymph nodes is mandatory for the intraoperative staging of lung cancer. This evaluation can be done either by frozen section FS or imprint cytology IC. In this prospective study we compared the diagnostic accuracy of IC of mediastinal lymph nodes performed during the operation and permanent section.
Materials and methods One thousand and fifty mediastinal lymph nodes from two hundred and fiftyfive patients were evaluated. To obtain lymph node samples from various stations cervical medistinoscopy was performed in twohundred and thirtytwo patients. Twenty percent of the patients had anterior mediastinotomy and the rest videothoracoscopy. The average time for preparing an imprint slide was approximately two or two and a half minutes. For the final pathological diagnosis both imprint and permanent section slides were reviewed and the results compared.
Results There were five false positive and eight false negative results. The sensitivity of IC was ninetythree point one percent and the predictive value of a negative result was ninetynine percent.
Conclusion Reviewing the literature and comparing the diagnostic accuracy of FS in other studies we concluded that IC for the evaluation of mediastinal lymph nodes in lung cancer is an accurate reliable simple and less time consuming method.

P-140

Ultrastructure of bronchial mucosa in chronic obstructive bronchitis

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The purpose of this investigation was to determine ultrastructural peculiarity of bronchial mucosa in the patients with chronic obstructive bronchitis (COB). The study was performed in bronchial biopsy samples obtained from 30 patients with COB.

Material was fixed in 2,5% glutaraldehyde, postfixed with 1% osmium tetroxide and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate. Morphological three types of COB have been reported, including chronic catarrhal, catarrhal sclerotic and sclerotic forms. Catarrhal COB was characterized with significant increasing of goblet cell count as compared to the bronchial mucosa in controls. Hyperplasia of endoplasmic reticulum and Golgi complex was observed. In the goblet cells, numerous secretory droplets were crowded together where they have been deposited from the surrounding cytoplasm. In ciliated cells, few cilia were seen in longitudinal section extending from the apical pole of epithelial cells, fragmentation of the endoplasmic reticulum and appearance of autophagosome were detected. In other types of COB, number of cell rows was decreased with different secretory activity of goblet cells. Interepithelial migration of lymphocytes with the damage of the basement membrane was described. Our findings demonstrated more aggressive changes of bronchial mucosa in sclerotic COB.

P-141

Chest trauma-induced rupture of benign mediastinal teratoma followed by a possible implantation of the tumor into the parietal pleura: a case report

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Introduction Mediastinal teratomas rarely rupture. An unusual case of chest trauma-induced rupture of benign mediastinal teratoma followed by a possible implantation of the tumor into the parietal pleura is reported.
Case presentation In a 26-year old woman, a pre-employment plain chest radiograph and CT of the chest revealed a large mediastinal tumor. Two smaller tumors were located on the left parietal pleura. On operation completely obliterated left pleural space and three well-encapsulated tumors were found. Microscope examination revealed three well-differentiated teratomas with no evidence of malignancy. Three years before the detection of the tumor the patient had a blunt chest trauma, with a left-sided chest pain and shortness of breath, which gradually disappeared. At the time the plain chest radiograph was analyzed as normal. Retrospectively an unusual mediastinal line, adjacent to the aortic arch, was disclosed. This line could be due to a small mass in the mediastinum. The left diaphragm was elevated, and a small left-sided pleural effusion could have been present. We suspect that teratomas located on the parietal pleura were caused by implantation of the tumor cells after the rupture of the mediastinal teratoma into the pleural space during blunt chest trauma.
Conclusion The chest trauma seen in presented case, could be at least a trigger, if not the only cause of the mediastinal teratoma rupture. The rupture was probably followed by both, obliteration of pleural cavity and possible implantation of benign tumor into the parietal pleura.

P-142

The Gorham - Stout Syndrome: presentation of a case with pleural effusion

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Gorham - Stout syndrome is a rare disorder of unknown aetiology characterised by a nonmalignant proliferation of vascular structures originating in bone with progressive bone destruction and often extending into surrounding soft tissues. Pleural effusion is uncommon, but usually fatal complication of this condition. We present a 28 years old male patient admitted to our departement because of huge left - sided pleural effusion. 10 years ago his left humerus was injured in a motorcycle accident. One month later osteolysis of the left humerus was diagnosed. Histological examination of the lytic bone revealed Gorham - Stout syndrome. Until recently there were no symptoms, then dyspnea occured and the patient was admitted to our departement. A huge left pleural sterile haemorragic exudate was found. X ray revealed destruction of the left humerus, the left scapula, the left clavicle and the first two left ribs. CT scan revealed no mediastinal mass, nor any destructions of vertebrae. The pleural effusion was drained, thoracoscopy was performed, the histology of pleura revealed only fibrosis and inflammation, there was no angiomatous tissue. The patient received radiation therapy of 40 G of the left side of the chest and the left upper arm. He also received 30 mg of pamidronat. 4 month after this therapy the pleural effusion does not reappear and no new osteolytic lesions occured.

P-143

p27kip1 and MIB-1 immunoreactivity in pleural malignant mesothelioma

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Introduction Malignant mesothelioma is an aggressive tumor with adverse prognosis (median survival 4-12 months) both in treated and untreated patients. In fact, only few patients survive more than two years. The identification of prognostic factors is critical to evaluate life-expectancy and crucial to approach novel therapeutical strategies. The study was performed to evaluate the use of MIB-1 and p27kip1 antigens as prognostic indicators of survival in malignant mesotheliomas.
Materials and methods A series of 122 surgical biopsies of patients with known follow-up were immunohistochemically examined. The results were compared with survival data and correlated with clinical and histological parameters. For immuno-

histochemistry, sections were stained for MIB-1 and p27kip1 antigens using ABC method. Number of positively stained nuclei was evaluated in consecutive high magnification fields (500 neoplastic cells were considered the minimum number to include the case in the study).
Results MIB-1, which is largely expressed in sarcomatoid MM ($p=0.02$), is more represented in patients with shorter (<12 months) then in subjects with medium ($12<\text{months}<24$, $p<0.05$) and longer (>24 months, $p<0.05$) survival. On the other hand, p27 is more expressed (52 ± 24.7) in patients with longer ($p<0.01$) then in subjects with shorter survival. Pearson's analysis showed no significant correlation between MIB-1 expression and p27, but this was predictive of survival in the entire population ($r=0.273$, $p<0.001$).
Conclusion The findings suggests that p27 and MIB-1 expression, although reliable attribute of MM, represent independent markers of prognosis in malignant mesothelioma.

P-144

Familial clustering of malignant mesothelioma without direct contact to asbestos. A genetic study

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Introduction Very few papers dealt with clustering of familial malignant mesothelioma (MM). Most of these, who developed mesotheliomas, presented work or domestic history of exposure to asbestos, but only in very few of them asbestos did not demonstrated any association. The aim of this study was to contribute to the hypothesis of a genetic predisposition in the pathogenesis of the mesothelioma.
Methods We report two cases of pleural malignant mesothelioma that affected two young sisters as part of a nine-member family with enviromental exposure to asbestos. They were affected by Marfan's syndrome and both developed pleural malignant deciduoid mesothelioma with long survival. We have performed comparative genomic hybridization (CGH) analyses of tumor samples to identify critical chromosomal regions involved in the onset of MM.
Results We found similar chromosomal changes in both cases and in particular we found losses at 6q12-q21 and 13q21.1-q22 chromosome.
Conclusions Early onset s. and same genetic impairments in the two sisters suggest that genetic factors may induce the onset of this cancer in those subjects who are exposed even to small environmental amounts of asbestos. However, the specific chromosomal changes we found might provide evidence for locations of relevant suppressor genes of familial MM.

P-145

Video assisted versus needle biopsy of the pleura in patients with pleural effusion

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Introduction Pleural effusion is quite a frequent finding that should be thoroughly investigated and diagnosed. Besides cytological examination of the pleural fluid, second most frequently used method is biopsy. This study was planned to investigate if video assisted thoracoscopy is mandatory where the case is diagnosed as inflammation and fibrosis with biopsy or the biopsy is non diagnostic.
Materials and methods In a four year period needle biopsies and video assisted thoracoscopic VATS biopsies were performed at the same session on thirtythree patients with pleural effusion. The biopsy material was processed for routine haematoxilyn eosin stain. Histochemical stains PAS, Alcian blue mucicarmin and immunhistochemical methods cytokeratin, carcinoembryonic antigen, vimentin were performed on all material and the results compared.
Results The pathological diagnosis of needle biopsies Group I were chronic inflammation and fibrosis in twentythree, malignant mesothelioma in six and granulomatous inflamation in four patients. When compared with VATS biopsy results Group II in group I with chronic inflammation one patient was diagnosed as having malignant mesothelioma, one patient had a metastatic carcinoma, nine patients had granulomatous inflammation.
Conclusion In patients suspected to have malignancy who are diagnosed as having chronic inflammation and fibrosis with needle biopsy VATS biopsy should be performed.

P-146

Solitary fibrous tumor of the pleura

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Introduction Solitary fibrous tumor of the pleura is a rare tumour and frequently arises from visceral pleura with a pedincule. In this study three cases with histopathological diagnosis of solitary fibrous tumor is discussed in respect to clinical radiological and pathological aspects of differential diagnosis.
Materials and methods Three patients who had symptoms related to a thoracic pathology were investigated through chest x ray, computerized tomogrophy and magnetic resonance imaging. Cytologic examination of sputum bronchoscopy, fine nedle aspiration of the mass lesion and finally resection through thoracoscopy or thoracotomy were performed. Routine haematoksilyn eosin stains, histochemical stains such as PAS and Van Gieson and immuno-histochemically CK, EMA, Vimentin, Actin, S 100 and CD 34 were done.
Results All three resection material were diagnosed as Solitary fibrous tumor of pleura. CK, EMA, Actin, S 100 were negative. Vimentin and CD 34 were strongly positive. Van Gieson showed the collagenous nature of the tumor.
Conclusion Definite diagnosis mostly can be reached after total exision of the tumour and detailed pathological

P-147

The expression of p-53 in tracheal mucosa of patients with laryngeal squamous cell carcinomas

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Introduction Analysis of immunohistochemical markers as an indicator of development and progression in tobacco component–induced tumors in the respiratory tract. However, although the inhaled smoke is passing through laryngo-tracheo-bronchial tree, cancers of trachea are extremely rare. The role of p-53 in cell growth regulation and its frequent overexpresion in tumors has lead to studies investigating its involvement in cancer. Mutation of the p53 gene occurs in approximately 45% of human cancers. p-53 expression increased consistently during the formation of squamous cell carcinomas of the larynx, trachea, bronchi and lungs.
Materials and methods Samples of tracheal mucosa from patients with laryngeal carcinoma (No =91) or non-malignant diseases (controls, No =20) were embedded in paraffin and stained with HE. p-53 was immunohistochemical performed on 6 sections in the tissue samples. The immunohistochemical staining was carried out using monoclonal mouse anti-human p-53 protein (Clone Do-7). Scores were determined microscopically by independent pathologist. At least 200 cells at the tracheal mucosa front in at least five regions were counted. The final results was expressed as the percentage of positive nuclei per 200 cells.
Results Correlation was found between p-53-posivity tracheal mucosa and TNM classification system: p-53 index: Control group=38%, Group T2=46%, T3=56%, T4=54%.
Conclusion The opinion that the presence of p-53 oncoprotein reflects mutation of the p-53 gene is commonly accepted. Other data published evaluating the relationships between p-53 and TNM–stage, G-stage, tumor size and site, age and sex, did not reveal any dependencies, although some studies have suggested such correlations. Our analysis had shown a correlation between TNM stage and p-53 index of tracheal mucosa, but is significant only between control group and T3, and control group and T4. Authors expect such results that show the correlation between prognostic factors in laryngeal malignant epithelium and tracheal epithelium.

P-148

Undifferentiated nasopharyngeal carcinoma (lymphoepithelioma). Report of four cases

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Background Nonglandular nasopharyngeal carcinomas are much more common in some areas of the orient than the other parts of the world. They are subtyped as keratinizing squamous cell carcinoma (type 1), nonkeratinizing carcinoma (type 2) and undifferentiated carcinoma (Lymphoepithelioma). We present on four undifferentiated nasopharyngeal carcinomas and detail clinical and histopathological characterisation of these rare cases.
Methods All our patients were male, white and 47-75 years old. Initial complaints were various: hearing loss, epistaxis, headache, and dysphagia. Examination of the nasopharynx showed us entirely normal features, or fullness, surface granularity, or obvious carcinoma. But, all our patients had cervical lymph nodes metastases, two of them bilateral. Involved lymph nodes were typically posterior of the sternocleidomastoid at the level of the angle of the jaw.
Results Histologically, lesions show cytologically uniform cells with ovoid vesicular nuclei prominent nucleoli and indistinct cell borders resulting in a syncytial pattern. An inflammatory infiltrate rich in lymphocytes and occasionally containing prominent eosinophils is usually a component of lymphoepithelioma, but diagnosis is based solely on the nature of the neoplastic epithelial component. Neoplastic cells are negative for LCA, but positive for cytokeratin and EMA.
Conclusion Undifferentiated nasopharyngeal carcinoma (lymphoepithelioma) is very rare. About 50% to 80% of patients are initially seen with cervical lymph node metastases from an occult primary. Such lesions may be different or impossible to distinguish from lymphoma on haematoxylin and eosin stained sections, particularly when the lesion appears as a lymph node metastasis from an occult primary. Ancillary methods like immunohistochemistry and molecular techniques or detection of serum antibodies against viral proteins may be necessary.

P-149

"Atypical" pleomorphic adenoma of the parotid gland

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Introduction Pleomorphic adenoma is a benign, epithelial tumor, composed of cells that demonstrate both epithelial and mesenchymal differentiation, with the absence of abnormal cytomorphologic features. We present the case of 31-years old white women, with a mass localized in the superficial, lower part of the right parotid gland.
Material and methods On clinical examination, the lesion was nodular, painless swelling, of smooth surface, measuring 40x30 mm in diameter. The tumour was excised, fixed in formaline, embedded in paraffin and the sections were stained with HE, PAS and ALCIAN blue.
Results The tumour was composed of both epithelial and mesenchymal-like tissue, with a complete capsule at the periphery. There was no neoplastic tissue within or extending through the fibrous capsule. On the other hand, the epithelial component expressed mild, limited, abnormal cytomorphologic features, such as enlarged, pleomorphic and hyperchromatic nuclei.
Conclusions Presence of prominent cytomorphologic atypias and capsular involvement in a pleomorphic adenoma causes concern about malignant transformation, but a lesion with mild or focally limited cytological atypias is designated as atypical pleomorphic adenoma and indicates an increase likelihood of malignant transformation if the tumor recurs.

P-150

Polymorphous low-grade adenocarcinoma of the minor salivary glands: immunohistochemical analysis and assessment of p53 expression and proliferative activity in two cases

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Introduction Polymorphous low-grade adenocarcinoma (PLGA) is a minor salivary gland carcinoma usually arising intraorally, primarily in the palate. These tumours may be confused histologically with cellular pleomorphic adenomas and adenoid cystic carcinomas. The aim of this study was to describe the immunohistologic features of PLGA, in comparison with those of PA and ACC.
Materials and methods This report describes the immunohistochemical distribution of epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), high-molecular-weight keratin (HMWCK), low-molecular-weight keratin (LMWCK), smooth muscle actin (SMA), vimentin (VIM) and glial fibrillary acidic protein (GFAP) in two cases of PLGA arising in the hard palate and the nasopharynx. Immunoprofile of a demonstrative case of PA and ACC were also evaluated. Immunoexpression of p53 oncoprotein and proliferative activity, measured by the expression of Ki-67 antigen and proliferating cell nuclear antigen (PCNA) were studied in all tumours, in aiding to distinguish benign from malignant lesions.
Results The tumor tissue in PLGA displayed diffuse and strong staining with HMWCK, LMWCK, VIM, and S-100. Cytoplasmic and luminal staining for CEA was seen in occasional cells. GFAP, SMA, and EMA displayed negative staining. Quantitative assessment revealed p53 positive staining in all tumor types. The percentage of tumor cells positive for PCNA staining was 80 % and Ki-67 antigen expression was 8.5 % in PLGA.
Conclusion PCNA and Ki-67 antigen, S-100, CEA and EMA immunoreactivity appears to be a potentially useful supplementary diagnostic tool in differentiating difficult cases of PLGA from ACC and PA.

P-151

Gene expression in major types of salivary gland carcinoma has similarities with gene expression in pancreatic and prostate carcinoma

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Gene expression profiles were determined in fresh-frozen samples of mucoepidermoid carcinoma (MEC), acinic cell carcinoma (ACC), salivary duct carcinoma (SDC), and adenoid cystic carcinoma (AdCC), using cDNA microarrays containing cDNA fragments of 1176 cancer-related human genes. The validity of findings was checked by means of real-time reverse-transcript polymerase chain reaction studies. Seventy-five genes were found to be more than 2.5 times overexpressed and 87 less than 0.4 times underexpressed than in samples of normal parotid gland. Five genes, namely fibronectin 1 (FN1), tissue metalloproteinase inhibitor 1 (TIMP1), biglycan (BGN), tenascin-C (HXB), and insulin-like growth-factor-binding protein 5 (IGFBP5) were overexpressed in all of the types of carcinoma studied. Sixteen genes, including mothers against decapentaplegic homolog 4 (MADH4/SMAD4) and prostate-specific membrane antigen (PSM/PSA) were underexpressed in all types of carcinoma studied. Average-linkage hierarchical clustering undertaken in relation to selected genes showed that high- and low-grade MECs formed one group of tumours, ACC/SDC another. Significance analysis of microarray findings identified 33 genes related to the MEC group, 10 associated with the ACC/SDC group. Our results indicate genes that may be involved in carcinogenesis in the salivary glands. Gene expression in normal and malignant salivary tissues was also found to be similar in several respects to gene expression in normal and malignant pancreatic and prostate tissues.

P-152

Rare tumours in the sinonasal tract

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Introduction Sinonasal neoplasms include numerous groups of different histogenetic types. The epithelial tumours have the highest prevalence among them in the daily routine, but we can encounter such lesions, which may cause diagnostic difficulties. The purpose of this study is to determine the least frequent tumours in our sinonasal biopsy files. We collected those kinds of tumours which occurred only once in our series.
Material and methods We reviewed our archive material of a 10-year period (1993–2002) Five cases were selected. The histologic slides were re-examined. HE, special stains, immunohistochemistry and electronmicroscopy were applied.
Results The cases in the order of their occurrence: 1. Fibrous solitary tumour, we supported our original opinion by CD 34 immunostaining performed retrospectively. 2. Chordoma, in addition to the morphologic features the S100 and cytokeratin co expression were pathognomic. 3. PNET, in this problematic case finally the CD99 positivity proved to be diagnostic. 4. Intestinal-type adenocarcinoma, the mucocele-like appearance and the highly differentiated colonic type epithelial structures reminiscent of adenoma caused our dilemmas. 5. Meningeoma, the immunohistochemical results were not convincing. We established the diagnosis by means of electronmicroscopy.
Conclusions The incidence of sinonasal neoplasms is low. The recognition of the unusual lesions in this region is a challenge for the pathologist. Ancillary techniques may be necessary in the diagnostic process.

P-153

Squamous cell carcinoma: diagnostically problematic variants

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Introduction The diagnosis of squamous cell carcinoma (SCC) is seldom problematic. Although it may cause difficulties for the pathologist to verify its uncommon varieties. The aim of this study

was to distinguish the rare variants of SCC from the conventional ones in our laryngeal tumour file and to present the most instructive cases.
Material and methods We reviewed our archive material of a 5-year period (1998-2002). Four cases were selected. The histologic slides of the preoperative biopsies and the surgical specimens were re-examined as well. HE, special stains, immunohistochemistry and electronmicroscopy were applied.
Results 1. „ Carcinosarcoma" with rhabdomyosarcomatous differentiation. This case represents a unique example of the spindle cell carcinoma category. 2. Verrucous carcinoma with verrucous hyperplasia. The interpretation of the latter term is a complicated issue. By virtue of our patient's follow-up we encountered this dilemma. 3. Papillary squamous cell carcinoma (PSCC). This 13-year long case history is a well-documented instance of a papilloma-papilloma with dysplasia-carcinoma sequence. 4. Adenosquamous carcinoma. It was not easy to justify the clinical suspicion of malignancy preoperatively. Furthermore we faced a differential diagnostic problem when we evaluated the removed larynx.
Conclusions In all the cases we managed to establish the definite diagnosis only by the thorough examination of the surgical specimens. Additionally we found special features as far as the morphology and histogenesis are concerned. So we can confirm the subheading of the corresponding AFIP fascicle for this tumour group: diagnostically problematic variants.

P-154

Intraocular leiomyoma: case report

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Introduction Intraocular leiomyomas are rare tumours probably arising from the ciliary body smooth muscle. They represent a diagnostic dilemma and must be differentiated from neural tumours and, more importantly, from amelanotic melanoma. They occur more frequently in women, usually between the third and sixth decades of life.
Case report We report the case of a 44 year-old woman with gradual loss of vision in her right eye. Slit-lamp examination and binocular ophthalmoscopy showed a large and pigmented ciliary body mass located in the inferior quadrants. A and B ultrasound examination revealed a multicavitary tumor with low internal reflectivity measuring 19x13.8x10.9mm. Enucleation of the affected eye was decided. Histopathological examination showed a solid neoplasm composed of interlacing and tightly packet of spindle-shaped cells with little intervening collagen. Nuclei were oval with blunted ends with no significant atypia. Mitotic activity was negligible. Immunohistochemical stains for smooth muscle actin, HHF-35, vimentin and desmin were positive. No stain was noted for S-100 protein, AE1-AE3 cytokeratin, CAM 5.2 cytokeratin, HMB-45, glial fibrillary acid protein, neurofilaments, neuron-specific enolase and synaptophysin. Final diagnosis was leiomyoma and the patient is free of disease two years after surgery.
Conclusions Intraocular leiomyomas are of clinical concern since they are difficult to differentiate from more aggressive neoplasms, especially amelanotic melanoma. In this particular case, the situation was even more complicated because the lesion was clinically pigmented. Once removed, the diagnosis was not difficult and was based on conventional histopathological examination along with confirmatory immunohistochemistry.

P-155

Correlation of various histopathologic classifications of nasopharyngeal carcinoma (NPC) with clinical parameters

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Introduction The aim of this study was to show a possible impact of various histologic classification of NPC on survival and correlations with clinical parameters.
Materials and methods Biopsies belonging to 36 NPC cases were histologically reclassified according to WHO and Cologne classification, and classification proposed by Hsu. Age, sex therapy modality and survival of each case were analysed. Presence of Epstein-Barr virus (EBV) was verified immunohistochemically.
Results Twenty-six of cases were male and 10 were female. Follow-up was available for 26 cases and ranged between 2 to 66 months. Eight cases died and 3 had recurrences. The recurrent tumour histology changed from type 3 to 2 in 2 cases, remained unchanged in 1. None of the clinical parameters were correlated with the survival. The type of therapy seemed to have no impact on survival. According to WHO classification 2 cases were type 1, 8 were type 2 and 26 were type 3 and it showed correlation with survival (p=0.0058). Lymphocytic infiltration, which, was added to WHO system, were seen by Cologne classification in 24 of tumours, but did not have an impact on survival. Cellular morphology and atypia suggested by Hsu's classification had no significance as well, although most of the tumours were classified as type A (%69,4). EBV infection was found to be present in 11of 29 cases. However no correlation with other parameters was found.
Conclusion In accordance to literature we concluded that WHO classification is the only parameter that has an impact on survival in NPC.

P-156

Vascular endothelial growth factor expression and microvessel count in laryngeal squamous cell carcinoma

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Introduction Vascular endothelial growth factor (VEGF) plays an important role in tumour growth and metastasis. The purpose of this study was to re-evaluate the prognostic value of VEGF and microvessel count (MVC) in laryngeal squamous cell carcinoma.
Materials and methods In this study, 27 patients were evaluated. Microvessel staining was performed with anti-factor VIII (F-VIII)

– related antigen. Paraffin embedded tumour specimens were stained with VEGF and F-VIII using immunohistochemical methods. VEGF staining was evaluated as negative (<%10 of tumour cells) or positive (>%10 of tumour cells). Staining intensity was graded mild, moderate and severe. For evaluation of F-VIII staining, average counts of the three most vascular areas on an x200 field were recorded. Statistically correlation between immunoreactivity and the clinicopathologic factors of laryngeal carcinomas was evaluated.

Results VEGF expression in the tumour cells was found positive in all cases (%100). Staining intensity was detected as severe of 9 cases, moderate of 11 cases, mild of 7 cases. In evaluation of F-VIII staining, average MVC of all cases was found 16,9. Statistically, no association was observed between the VEGF expression, F-VIII of carcinoma cells and histologic grade, TNM stage, tumour size and tumour site. Association was observed between the VEGF expression and MVC (P=0,007). In additional, significant correlation was observed between the VEGF percentage of positive tumour cells and staining intensity (P=0,000).

Conclusion Our data suggest that in laryngeal squamous cell carcinomas, VEGF expression and MVC of carcinoma cells are not prognostic markers.

P-157

Acinic cell adenocarcinoma: presentation of five cases

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Five cases of acinic cell adenocarcinoma, diagnosed in our laboratory during a period of 10 years, are presented. Four of them concerned salivary gland tumours of the parotid gland, the main site of their development, and the fifth was found as an endobronchial mass. The patients ranged from 28 to 76 years of age and the greatest stromal diameter varied from 0,6 to 1,5 cm. Microscopically all neoplasms had the typical morphological features consistent with acinic cell adenocarcinoma. This neoplasm has distinct clinicopathological features and a rather indolent biological course, in spite of its malignant potential, responsible for the prevalence of the term carcinoma instead of “stromal”. A broad histo- and immunohistochemical investigation was performed confirming the diagnosis. A review of the available reference is reported.

P-158

Relationship of p53 oncoprotein expression with histological grade and outcome of patients with oral squamous cell carcinoma

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Introduction An alteration in the p53 tumour suppressor gene is the most frequent genetic abnormality in human cancers.

Materials and methods Overexpression of the p53 oncoprotein and histological differentiation were investigated in 28 routinely formalin-fixed and paraffin embedded squamous cell carcinomas of the oral cavity. P53 oncoprotein was detected in all specimens with the monoclonal antibody DO-7. P53 oncoprotein expression was scored in the following way: score 0 <10%, score 1 10-50%, score 2 >50% positive cells.

Results and conclusions Using histological differentiation, all specimens are divided in three groups: group I - well-differentiated (6 cases), group II - moderately differentiated (12 cases) and group III - poorly differentiated carcinomas (10 cases). In the first group 33% specimens were negative, 67%highly positive for the p53 oncoprotein. In the second group, positive detection of the p53 oncoprotein was observed as low in 66,7% of cases, moderately in 25% and highly positive in 8,3%. In the third group 60% expressed low positivity for the p53 oncoprotein and 20% of the cases had moderate and high positivity respectively. Patients' survival was monitored for a five years. In the group with the highestp53 oncoprotein expression 7,14% of the patients survived 5 years or longer and 17,85% survived less than 5 years. Mutation in the p53 tumour suppressor gene is highest in the well-differentiated squamous cell carcinomas, and its expression decreases in poorly differentiated carcinomas.

P-159

Immunohistochemical prognostic factors in laryngeal squamous cell carcinoma

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Introduction The molecular mechanisms of growth and progression of laryngeal carcinoma are not studied well. The aim of the present study was to investigate the prognostic significance of over expressions of p53 protein, cyclin D1, c-erbB2, and markers of cellular proliferation (Ki-67 and PCNA).

Materials and methods Primary tumours were studied on a series of 75 patients. All patients were treated between the years 1991 and 1998. The expression of p53 was analysed with monoclonal DO7 antibody, cyclin D1 (clone DCS-6), c-erbB2 (polyclonal antibody Hercep Test), proliferate activity with Ki-67 (clone MIB-1) and PCNA (clone PC10)

Results Overexpression of the p53 protein was found in 36% of patients, cyclin D1 in 47%, and c-erbB2- in 13% of patients with laryngeal squamous cell carcinomas. The reverse relationship of cyclin D1 with 5-year survival of patients ($p<0.05$) was revealed. Overexpression of the p53 protein was correlated with lymph node metastasis ($p<0.05$). The value of PCNA index in patients with metastasis is significantly higher than those in patients without metastasis ($p<0.05$). The PCNA index was correlated with the stages of the TNM. High level of the Ki-67 index and the PCNA index really decreases 5-year survival in patients. No significant correlations were found between positive p53, cyclin D1, c-erbB2, Ki-67 and PCNA immunostaining and anatomical localization and histological grade.

Conclusions Immunohistological examination of c-erbB2 is not a valuable prognostic factor in laryngeal carcinoma. High proliferation index (Ki-67 and PCNA) and expression of cyclin D1 correlate with worse prognosis of the disease. Overexpression of the p53 protein is correlated with progression of laryngeal cancer.

P-160

Gingival cell answer in patients with diabetes type I associated with parodonthal disease

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Introduction Parodonthal disease associated with diabetes mellitus is a special entity present in a new classification of parodonthal diseases. The pathogenic mechanisms important for developing parodonthal disease in diabetics are the disturbances in the metabolism of collagen, changes of the gingival net of the microvascular elements and gingival basement membrane and disturbance in the function of the polymorphonuclear leukocytes. The aim of this investigation was to establish predominant cell type in gingiva of patients with diabetes type I and parodonthal disease.
Material and methods Out of 100 patients that were included in our study, 52 had diabetes type I and 48 were non-diabetics. Biopsies of gingival tissues were performed. The material was stained by HE, PAS, and van Gieson.
Results The gingival epithelium showed papillomatosis, acanthosis and necrobiosis. We identified infiltrates of lymphocytes, plasmacytes, fibroblasts as well as hyalinized collagen fibres.
Conclusions Gingival answer in patients with diabetes type I with parodonthal disease is imunodeficient and combined with deep disorders of metabolism in cells, vessels and epithelium.

P-161

Peripheral giant cell granulomas of the jaws - a clinico-pathological study of 91 cases

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Introduction The peripheral giant cell granuloma (PGCG) is reactive lesion that is confined to alveolar and gingival mucosa. The aim of this study was to present epidemiological data and histopathological features of PGCG.
Material and methods All biopsies submitted from January 1994 to December 2000 at the Department for Oral pathology Faculty of Stomatology Belgrade were reviewed and the cases diagnosed as PGCG were included in this study. All section studied were stained with haematoxylin and eosin. Following parameters were analysed: sex and age distribution, epithelium condition, demarcation of the lesion, haemorrhage zones, distribution and types of giant cells and inflammatory infiltrate.
Results Females were found to be more commonly affected than males (1.22:1). All age groups were found to be affected, with a peak at 5th decade (female) and at 1st decade (male). Most of the lesions were clearly demarcated (84%). The covering epithelium was ulcerated (49%) and hyperplastic (26%), while intact epithelium was present in 9 lesions (25 %). Interstitial haemorrhage was found in all lesions, while hemosiderin deposits were found in 86 lesions. Giant cell arrangements were diffuse in 94.5% lesions, and focal in 5.5%. Inflammatory infiltration consisted of: lymphocytes, plasma cells, and polymorfonuclear leucocytes in 54 PGCG (59%).
Conclusions Giant cell lesions of the jaws are a diverse group of lesions, which are little understood. Fibrovascular stroma and multinucleated giant cells represent the most important but not pathognomonic elements for diagnosis. Key words: peripheral giant cell granuloma, histopathologic features

P-162

Vimentin and factor VIII: expression in benign lesions of the vocal folds

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Object Precisely histopathologic diagnosis of benign lesions of the vocal fold is very important for therapy.
Material and methods 30 tissue samples benign lesions of the vocal folds were analysed by immunohistochemical methods with monoclonal antibodies for Vimentin and Factor VIII.
Results Using this specific monoclonal antibodies we concluded that the subepithelial tissue of vocal fold polyps contains vascular spaces lining with endothelial cells (Factor VIII positive). The subepithelial tissue of Reinke's oedema contains fissured space lining with cells, which are Vimentin positive.
Conclusion In this way, using immunohistochemistry for monoclonal antibodies, we can differentiated between Reinke's oedema and polys of the vocal folds.

P-163

Warthin's tumor with multiple granulomas: a search for the cause

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Introduction Warthin's tumor (WT) is a well-recognized benign neoplasm of salivary gland. Granulomatous inflammation occurring in WT is a rare phenomenon. It has been suggested that prior fine needle aspiration (FNA) biopsy might be the triggering factor. Mycobacterial infection has also been reported as a cause of granulomatous change seen in WT. We report six cases of WT with granulomatous inflammation to analyse the possible relationship to prior FNA.
Materials and methods A computerized search of pathology file of Chang Gung Memorial Hospital yielded 382 cases of WT from 1983 to 2002. Granulomatous inflammation was described in six cases (1.6%). FNA cytology was performed in two cases before surgical excision. Clinical history, histology and cytology slides were reviewed. Ziel-Neelsen stain was performed.
Results All tumours occurred in parotid gland. There were 5 men and 1 women. Their age ranged from 54 to 82 yrs (mean 68) and the tumor size ranged from 2 to 5 cm (mean 3) in maximal dimen-

sion. All six cases showed typical features of WT accompanied by multiple granulomas composed of epitheloid histiocytes and multinucleated Langhans giant cells with or without necrosis. No acid-fast bacilli were found in any of these cases. History of prior FNA was identified in two cases. Both specimens also showed granulomas and features of WT.
Conclusions The pathogenesis of granulomatous inflammation in WT remains speculative. Prior FNA has been suggested to be the cause. However, this hypothesis cannot be supported by our observation of granulomas in the cytology specimens of two of our cases. It is most likely an immunologic reaction to the tumor, but a Ziehl-Neelsen stain should be performed to exclude the possibility of mycobacterial infection.

P-164

Malignant peripheral nerve sheath tumor masquerading as an allergic nasal polyp

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Introduction Approximately 14% of all malignant peripheral nerve sheath tumours (MPNST'S) occur in the head and neck. Of these, 59% are sporadic. The mean age at presentation is 43 years. Sinonasal tumours are extremely rare and whilst they share the morphology of MPNST'S at other locations, they tend to be of low grade at this site. We describe a unique case of a high grade MPNST clinically simulating an allergic nasal polyp in an otherwise healthy child without identifiable risk factors.
Methods A 13 year old Black boy presented with an obstructive nasal mass, which was first felt 6 months earlier. The clinical diagnosis was an "allergic polyp" and the mass was excised without antecedent roentgenographic investigations.
Results The 7x4x3cm tumor was unencapsulated but circumscribed, lobulated and friable. Histologically, predominantly high-grade sarcomatoid and epithelioid areas were seen. However, differentiating features of peripheral nerve sheath tumor were also found. These included alternating hypercellular and hypocellular areas, palisading nuclei, perithelial accentuation of cells and serpentine, buckled nuclei. In low-grade areas, axons were demonstrated by silver staining. Of the broad panel of immunohistochemical stains done, only S100 protein positivity was observed. The latter was uncharacteristically prominent.
Conclusion The young age of the patient, the tumor location and clinical presentation as an allergic polyp, the predominantly high grade microscopic appearance and the strong S100 protein positivity of this MPNST are unusual and hence worthy of documentation.

P-165

Follicular dendritic sarcoma in a Black teenager – report of a South African case and literature review

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Introduction The follicular dendritic cell (FDC) sarcoma is thought to differentiate towards the dendritic reticulum cell of the lymphoid follicle, possibly originating from a pluripotential stem cell. The mean age at presentation is 44years. Histologically the hallmark of the tumor is the intimate admix of a usually low-grade sarcomatoid proliferation with predominantly B-lymphocytes. A variety of architectural patterns may be observed in a single tumor thus simulating a variety of other fusocellular tumours. The use of diagnostic immunohistochemistry as well as electron microscopy, are often necessary. We describe the clinical and pathological features of a tonsillar follicular dendritic sarcoma, highlighting aspects that are particularly uncommon.
Methods An 18 year old Black male from a rural African village presented with a tonsillar mass that had evolved over a 12-month period.
Results At surgery, a pedunculated approximately 7 x 7 cm mass was excised in fragments. Histologically, the defining characteristics of FDC sarcoma were present. Unusual features included conspicuous "thymoma-like" lobulation, foci of epithelioid change as well as a prominent plasmacytic infiltrate. The immunohistochemical profile of the tumor confirmed the diagnosis.
Conclusion The race and young age of this patient as well as the presence of unusual histological features makes our case of FDC sarcoma, an already enigmatic entity, particularly fascinating reporting.

P-166

Natural killer cell immunohistochemical detection in laryngeal carcinoma

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Introduction Natural Killer (NK) cells may provide the first line of defence against many tumours. Cells bearing the NK phenotype along with CD8+ cells have been reported to be the most frequently encountered in laryngeal cancer, mainly within tumour mass. NK cells activity in peripheral blood samples of patients with laryngeal carcinoma seems to have a favourable prognostic effect on survival. We conducted this study to evaluate the distribution of NK cells in tissues of laryngeal carcinomas.
Material and methods The study included 30 males and 1 female with previously untreated epidermoid carcinoma of the larynx (mean age, 61.32 years; range, 42-75 years). After mean follow-up of 24.067 months (range, 4-36 months), 13 patients (41.9%) had relapsed. NK cells were identified by their immunohistochemical expression of monoclonal antibodies to CD16, CD56 and CD11b antigens on frozen tissue sections. NK cell presence was scored on a semiquantitave scale and possible associations were investigated between this presence and various clinicopathological variables including the expression of several immunohistochemical markers.
Results High CD16+ NK cell presence was detected in 7(22.6%) carcinomas while low and intermediate CD16+ NK cell presence was noticed in 7(22.6%) and 17(54.8%) tumours respectively. The majority of CD16+ cells expressed one or both of the other NK cell markers. NK cell presence was statistically unrelated to patients' age, tumour location, tumour maximum diameter, tumour stage, proliferation Ki-67 index and p53 accumulation in cancer cells. As far as the prognostic impact of NK cell tissue presence is concerned, using log rank test, this parameter was not related to patients' disease free survival. Interestingly, high NK cell presence was more often detected in poorly differentiated carcinomas by

comparison to well and moderately differentiated ones; this difference reached statistical significance (p=0.031).
Conclusion Poorly differentiated laryngeal cancer cells appear to be more capable of enhancing or altering the target antigens recognized by NK cells than well and moderately differentiated cancer cells.

P-167

Primary large cell neuroendocrine carcinoma of the larynx

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Introduction The 1999 World Health Organization (WHO) classification of pulmonary neuroendocrine tumours recognizes typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma (LCNEC), and small cell carcinoma. Recently, LCNEC has been reported in extrapulmonary sites such as the bladder, gallbladder, and the ampulla of Vater. Aims: Using the strict morphologic criteria for LCNEC set forth by the 1999 WHO classification, we report such a tumor in the larynx.
Materials and methods Chart review revealed a 74 y.o. male with a history of colon cancer who presented with otalgia. Direct laryngoscopy with biopsy revealed an invasive poorly differentiated carcinoma. Laryngectomy with bilateral modified neck dissections was performed. The tumor invaded the pre-epiglottic space with metastases to bilateral lymph nodes (AJCC T3N2c). Histologically, the tumor cells were large with a low nuclear:cytoplasmic ratio, vesicular chromatin, and prominent nucleoli. The cells were arranged in organoid and trabecular patterns in a background of extensive necrosis and mitotic figures. Ultrasound biopsy of the liver revealed metastatic disease. The patient died within weeks of surgery. The cause of death was unknown. No post-mortem examination was conducted. Immunohistochemical expression of anti-keratin AE1/AE3, chromogranin, and synaptophysin was evaluated. Electron microscopy was performed.
Results The tumor cells stained with anti-keratin AE1/AE3, chromogranin, and synaptophysin. Electron microscopy revealed dense core granules. These findings, along with the morphologic criteria, fit with a LCNEC.
Conclusion LCNEC is a rare tumor in the larynx. Recognition at this site is essential so that proper patient management can be initiated.

P-168

Analysis of blood serum lymphocytic phenotypes in chronic hepatitis C infected patients with advanced fibrosis and periodontium diseases

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Introduction Few studies focus on periodontium diseases in the course of chronic viral hepatitis C. The aim of this study was analysis of the relationship between the stage of periodontium disease and a number of lymphocytic phenotypes in blood serum of patients infected with chronic hepatitis C and advanced fibrosis.
Materials and methods The study included 28 patients (mean age of 57,3 years), whose blood serum revealed the presence of HCV-RNA, demonstrated with RT-PCR method, while biopsies helped to reveal chronic hepatitis with septal fibrosis or cirrhosis. Periodontium inflammation was assessed according to the following criteria: inflammation limbus, swelling, gingival pocket depth, presence of supragingival and subgingival calculus and gomphiasis. Each criterion was assigned 0 or 1 point, cumulatively from 0 to 4 points. Flow cytometry analysis included monoclonal antibodies: CD3, CD4, CD8, CD3/CD16/CD56, CD19 provided by Becton-Dickinson. Analysis of morphological parameters and fluorescence was performed with CellQuest computer software.
Results All patients with advanced liver fibrosis were found to show signs of advanced periodontum diseases (mean cumulative value of 3,25 points). The diseases most frequently encountered included: bleeding and calculus (N= 28), deep gingival pockets (N=20) and gomphiasis (N=18). The degree of periodontal disease depended on the total number of T lymphocytes ($p<0,05$) and on the number of T helper lymphocytes ($p<0,01$). No significant dependencies were found between the degree of a periodontal disease and NK cells, B-lymphocytes or T suppressor.
Conclusion Degree of periodontum disease in patients infected with hepatitis C is dependent on low values of T and T helper lymphocytes in blood serum.

P-169

Adenomatous ductal proliferative foci accompanied the Warthin tumour in parotid gland

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Adenomatous ductal proliferation (ADP) and intercalated duct hyperplasia (IDH) which are two new similar disease concepts in the field of salivary gland have been reported recently focusing on the relationship of precursor lesion to some salivary gland tumour, differential diagnosis, and possibility of dedifferentiated lesion. We report here a case of Warthin tumour accompanied by the foci of the adenomatous ductal proliferation in the parotid gland and these epithelial foci exhibited the histological and immunohistochemical findings resembling tubular type of basal cell adenoma. A 57-year-old female received the resection of Warthin tumour in the parotid gland, which was diagnosed as a benign parotid gland tumour. Macroscopically, this tumour showed two small legions in the parotid area, 13 X 7 mm and 7 x 3 mm in sizes respectively. The bigger legion was a typical Warthin tumour consisted of papillary epithelial projections into cystic spaces associated with variable number of lymphocytes. Papillary projections were lined by eosinophilic two cell layers having luminal columnar cells and basal polygonal to triangular shaped cells. On the other hand, histologically, the smaller legion resembled tubular type of basal cell adenoma. This lesion showed ductal proliferation with some admixed acinar cell complexes having a fibrous capsule in its half area.

Ductal proliferation consisted of bilayered structure (inner luminal cells were strongly positive for cytokeratin and weakly positive for S-100 protein). Some stromal areas around the ductal components appeared to be cellular with spindle shaped cells strongly positive for S-100 protein immunohistochemistry.

P-170

Malignant rhabdoid tumour (proximal epithelioid sarcoma) of the tongue: report of a case

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Introduction Malignant rhabdoid tumour of the oral cavity is very rare. To our knowledge, there has been one reported case of malignant rhabdoid tumour of the tongue so far.
Patient and methods A case of an 18-year-old female with malignant rhabdoid tumour of the tongue is presented. A patient presented with the induration of the anterior third of the tongue of 16 months duration. The surface was bulging, berry-shaped and bleeding, suggestive of an animators lesion. The radial excision of the tongue was performed.
Results Histopathological analysis revealed a solid tumour measuring 1cm in the largest diameter. The tumour was composed of epithelioid, eosinophilic cells with "rhabdoid" features; some of these cells contained rounded eosinophilic cytoplasmic inclusions that displaced the nuclei to the periphery. Most of the cells showed strong diffusely distributed vimentin immunoreactivity and focal epithelial membrane antigen and keratin immunoreactivity. Angioinvasion was present at the periphery of the tumour. Following pathological report re-excision of the tongue was done and the patient received chemotherapy. Seven month after the diagnosis, respectively, the patient showed no signs of tumour recurrence or metastases.
Conclusion Although there has been a contradiction whether malignant rhabdoid tumour represents a distinctive clinicopathological entity or a phenotypic pattern shared by heterogeneous neoplasms, most of the authors support the diagnosis of "rhabdoid tumour" because the morphological pattern is attended by aggressive biological behaviour.

P-171

A case of sarcomatoid salivary duct carcinoma ex pleomorphic adenoma in buccal mucosal region

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Salivary duct carcinoma (SDC) is an established aggressive salivary gland neoplasm resembling intraductal and infiltrative ductal carcinoma of breast. Diagnostic criteria and concept of SDC has been expanded along with subtype and new entity case reports such as low grade SDC and sarcomatoid SDC. Here we report a special case of sarcomatoid SDC ex pleomorphic adenoma (PA) arising from buccal mucosal region. A 32-year-old female noticed a painless swelling of 20 x 20 mm at left buccal mucosal area, which exhibited elastic hard lesion without surface ulceration. Prior to surgical operation, biopsy tissue revealed histological characteristics of typical PA without prominent cellular atypia. After operation, this stromal relapsed 3 times in 9 months. Although histologically greater part of primary tumor showed typical features of PA with two-cell layered ductal structures, a transition from ductal epithelia to atypical or malignant neoplastic epithelia was observed. These malignant neoplastic epithelia were arranged in intraductal infiltrative pattern with cribriform (Roman bridge-like) and/or solid nests of large polygonal eosinophilic cells and small central necrosis variably. Although a part of primary tumor showed sarcomatoid appearance characterized by pleomorphic and atypical spindle cells with few carcinomatous (ductal and solid) nests but all recurrent lesions exhibited atypical spindle and multinucleated giant cells without epithelial elements. Immunohistochemical findings for SDC component: cytokeratin (++), S-100 protein (-), smooth muscle actin (-), vimentin (-), PCNA (++), sarcomatoid area of primary tumor: cytokeratin (- to +), S-100 protein (-), vimentin (++), smooth muscle actin (-), PCNA (+++), a-antichymotripsine (+), recurrent sarcomatoid area: cytokeratin (-), S-100 protein (-), smooth muscle actin (-), vimentin (++).

172

Ameloblastic carcinoma with lymph node metastases

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Background Malignant epithelial odontogenic tumours are extremely rare. Although locally aggressive and infiltrating, with high recurrence rate, ameloblastoma is a benign odontogenic tumour. Malignant form of ameloblastoma may present as ameloblastic carcinoma or malignant ameloblastoma. Ameloblastic carcinoma exhibits cytological features of frankly malignant epithelial cells along with recognizable ameloblastoma.
Patient and methods A case of a 47-year-old male with ameloblastic carcinoma of the mandible is presented. A year ago, presenting symptom was discomfort in the area of his left mandibular second premolar and first molar teeth. Nine months later physical examination revealed an expansion of the mandible and destruction of its lingual cortex, accompanied by marked exulcerated lesion of sublingual mucosa extending from medial line to the retromolar area. Panoramic radiograph revealed a large radiolucent lesion causing bony destruction of the mandible. A biopsy was done and the pathological report indicated a squamous cell carcinoma. Three months later patient presented at maxillofacial department with further local progression over the midline. Examination of the neck revealed no lymphadenopathy.
Results Patient underwent segmental mandibulectomy from right premolar region up to the left angular region and bilateral modified neck dissection. Pathological report indicated a ameloblastic carcinoma with positive neck nodes bilaterally.
Conclusions Ameloblastic carcinoma with metastasis is a rarity, and a case with lymph node metastases at the admission has not been found in the available literature. Further reporting of this highly aggressive neoplasm is encouraged.

P-173

Prognostic significance of cathepsin D intratumoral heterogeneity in laryngeal squamous cell carcinoma

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Introduction Our previous investigations demonstrated prognostic significance for cathepsin D immunoreactivity in laryngeal squamous cell carcinoma (LSCC). We also demonstrated heterogeneity for AgNOR and DNA content in different areas of LSCC showing prognostic potential for DNA content of invasive tumour border. As this heterogeneity is a potential source of highly variable results we decided to investigate cathepsin D parameters in different microscopically defined tumour areas of LSCC.
Patients and methods 100 total laryngectomy specimen have been immunostained with anti-cathepsin D antibody (DAKO). Three distinct regions were analysed: transformation zone, invasive tumour margin and tumour center. Immunoreactivity in tumour and stromal cells was accessed by scoring (0 - 4) in at least 200 cells respectively. Tumour and stromal cell positivity was accessed in the most positive area of tumour staining of the respective tumour region.
Results and conclusion Significant difference of immunostaining was observed between tumour invasive margin and the other regions. In most cases the invasive margin was the most prominently stained region for tumour as well as for stromal cells. Most patients showed stronger staining of stromal than tumoral cells. Only patients with survival less than 30 months showed stronger tumour than stromal cell staining in invasive margin. From this we can conclude that the basis for cathepsin D prognostic value in laryngeal SCC lies in the stromal/stromal cell correlation of cathepsin D immunoreactivity of the invasive stromal margin.
Literature Seiwerth at al.: Immunohistochemical analysis and prognostic value of cathepsin D determination in laryngeal squamous cell carcinoma. J Chem Inf Comput Sci. 2000 May-Jun; 40(3):545-9.

P-174

Relationship between clinical and immunohistological properties of the middle ear cholesteatoma

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Introduction Middle ear cholesteatoma is characterized by proliferation of the keratinised squamous epithelium and accumulation of keratin debris into the middle ear cleft and mastoid. By aggressive growth it leads to the destruction of the mucous membrane, middle ear ossicles and nearby bony structures. Various inflammatory and immunocompetent cells as well as cytokines are involved in activation of epidermis and subepithelial tissue and accumulation of keratin debris in cholesteatoma. In the matrix of cholesteatoma as well as in the epidermis of the skin, Langerhans and Merkel cells are identified. The aim of this study was to elucidate the role of Langerhans and Merkel cells in mechanisms of cholesteatoma growth.
Material and methods Pathohistological and immunohistochemical studies were performed in 26 cholesteatoma specimens compared to the ear canal and retroauricular skin. Mononuclear antibodies against cytokeratin 20, which label Merkel cells and CD1 molecule with expression on Langerhans cells, were studied immunohistochemically.
Results and conclusion Correlation of the presence of Merkel and Langerhans cells as well as the extent of mononuclear cellular infiltrate in the perimatrix according to the degree of ossicular defect and the destruction of the bony walls were made. We found that these cells are predominantly seen in the cholesteatoma with aggressive and destructive growth. They were seen in the less degree in the skin.

P-175

The role of high-resolution flow cytometry in the grading of mucoepidermoid carcinomas

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Introduction The grading of mucoepidermoid carcinomas (MEC) is based on subjective microscopic evaluation of the prevalence of cell types as well as features of atypia and aggressiveness. A more objective, modified grading system based on the allocation of numerical scores to histological features was recently proposed by Brandwein et al 2001. Our study was aimed at evaluating the role of high-resolution DNA flow cytometry in the grading of MEC.
Material and methods Sixty-eight cases of intraoral- and major salivary gland tumours diagnosed as MEC were retrieved. The diagnosis of all cases was reviewed and the grading system proposed by Brandwein et al applied. Flow cytometry was performed on 50 μm sections of the formalin-fixed paraffin-embedded tumour blocks. The nuclei of the cell suspension were stained with DAPI solution (4`,6-diamidino-phenylindole) and at least 10 000 events from each case were analysed using a PAS III flow cytometer equipped with a high-pressure 100 W mercury lamp.
Results and conclusion Sixty-eight cases of MEC were retrieved and 44% were graded as high, 26% as intermediate and 30% as low. Ninety-four per cent of the high-grade MEC showed aneuploid DNA cell populations while 95% of the diploid tumours were graded as intermediate or low. Forty-four percent of our sample was graded as high, 26% as intermediate and 30% as low. Ninety-four per cent of the high-grade MEC showed aneuploid DNA cell populations while 95% of the diploid tumours were graded as intermediate or low. This study showed that high-resolution DNA flow cytometry of archival paraffin-embedded tissue is accurate in the grading of MEC.

P-176

Malignant melanoma of the head and neck mucosa: review and revision on six cases

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Introduction Malignant melanoma is a well recognized cutaneous

malignancy with poor prognosis, but occasionally arises in the various mucosa of general site including nasal or paranasal sinuses and oral cavity. It shows wide variety in cellular morphology and histological architecture and, therefore, can be easily disregarded as other neoplasm in such instance above. We describe herein a series of malignant melanoma arising in the head and neck mucosa, which diagnosis was corrected in our review, and discuss its histological and immunohistochemical characteristic as well as important clues for diagnosis.
Materials and methods Six cases were selected from the pathology files of Kurume University Hospital. Histological sections were routinely processed, stained with H&E, and observed with a light microscope. Immunohistochemistry and special stains were done as needed. Among these, two arose in ethmoid sinus and the others in nasal cavity, inferior nasal choncha, tongue and palate, respectively. Age ranged between 62 and 88 years old (average 74.8).
Results and conclusion The tumours showed undifferentiated morphology with anaplastic or sarcomatoid appearance and cellular population included ovoid, polygonal and spindle cells, occasionally intermingled with those with rhabdoid or plasmacytoid features. Immunoreactivity for HMB45, Melan-A and CD63, know as melanoma-related markers, considerably varied for each case, while that for S100 protein was relatively stable and useful for initial screening. Melanin deposition was easily passed by, especially if it appeared in the stroma rather than neoplastic cells themselves, but quite important as well as intraepithelial spread of atypical melanocytes. If resected together, also mucosal tissue should be examined carefully.

P-177

Tenascin expression in salivary gland tumors: an immunohistochemical study in 33 cases

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Introduction Tenascin is an extracellular matrix glycoprotein, which interacts with other extracellular matrix proteins and cells. It is reported that tenascin expression is detected in different localizations of normal salivary gland tissue and in most of salivary gland tumours. It is accepted as an indicator of invading potential in a report. The aim of this study is to detect whether tenascin expression patterns relate with type and malignancy of salivary gland tumours.
Material and method 33 cases of salivary gland tumours: 8 Warthin's stromal, 18 mixed stromal, 1 myoepithelioma, 1 basal cell adenoma, 1 basal cell adenocarcinoma, 2 acinic cell carcinoma, 1 adenoid cystic carcinoma, 1 malign mixed stromal were included in our study. Antibody anti-tenascin was applied to the paraffin sections.
Results Tenascin expression was observed in all tumours. Most invasive expression was observed in mixed tumours, generally in extra cellular matrix localization and in the pattern of coarse clusters. Warthin's tumours demonstrated dominantly epithelial tenascin expression, sometimes in globular pattern. In malignant salivary gland tumours tenascin expression is relatively rare compared with benign ones.
Conclusion Our findings suggest that tenascin expression may show variations in patterns and localizations in different kind of salivary gland tumours. Tenascin expression is not a feature of invasive potential but may be responsible of epithelial mesenchymal interactions, stromal growth, cell differentiation and production of mixoid and chondroid matrix.

P-178

Links between nucleolar and extracellular matrix proteins: tenascin and Ki-67 in vocal cord biopsy

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Introduction It is postulated that cancerogenesis goes through interchange of differentiation and proliferative processes. The aim of this study was to investigate morphogenesis and proliferation in the epithelial compartment of hyperplastic, dysplastic and neoplastic vocal cord mucosa with simultaneous expression of tenascin and Ki-67.
Materials and methods 48 vocal cord biopsies were analysed: 17 invasive squamous cell carcinomas, 10 in situ squamous cell carcinomas, 10 hyperkeratoses with low dysplastic change, 5 papillomas and 6 nodules with hyperkeratosis. Immunohistochemistry was performed for each marker separately as well as simultaneously for both markers.
Results Tenascin was expressed in differentiated and neoplastic tissues, decorating the membrane/cytoplasm of cells in carcinoma and dysplastic mucosa, but only the intercellular epithelial junctions in papilloma and hyperkeratosis. Ki-67, a nucleolar protein, decorated nuclei, nucleoli and chromosomes of cells engaged in mitosis in homotypic and heterotypic bioptic tissue. A double staining for tenascin and Ki-67 revealed synchronous decoration of cytoplasms and nuclei in invasive squamous cell carcinoma (17/17), in cells of in situ squamous cell carcinoma (6/10) and occasionally in cells of dysplastic hyperkeratosis (2/10). Simultaneous staining was not found in homotypic tissue. Neoplastic cells in mitosis had tenascin stained membranes/cytoplasms or were tenascin negative. In dysplastic hyperkeratosis basal cells in mitosis had rarely tenascin positive membranes (1 biopsy only).
Conclusion The observation that tenascin (a marker of morphogenesis) and Ki-67 (a marker of proliferation) express simultaneously in neoplastic epithelial cells makes a difference regarding homotypic proliferation.

P-179

RTPCR detection of ret/PTC 1 rearrangement in a case of osteoclastoma like anaplastic thyroid carcinoma

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Introduction Anaplastic thyroid carcinoma with an osteoclastoma like component is a rare type of thyroid neoplasm. The origin of the osteoclastoma like element has been a source of controversy, with conflicting theories emerging from the literature, some supportive of an epithelial origin with others favouring macrophage/histiocytic lineage. Ret/PTC is a fusion transcript associated with papillary thyroid carcinoma but not previously described in a tumour of this type. Our aim was to examine more closely the osteoclastoma like component and assess the histogenesis of this tumour.
Materials and methods Electron microscopy, histochemistry and

a broad immunohistochemical panel were performed. In addition pure populations of each constituent cell type comprising the tumour were isolated using laser capture microdissection. After RNA extraction, each of these was examined by RTPCR for chimeric transcripts of ret/PTC-1 and ret/PTC-3.
Results Electron microscopy, histochemistry and immunohistochemistry were supportive of a macrophage origin for the osteoclastoma element. However, chimeric transcripts of ret/PTC were detected in the microdissected bland mononuclear cells of the osteoclastoma component. Ret/PTC could not be detected in the microdissected multinucleated giant cell component.
Conclusion We present a case of osteoclastoma-like anaplastic carcinoma of the thyroid and we demonstrate, for the first time, ret/PTC chimeric transcripts (ret/PTC-1) within the mononuclear cells of the osteoclastoma component thus shedding further light on the controversy regarding the histogenesis of this tumour.

P-180

Proliferative activity in oral peripheral and central giant cell lesions – immunohistohemical expression of p53, PCNA and Ki-67 - first report

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Introduction Aim of the study was to define a correlation between the index value of p53, PCNA and Ki-67 in several types of oral mucous epulides and to evaluate their proliferative activity.
Methods Histopathologic studies were performed in a group of 56 patients. Monoclonal antibodies p53 (DAKO) – clone DO-7, subclass Ig-2b Kappa, method ABC (code NPO 10) were applied in the immunohistologic analysis. Nuclear antigen of proliferating cells, PCNA, was marked thanked to monoclonal antibody PC10 (DAKO), subclass IgG2a Kappa. Nuclear antigen Ki-67 was marked with the use of mouse monoclonal antibody MIB-1 (DAKO), class Igg-1 Kappa, code N1633. The p53, PCNA and Ki-67 indices were defined as a percentage of positive reactions to 1000 cells in high power fields.
Results No expression of the protein coding gene, p53, which is present in a great number of neoplasms was observed. It can be a proof of their non-neoplastic origin. Analysing preparations with PCNA, one can observe great proliferative activity in the epulides of the studied types. In case of Ki-67, a small proliferative activity is observed. The average indices for women are: IEg 4.4, IEf 2.6, IEi 3.2. For men IEg 1.8, IEi 2.1. At this stage of our study, due to small differences in each epulis type, no far-reaching conclusions can be drawn. Further studies of CD68 and MDM-2 protein in the same group of patients seem to be justified since in the authors' opinion, they will explain biological behaviour of that type of tumours.
Conclusions Great proliferation activity of the cells in all types of epulides analysed with PCNA, with low Ki-67 and lack of p53 activity, was observed.

P-181

Adenoid cystic carcinoma and polimorphous low grade adenocarcinoma of salivary glands. A morphometric study

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Introduction adenoid cystic carcinoma (ACC) and polymorphous low grade adenocarcinoma (PLGA) have a relatively high rate among salivary glands carcinomas. These tumours share several morphologic, histogenetic and cytogenetic features regarding their morphology. Nevertheless they present a different biological behaviour: PLGA is generally considered only a locally aggressive tumour whereas ACC, though in a long time, can cause recurrences and distant metastases. In this study five cases of ACC and four cases of PLGA are compared in order to identify morphological differences between epithelial cell nuclei useful for their discrimination.
Material and methods all the biopsies were formalin fixed, wax embedded and haematoxylin-eosin stained. 30 nuclei of PLGA and 30 of ACC were submitted to the SAM (Shape Analytical Morphometry) software system, which enables us to express numerically both dimensions (area, perimeter and diameter) and nuclear contour irregularities and shape distortions. Each selected nucleus was photographed at 1000X. For each nucleus 14 variables were obtained; all of them were submitted to univariant statistical comparisons by considering the total number of studied nuclei for the two lesions. Moreover, a multivariate test was performed.
Results nuclei of PLGA resulted significantly larger and with a more regular shape than nuclei of ACC. Multivariate analysis discriminated the 85% of nuclei by utilizing 3 variables: nuclear area and 2 variables obtained by the nuclear asymmetry evaluation.
Conclusion Morphocytological differences could have a discriminating power between PLGA and ACC, they seem to be more important than architectural differences and could be related to their different biological behaviour.

P-182

Oral synovial sarcoma – a report of two cases and a review of the literature

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Introduction The head and neck region constitutes the second most common site of involvement for synovial sarcomas, accounting for up to 9% of all cases. Intra-oral synovial sarcomas are rare, with only 29 cases having been reported. The aim of this study is to report 2 additional cases of intra-oral synovial sarcoma, which occurred in the floor of the mouth and retromolar areas respectively in middle-aged black males and to review the clinico-pathologic features of all previously reported intra-oral cases.

Materials and methods The current cases were immunohistochemically stained with EMA, CD99, bcl-2, vimentin, CAM5.2, MNF116, CK7, S-100 protein, HBA71, CD34 and calretenin; and subjected to ultrastructural studies. The literature was reviewed over a period of 40 years, examining a total of 31 cases of oral synovial sarcoma.
Results The intra-oral cases differ from lesions occurring in other sites only in respect of a more marked male predilection, and a generally painless presentation. This is the first report demonstrating calretinin immunopositivity in intra-oral synovial sarcomas. Even though in the oral cavity possible earlier detection, easy accessibility and small size renders them more amenable to surgical excision, their biological behaviour remains aggressive with a poor long-term prognosis.
Conclusion This study clearly demonstrates the histomorphologic, immunohistochemical and cytogenetic similarity of intra-oral synovial sarcomas to that of synovial sarcomas in general. The unusual intra-oral location often results in these lesions being misdiagnosed. These tumours should be considered, as both primary and metastatic tumours, in the differential diagnosis of intra-oral spindle cell malignancies.

P-183

Thirty nine years long follow up of the patient with dermatofibrosarcoma protuberans

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Background Dermatofibrosarcoma protuberans (DFSP) is an infiltrative, aggressive, nodular stromal with a great potential to relapse, that appears early in the life, in 15% of cases at the head and neck region. During last ten years there were five patients with the DFSP at the Clinic for Maxillofacial Surgery of the Military Medical Academy that makes 9.1% of all cases with mesenchimal malignancy. Here we present the most extreme case of the DFSP.
Case report S.S. was 8 years old when she injured a small subcutaneous stromal at the right side of her forehead. Ten years later it was 5 cm wide and excised for the first time, as well as two years later when the relapse of the same size, this time involving the bone, was removed and the patient went through radiation therapy. After four corrective operations in a meantime, at her age of 26 and 28 the relapse 6 cm wide from the right frontal region was removed. Three years later, at her age of 31, stromal sized 5 cm was excised from her right buccal mucosa. Two years later, at her age of 33, local relapse was widespread into the right parotid gland, superior eyelid and frontotemporoparietal region including dura. Two years after extensive ablative surgery and delicate reconstruction with scary compromised local cutaneous flaps, the relapse from the previously hardly mutilated right forehead region together with dura was removed. Six years later, at her age of 41, the right orbit was exenterated together with excision of adjacent temporoparietal tissues. One year later the local relapses from the right infraorbital and buccal region were removed. Two years after another stromal 5 cm sized from the periorbital region was removed. After two years, at her age of 46, the lesion from her cheek was removed. It has been her tenth, but obviously not the last surgery. Almost one year later there were no signs of local relapse or regional metastases at her routine postoperative check up.
Conclusion In spite of such a great mutilation of face and psychological disturbances connected with it, this is a case of successful surgical management of the DFSP. Few times some differential diagnostic dilemmas appeared, but careful and detailed revision of all specimens definitely confirmed the DFSP.

P-184

Multiple and multifocal malignancy - a case report

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Background It is well known that one either benign or malignant stromal can appear multifocally, as well as the fact that histologically different tumours can exist coincidentally. There is just a few papers concerning on how many different concomitant tumours can appear in the human body. This is a case of 78 years old male with several tumours of different malignancy degree and morphologically benign or semimalignant cutaneous lesions.
Case report During previous 27 years three times he underwent surgical excision of epitheliomas of the scalp, body and limbs. The third one has been removal of the epithelioma of the right frontoparietal region. At his age of 78 because of the stromal sized 4 cm in the right parotid salivary gland, followed by paralysis of the frontal and paresis of buccal branches, along with laboratory finding of 22.48 leukocytes, right-sided radical parotidectomy and suprahyoid dissection of the neck has been performed. Pathohistologic analysis confirmed malignant mixed stromal of the parotid salivary gland. Postoperative irradiation therapy has been applied in 21 fraction and total dose of 45 Gy. Nine months later the patient has been operated again because of an exulcerated, 5 cm diameter cutaneous lesion of interscapular region, which has proved to be the infiltrative squamous carcinoma G-2 grade. At the same time the chronic lymphoproliferative disease in the form of chronic lymphocytic leucosis A grade has been diagnosed (although the reactivity following the neoplastic process could not be excluded), but specific haematological treatment has not been indicated. Nine months later nine cutaneous facial lesions have been removed: malignant mixed stromal of the left auricle, senile keratosis from the left temporoparietal region, chronic dermatitis from the forehead, squamous carcinoma G-2 and seborrhoic keratosis one inch inferiorly, two lesions, sebaceous adenomas from the right zygomatic region, corneal squamous carcinoma G-1 from the right parietal region, baseocellular carcinoma from the left one and adenoid type of baseocellular carcinoma from the right supraclavicular region. The patient has been operated without complications and came five times for monthly control in a good general condition, without relapse, regional or distant metastases. Later on we ascertained that the patient suddenly died during a sleep seven months after surgery.
Conclusion Pathohistologic examination of removed tissues and revision of the former specimens undoubtedly excluded the spreading of the one and same stromal in different regions, but proved multifocal appearance of tumours of the same embryologic – ectodermal origin in very different structures.

P-185

Melanoma of the oral cavity - a case report

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Background Melanoma is a capricious malignancy followed by reputation of aggressive growth and dissemination, able to originate from any tissue containing melanin. In less than 2% it appears at mucous membranes. Here we present a case of such localization.
Case report During last ten years there were 47 patients with primary cutaneous head and neck melanoma who underwent surgery because of secondary deposits at the neck, but only one patient, R.M., 52 years old (pale skin, dark hair and eyes, never working outside), who appeared four years ago with inoperable intraoral melanoma involving tongue, base of mouth, mucosa of the left cheek, retromolar region, tonsillar fossa and soft palate, including a single pendular mass from the left palatoglossal arc that almost completely narrowed his isthmus faucium. The first pigmentation he noticed eight months before his first admittance suffering of disturbed swallowing for few weeks. Although there was no sign of regional or distant metastases, only the last-mentioned mass has been removed and pathohistologically verified as melanoma. Patient was able to breathe normally, and continuing with his previous way of life. He received DTIC during the first four postoperative months. One year later, smaller mass from the same location has been removed again. Routine laboratory analyses, radiographic examination of the lungs, ultrasonographic examination of the neck and abdomen never revealed any changes. The third reduction under the same circumstances took place one year later and has been followed with minimal doses of adjuvant immunotherapy.
Conclusion This is a rare case of such a large, inoperable intraoral melanoma that has been three times reduced at the place of its exophytic growth into throat, otherwise demonstrating no signs of regional or distant spreading, as well as any disturbances in patient's general condition during last four years.

P-186

CD34, alfa-smooth muscle actin and TGFbeta1 expression in stromal cells in epithelial hyperplastic lesions and squamous carcinoma of the larynx

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Introduction Recent studies have shown that stromal reaction in cancer has an important diagnostic and prognostic significance. CD34-positive stromal cells and myofibroblasts may play an important role in host response to cancer. The aim of this study was to analyse the expression and significance of CD34, alfa-smooth muscle actin (SMA), and TGFbeta1 in epithelial hyperplastic lesions (EHL) and squamous carcinoma (SC) of the larynx, and tried to reveal the mechanism of myofibroblast formation in SC.
Materials and methods We investigated samples of 42 resected larynxes with SC, 12 laryngeal biopsies of EHL and SC, and 6 samples of normal laryngeal mucosa. Immunohistochemistry was performed with antibodies against SMA, CD34, CD31, TGFbeta1 and TGFbeta1 receptor. The expression of TGFbeta1 mRNA was detected by RNA in situ hybridisation.
Results Stroma of normal mucosa and EHL contained scattered CD34-positive cells, but there were no SMA-positive myofibroblasts. Stroma of SC contained SMA-positive myofibroblasts but there were no CD34-positive stromal cells. In normal mucosa and EHL, TGFbeta1 and its receptor were present in epithelial cells and scattered stromal and inflammatory cells. In SC, TGFbeta1, its receptor and TGFbeta1 mRNA were found in stromal cells, inflammatory cells and some stromal myofibroblasts.
Conclusions Disappearance of CD34-positive cells and appearance of SMA-positive myofibroblasts in the stroma is associated with transformation of laryngeal EHL to SC. This pattern of stromal reaction is limited to invasive carcinoma and should be regarded as an additional marker of invasion. Our results also support the hypothesis based on experimental studies that TGFbeta1 may be one of the mechanisms of transformation of stromal cells to myofibroblasts in SC.

P-187

FHIT gene alterations in squamous cell carcinoma of larynx and hypopharynx

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Introduction Loss of heterozygosity (LOH) on short arm of chromosome 3p is frequently found in squamous cell carcinoma of larynx and hypopharynx (LHSCC). This is a region where potential stromal suppressor gene, frequently changed in pulmonary cancer, the FHIT gene, is located. We wanted to determine whether there is presence of molecular and/or immunohistochemical changes in FHIT gene in 69 patients with LHSCC.
Materials and methods DNA was isolated from stromal and adjacent normal tissue using standard procedure. Non-isotopic LOH analysis was performed with micro satellite markers D3S1007, D3S1038, D3S1233, D3S1285, D3S1300. Formalin fixed tissue samples were stained immunohistochemically with antibody against FHIT protein and reactions scored according to % of nuclear positivity in stromal cells.
Results LOH at 3p was detected in 42 (60.9 %) and microsatellite instability (MSI) in 4 (5.8%) tumours, which were mostly moderately differentiated (60.9%). High expression of protein was found in 18, moderate in 14 and low in 18 tumours. Immune reaction was negative in 19 cases. There was no correlation between presence of LOH and/or MSI, results of immunohistochemistry and stromal grade.
Conclusion Our results show that alterations of FHIT gene are frequent in LHSCC. They are most commonly reflected by LOH, the most important mechanism in LHSCC cancerogenesis. The absence of correlation beween LOH and immunohistochemistry is probably caused by molecular changes, reflected in altered FHIT protein expression, which can not be detected with LOH analysis. Further studies are needed to determine the possible significance of FHIT gene in pathogenesis of LHSCC.

P-188

Two loci PCR test for prognosis of colorectal cancer

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Introduction Deletions of chromosomes 17p and 1p are usual in human colorectal cancer (CRC). Breakage of tumor suppressor genes located there is commonly considered as a cause of tumor progression. Loci YNZ22 (17p13.3) and Alu-VpA/MycL1 (1p34.3) may serve as markers of 17p and 1p loss. The aim of this study was to test whether lesions of named loci may be used as prognostic signs in CRC.
Materials and methods Losses of heterozygosity (LOH) of named loci in DNA isolated from 50 primary colorectal adenocarcinomas were tested by PCR.
Results YNZ22 LOH was found in 33% (13/39) of informative tumors and was 3 times more often in men than in women. This lesion was associated with moderate or low histological differentiation of tumors and ploidy >3n of tumor cells. Twenty two percent (11/50) of tumors showed Alu-VpA/MycL1 instability, and 14% (6/43 informative) lost Alu-VpA/MycL1 allele. LOH of this locus was associated with moderate or low differentiation. Instability of Alu-VpA/MycL1 was associated with proximal localization of tumors. By Kaplan-Meyer assessment, LOH of any one of the two loci correlates with development of CRC relapses or metastases (RM) in 30 months after tumor resection. In group of patients with any of the two LOH in tumor, 67% (8/12) had RM in this period, while in group whose tumors retained both loci heterozygous this index was 20%.
Conclusion We propose to make up group of CRC patients with RM risk in 30 months after surgery relying upon PCR test of loci YNZ22 and Alu-VpA/MycL1.

P-189

Age dependence of DNA preservation and retrieval relationship to fixative type

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Introduction Permeation of fixatives into tissue for optimal DNA preservation for molecular pathology studies is related to tissue type and fixative but also to glycosaminoglycan content of the ground substance. This may alter with age, warranting further study. The aim of this study was to assess nuclear DNA preservation related to age and fixation agent.
Materials and methods Fresh standardised testicular tissue samples from dogs 0 - 6 months (I), 6 months - 2 years (II) and 3 years and over (III) were used for immediate DNA extraction (T0), extraction after 24 hours (T1) and after 14 days (T14) of immersion in formaldehyde-based and formaldehyde-free, commercially available fixatives. DNA extraction used standard protocols, commercially available kits (QIAamp minikit, Qiagen, the Netherlands), standard primers for the betaglobin gene preserved between species (MYO9/MY11, Digene/Abbot, USA) and quantitative PCR (RealTime Light Cycler, Roche, 45 amplification cycles).
Results and conclusions In formaldehyde free fixatives, initial loss of extractable DNA in T1 samples of young (I) animals was 15% as compared to 20% in older (II+III) animals ($p<0.05$, student T-test). The same, with a significantly poorer result ($p<0.01$) was found in formaldehyde based fixation, with loss of 20% in young (I) as compared to 30% ($p<0.01$) in older (II+III) animals. At 14 days, further loss to 45% of T0 values occurred in all 3 age groups ($p<0.001$), with formaldehyde free fixatives retaining the proportional age associated difference. In formaldehyde based fixatives at T14, loss to 5% of T0 values was found ($p<0.001$), again without statistically significant differences between age groups.

P-190

The G870A polymorphism of CCND1 correlates with susceptibility and prognosis of breast cancer in Taiwan

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A frequent G870A polymorphism of CCND1 gene located on the splice donor site on exon 4-intron 4 boundary is implicated in influencing cyclin D1 mRNA splicing and production of two distinct mRNA transcripts. Although cyclin D1 is believed to perform an important role in cell cycle progression, this G870A single nucleotide polymorphism (SNP) seems to be a critical modulator of its functions. It has been reported that the prognostic significance and mRNA splicing regulatory effect of G870A varies with tumor type and ethnic diversity. To explore the association between the CCND1 G870A polymorphism and the breast cancer susceptibility as well as other prognostic parameters, we employed 103 infiltrating ductal carcinoma (IDC) cases and 215 native controls in this study. There is obviously correlation between the genotype distribution of these two groups ($p=0.038$), and the AA genotype reveals a higher association and risk than the AG+GG genotypes ($p=0.011$ with c2 test and OR 1.93, 95% CI 1.16-3.21, $p=0.012$). Moreover, there is no statistical significance found between genotype or allele frequencies and tumor stage, grade, nodal involvement, or patient'¡s age. However, the trend tests of CCND1 G870A genotype distribution or allele frequencies to the increasing of tumor grade or tumor stage are significantly different when comparing the A vs. G allele ($p=0.01$ in both tumor grade and stage) or AA vs. AG+GG genotype groups ($p=0.04$ and 0.05 in tumor grade and stage, respectively) but no significance when comparing GG vs. AG+AA or AG vs. AA+GG genotypes. These results suggest that CCND1 G870A polymorphism correlates with IDC susceptibility, and 870A allele reveals an increased risk in carcinogenesis of IDC and worse prognosis.

P-191

The new HOPE*-fixative as an enhancement in molecular pathology

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During the last years the growing demand for the detection of

prognostic and diagnostic biomarkers has been slowed down to a large degree by the use of formalin and its influence onto antigenic structures and nucleic acids, although other reasons also exist. With regard to morphology a limitation in the possibilities, compared to frozen sections, takes place. With the introduction of the HOPE-fixation to date both has become possible; substantially enlarged possibilities concerning the detection of DNA, RNA and proteins together with a formalin-like morphology. This will be demonstrated by presenting several examples like preservation of nucleic acids (DNA, RNA), the detection of those by PCR and RT-PCR, in situ hybridization and the preservation and delectability of proteins by immunohistochemistry. We conclude that HOPE-fixation opens up new possibilities, especially within the growing number of diagnostic and prognostic biomarkers. *Hepes-glutamic-acid-buffer-Organic-solvent-Protection-Effect

P-192

Prognostic value of HPV viral load in cervical cancer evaluation of real-time PCR, conventional PCR and signal amplified in situ hybridization

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Introduction High-risk human papillomaviruses (HPV) represent a major risk factor for the development of cervical cancer. Quantitative Real-Time PCR for viral load determination of HPV in archival cervical cancer specimens could be of clinical and prognostic relevance. The aim: We investigate the prognostic value of HPV status and viral load. Furthermore, we compared the applicability and sensitivity of three molecular HPV-detection methods.
Materials and methods All examinations were carried out from formalin-fixed, paraffin-embedded tissue of 164 cervical carcinomas. HPV status was detected by signal amplified chromogenic in situ hybridization (SA-CISH), conventional polymerase chain reaction (PCR), and detection and additional quantitation was performed using Real-Time PCR.
Results Patients with low viral load of HPV 16 in stage I carcinoma had a significantly better prognosis than HPV 16-negative patients (p=0.037). Additionally, high viral load of HPV 16 in patients indicating a higher stage carcinoma strongly tended to benefit prognosis compared to HPV-negative patients. Our evaluation of the molecular HPV-detection methods showed a higher sensitivity of Real-Time PCR and conventional PCR compared to SA-CISH.
Conclusion It appears that cervical cancer patients can be distinguished in a HPV 16-negative subgroup revealing a worse prognosis, and a lower-stage subgroup with low viral load, respectively a higher-stage subgroup with high viral load, both indicating a better survival, maybe due to an altered immune status caused by HPV-infection. These findings are crucial to accord each patient the individual therapy, and point out the importance of quantifying viral load and the necessity of Real Time-PCR in HPV-diagnosis.

P-193

Laser capture microdissection in 2-D coculture models as a novel tool to study tumor-stroma-interactions

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Introduction Cellular adhesion, migration and invasion are essential processes in tumor progression and do include actions by malignant cells as well as by the stromal microenvironment. In vitro, these interactions can be studied in coculture models. In conventional coculture systems, the different cell types are grown together in glass-slide based chambers with medium. In situ analysis can be easily performed, however, for mutational or gene expression analyses the cell compartments have to be dissociated and sorted. We present a novel technique for co-culturing fibroblasts and carcinoma cells and separating them without cross-contamination.
Methods Our method is based on cell cocultivation on a 1.35 µm thin membrane followed by laser microdissection of tumor and stroma cells. For identifying the tumor cell compartment, immunolabeling for the laminin gamma 2 chain was performed, a marker that is expressed only in epithelial tumor cells. DNA and RNA was isolated from the microdissected cells. RNA quality was tested by RT-PCR for a housekeeping gene transcript and for the laminin gamma 2 chain gene transcript.
Results Co-cultivation on the membrane did not influence growth behaviour and cell morphology. The RNA quality from the microdissected co-cultured and immunostained cells could be successfully proved by RT-PCR. Laminin cDNA was amplificable only in tumor cells and not in the co-cultivated fibroblasts indicating no cell-cross-contamination during microdissection.
Conclusion Our technique provides a suitable tool to study tumor - stroma interactions at the DNA or RNA level for expression profiling and genetic analyses.

P-194

P53 expression in prostate cancer and its correlation with microvessel density

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Introduction Angiogenesis is important in the development and progression of all cancer types, including prostate adenocarcinoma (PCa). Increased vascularization may be influenced by p53 status. The aim of this study was to evaluate MVD in prostate cancer and its relationship with p53 nuclear accumulation.
Methods paraffin-embedded tissue from 61 patients with well-, moderately and poorly differentiated prostate carcinoma was examined immunohistochemically using monoclonal antibodies against CD31, VEGF and p53 (clone DO-7). A modified semiquantitative Weidner score and computerized image analysis was used to assess MVD.
Results expression of immunohistochemically detected p53 protein was found in 10 of 61 cases (16%). P53 nuclear accumulation was observed in two different groups of cancers – poorly differentiated PCa with high VEGF expression and high MVD, with high

level of serum PSA (6 cases) and moderately differentiated PCa without significant microvessels with low serum PSA (4 cases). We suppose p53 mutation in the first group of patients which leads to increased angiogenesis as the result of loss of wild-type p53-induced anti-angiogenic control. In our opinion p53 detection in the second group of p53+ patients is the result of overexpression due to stabilization but not mutation.
Conclusion p53 overexpression is observed due to either mutations or protein stabilization. Increased angiogenesis seems to be influenced by p53 mutations, while low MVD is observed in cancers with non-mutated p53. Immunohistochemistry alone is not able to detect p53 mutations, additional techniques are need for this purpose, especially in patients with p53 nuclear accumulation.

P-195

Malignancy-associated X chromosome LOH in foregut endocrine neoplasms: further evidences in lung tumors

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Introduction association of X chromosome allelic losses (LOH) with tumor malignancy has been found in foregut-derived gastro-pancreatic endocrine neoplasms, but not in midgut carcinoids. Also lung endocrine tumors originate from the embryological foregut. They are classified into "typical" carcinoids (TC), "atypical" carcinoids (AC), large cell neuroendocrine carcinomas (LCNEC) and small cell lung carcinomas (SCLC), in order of increasing malignancy The aim of this study was to investigate X chromosome LOH and its association with malignancy in a series of endocrine tumors of the lung.
Materials and methods DNA extracted from 9 TCs, 17 ACs and 4 LCNECs from 30 female patients was PCR-amplified using fluorescently-labeled primers for 18 microsatellite markers spanning the whole X chromosome. For LOH evaluation, amplimers were analysed in an automatic DNA sequencer (CEQ2000, Beckman Coulter). All samples were formalin-fixed, paraffin-embedded and DNA from tumoral and non-tumoral adjacent tissue was available
Results allelic losses were absent in TCs, while LOH on X chromosome was found in 6 of 17 ACs (35%) and in 3 of 4 LCNECs (75%). Allelic losses were generally wide, involving all or most informative markers investigated
Conclusions allelic losses on X chromosome, absent in benign "typical" carcinoids, progressively increased their frequency from intermediate-grade "atypical" carcinoids to high-grade LCNECs. Therefore the observation that X chromosome LOH is associated with malignancy, already demonstrated in gastro-pancreatic endocrine tumors, is extended to another group of foregut-derived endocrine neoplasms, the lung endocrine tumors

P-196

Withdrawn

P-197

A new way of mycobacterial DNA-extraction for PCR targeting mycobacterial DNA in formalin-fixed, paraffin-embedded tissues

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Introduction Because of the withstanding mycobacterial cell-wall, DNA extraction for PCR-based detection testing is an obstacle in molecular diagnostics for mycobacteria. In formalin-fixed, paraffin-embedded (FFPE) tissues the DNA is fragmented by the fixation and embedding process. To get suitable DNA for PCR, the bacterial cell-wall has to be opened. Since currently used methods to open the bacterial wall might further increase DNA-fragmentation, we looked for a new, milder way to open up the mycobacterial cell-wall. Aims: As cut up mycobacteria in are regularly visible in Rhodamin-Auramin or Ziehl-Neelsen stained sections of FFPE tissue, we intended to open up the mycobacterial cell-wall by cutting very thin (1-2 µm) sections by microtome. In this mechanical opening there should be only little or no further fragmentation of DNA.
Materials and methods We investigated 28 formalin-fixed, paraffin-embedded tissue samples. From each paraffin-block four times 30 one to two µm sections in three steps and two 15µm sections were cut and put to 1.5 ml reactiontubes. One tube of the thin sections and the thick sections were exposed to heat/cold shock-treatment, one tube had a proteinase-K treatment for only three hours instead of overnight, and the thin cuts in another tube were treated with a Riboliser. DNA amount and purity measured by spectrophotometry after extraction. PCR was performed with routinely used 16SrDNA-Primers.
Results Over all we obtained best results with cutting up thin sections and proteinase-K treatment overnight. This tissue yielded the best purification, the highest DNA amounts and strongest bands in agarose gelelectrophoresis. Second best were thick and thin sections with heat/cold shock-treatment and proteinase-K treatment overnight.
Conclusion The mechanical opening of the mycobacterial wall using a standard microtome is a feasable way to obtain higher DNA-amounts –purity and -complexity resulting in stronger signals following PCR.

P-198

Impact of tumour suppressor gene PTEN on cell cycle and apoptosis in prostate cancer cell lines

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Introduction The tumour suppressor gene PTEN encodes a dual specifity phosphatase that recognizes lipid and protein substrates.

Aims: To study the influence of overexpressed PTEN on cell cycle progression and apoptosis.
Material and methods Prostate cancer cell lines DU-145 and PC-3 were transfected by PTENpxmyc and its mutation forms. Effect on the cell cycle was analysed by using bromdeoxyuridin (BrDU) incorporation and in situ immunocytochemistry.
Results The results are based on transient transfection of wt PTEN and its constructed point mutations with altered phosphatase activity in the prostate cancer cell lines DU-145 with mutated p53 and pRb and PC-3 with mutated p53 and wt pRb. Overexpression of the wt PTEN with intact phosphatase activity or its mutation G129E with altered lipid and functional protein phosphatase activity caused a block in G1-S transition in both tumour cell lines. Overexpression of the wt PTEN in DU-145 also led to an increase number of cells with nuclear changes which are characteristic for apoptosis.
Conclusion In tested prostate cancer cell lines PTEN blocked cell cycle by p53 and pRb independent manner. This result is not in agreement with already published mechanism in some breast cancer cells. Supported by grants IGA MZ CR NC/6779-3, GACR 204/01/0488 and MSM 151100001

P-199

Gene response to bicalutamide in prostate carcinoma cell lines LNCaP and DU-145 with respect to telomerase activity

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Introduction The progression of prostate cancer from androgen-responsive to an androgen-unresponsive status remains the greatest problem in the treatment of this disease. Aims: To study the relationship between telomerase activity and gene expression after bicalutamide treatment in prostate cancer cells.
Material and methods Androgen-responsive (LNCaP) and androgen-unresponsive (DU-145) prostate cancer cell lines treated with 80 μM bicalutamide for 24 hours were analysed by Affymetrix HG-Focus Array Chips and their expression pofile was compared with control.
Results The decrease of telomerase activity which was associated with increase of p21waf1/cip1 and decrease of dyskerin and hsp90 were found in LNCaP cells only. DNA damage-responsive genes were upregulated in both cell lines. In LNCaP cells, the increase of p21waf1/cip1 was associated with induction of p53-dependent gene expression (GADD45α, bax, mdm-2 and TRAIL-R2). We have also found induced transcription of pro-apoptotic genes represented by BNIP3-like, Bcl-10, CBX4 and TRADD and the decrease of anti-apoptotic protein, survivin. In DU-145 cells, we observed an increase of mt p53, TRAIL-R2, GADD34, GADD45α expression together with a decrease in expression of survivin and the pro-apoptotic BNIP3 gene.
Conclusion The decrease of telomerase acivity in LNCaP cells after bicalutamide treatment could be explained by changes in expression of p21waf1/cip1, dyskerin and hsp90. Bicalutamide upregulates DNA-damage genes in androgen-responsive as well as androgen-unresponsive prostate cancer cell lines. This work was supported in part by MSM151100001 and NC/7497-3.

P-200

Involvement of Bcl-2 family proteins in induction of cell cycle arrest by bicalutamide in human prostate carcinoma cells

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Introduction Bicalutamide, an androgen antagonist used in the prostate cancer treatment, has a significant cytostatic effect on cancer cells. The aim of this study was to analyse the changes of Bcl-2 family members after bicalutamide treatment in androgen-responsive (LNCaP) and androgen-unresponsive (DU-145) prostate cancer cells.
Material and methods We examined mRNA and protein expression of the Bcl-2 family members by DNA-microarray (Affymetrix) and Western blotting.
Results Bcl-2 protein was expressed in LNCaP cells but not in DU-145 cells. Bax protein was present at similar levels in both cell lines. Bicalutamide induced decrease of Bcl-2 in LNCaP and slight increase of Bcl-2 in DU-145 cells, but the level of Bax decreased only in DU-145 cells. These changes were not accompanied by changes in levels of mRNA. Bcl-2 alpha mRNA and Bax mRNA were not detectable in both untreated cell lines. Following bicalutamide treatment, LNCaP cells showed increased transcription of Bax, Bcl-W, BNIP3, BNIP3L and BAG2, and a decreased transcription of Bik, Mcl-1 and Bid. DU-145 cells responded only by a decrease in BNIP3, BNIP3L and BAG4 mRNA levels. In addition, we have found induction of mt p53 transcription in DU-145 cells and transciptional activation of genes upregulated by p53 in both cell lines.
Conclusion Bicalutamide induces different levels of pro- and anti-apoptotic proteins of Bcl-2 family in these two cell lines. The effect of bicalutamide can be mediated by p53-driven pathway independently of hormone receptor status. This work was supported in part by MSM151100001, NC/6200-3 and NC/7497-3.

P-201

Comparing the toxicity and potential carcinogenicity of two antiestrogens. A short-term study with microarray analysis in female rat liver

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Introduction Tamoxifen and toremifene are triphynylethyle antiestrogens used in the treatment of breast cancer. Tamoxifen is genotoxic hepatocarcinogen in the rat, whereas toremifene is not. In humans, long-term tamoxifen treatment causes an increased risk of endometrial cancer. The major difference between the genotoxicity of the two drugs is the formation of liver DNA-adducts in

tamoxifen-treated rats. Differential gene expression in rat liver after administration of antiestrogens was studied by microarray analysis.
Materials and methods Equimolar doses of antiestrogens were given p.o. to six week old female Sprague-Dawley rats for 10 days. Total RNA was isolated using TRIzol Reagent. Equal amounts of total RNA from four animals in the same group was pooled and then purified from 1 mg total RNA using 35 mg oligo(dT)-cellulose. Our cDNA array contained approximately 4000 cDNA clones selected from the TIGR Rat Gene Index. The samples were hybridized to rat cDNA array scanned with a GMS 418 scanner (Afflymetrix). Image analysis was performed using the GenePix Pro software. Normalization was performed using Lowess normalization. The results from rat cDNA array were evaluated according to 'Significance Analysis of Microarrays' (SAM).
Results SAM plot for tamoxifen vs. control revealed 445 significant genes from which 183 were positive significant (up-regulated, red) q < 5 %, 165. Particularly the NADPH-cytochrome P450 oxidoreductase and microsomal epoxide hydrolase were upregulated genes which are apparently related to tamoxifen-induced carcinogenesis. SAM plot for toremifene vs. control revealed 443 significant genes from which 129 were positive significant q < 5 %, 35.
Conclusion Microarray technology gives important information of the toxicity as well as carcinogenicity of antiestrogens in experimental conditions.

P-202

Expression analysis of RET and its GDNF/GFRalpha1 ligand complex in normal gastrointestinal tract and derived tumors

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Introduction The RET proto-oncogene tyrosine kinase receptor is activated by a ligand complex comprising glial cell line-derived neurotrophic factor (GDNF) and GDNF family receptor alpha-1 (GDFRalpha1). They are expressed in multiple organs during development and also in some types of human tumors in adults. The aim of this study is to investigate RET, GDNF and GDFRalpha1 expression in normal tissue and tumors derived from the gastrointestinal tract, circumstance that we have not found it published in the literature.
Material and methods RET, GDNF and GFRalpha1 expression was analyzed in samples of fresh frozen and formalin-fixed tissues from tumoral zone, and adjacent non-affected tissue of biopsies from 64 patients with gastrointestinal cancer using rt-PCR and immunohistochemical methods.
Results Presence of RET, GDNF and GFRalpha1 transcripts in most of normal tissues was confirmed by rt-PCR. Immunohistochemical study showed positive staining in citoplasms of glandular epithelium of the mucosa, ganglionar cells and muscular fibers (only GDNF) of esophagus, stomach, duodenum, colon and rectum but not in jejunum and ileum. Gastric oxyntic cells were negative. Sixty four gastrointestinal tumors (62 adenocarcinomas and 2 cases of GIST) were also studied. By immunohistochemistry, 90% of cases showed citoplasmic immunoreactivity for GDNF; 87% for RET and 96% for GFRalpha1. These findings were confirmed by rt-PCR. There was not association with microscopic type, size, grade or Duke stage.
Conclusions Our study shows RET, GDNF and GFRalpha1 positive protein and mRNA expression in normal tissues and in the majority of tumors derived from the gastrointestinal tract. There is no association with microscopic type, size, grade or lymph node metastasis. This expression gives no prognostic information in the clinical management after two years follow-up of our patients.

P-203

Expression analysis of RET and its GDNF/ GFRalpha1 ligand complex in breast cancer

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Introduction The RET proto-oncogene tyrosine kinase receptor is activated by a ligand complex comprising glial cell line-derived neurotrophic factor (GDNF) and GDNF family receptor alpha1 (GFRalpha1). They are expressed in multiple organs during development and also in some types of human tumors in adults. In this study we evaluated the expression of RET, GDNF and GFRalpha1 in normal mammary tissue and different types of breast cancer.
Material and methods RET, GDNF and GFRalpha1 expression was analyzed in samples of fresh frozen and formalin-fixed tissues from tumoral zone, and adjacent non-affected tissue of mastectomies from 31 patients with different types of breast cancer (24 DIC, 1 LIC, 3 medullary carcinoma, 1 mucinous carcimoma, 1 papillary carcinoma and 1 fibrosarcoma) using rt-PCR and immunohistochemical methods.
Results Normal breast ductal epithelium express RET, GDNF, and GFRalpha1. By immunohistochemistry, 54,8% of breast tumors showed citoplasmic immunoreactivity for RET, 83,8% for GDNF and 76,0% for GFRalpha1. rt-PCR confirmed mRNA expression in 88,4% of cases for GDNF, 42,3% for RET and 96,0% for GFRalpha1. By chi-square analysis, RET expression was associated with less tendency to the regional lymph nodes metastases ($p<0,001$) and low grade differentiation ($p<0,005$). There was not association with microscopic type, size or stage.
Conclusions We found that RET and its GDNF/GFRalpha1 ligand complex are expressed in normal breast tissues and in a high percentage of different types of breast tumors using immunohistochemistry and rt-PCR. Univariant analysis also revealed that only RET expression was associated with less tendency to the lymph nodes metastases and low grade DICs (highly differentiated tumors). The short time follow-up of our patients (less than two years) gave no information about clinical outcome.

P-204

Telomerase activity in chronic B-lymphocytic leukemia

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Telomerase, the enzyme that elongates telomeres, contains an RNA template complementary to TTAGGG repeats that permits de novo

synthesis of telomeric DNA onto chromosomal telomeric ends. Recent findings have suggested that activation of telomerase is one of the most common and fundamental steps in cancer genesis, although reactivation or telomerase up-regulation alone might be insufficient for cells to proliferate indefinitely. Telomerase expression seems to be concomitant with the attainment of immortality of cancer cells, but there are contradictory reports about telomerase activity in early and late B-CLL stages. In order to investigate the telomerase activity in patients with CLL and its correlation with the clinical stage, 47 frozen blood lymphocytes samples from patients with CLL (32 males, 15 females), and 47 age matched controls (32 males, 15 females) were investigated for telomerase activity using the 'Telomerase PCR ELISA-plus kit' from Roche, based on the Telomeric Repeat Amplification Protocol. Binet and Rai stages were evaluated at the time of sample collection, and re-evaluated after 14-16 months. Results were analyzed with a commercial statistic software package. The study showed that peripheral lymphocytes telomerase activity from B-CLL patients is significantly higher (3,9 times; $p<0,01$) compared to normal lymphocytes. The age and sex of patients does not influence the telomerase activity, while it is age dependent in healthy controls (Pearson Correlation – 0,543; $p<0,01$). The study also showed that telomerase activity is increased more significantly in advanced stages of the disease, and correlates with the disease course.

P-205

P63-driven nuclear accumulation of β–catenin is not a frequent event in human neoplasms

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Introduction DeltaN-p63 isoforms may act as oncogenes, owing to their ability to bind to p53-reporter genes without inciting their transcription, thus blocking the p53-driven cell cycle arrest and apoptosis. A novel mechanism, linking p63 and Wnt pathways has recently been proposed. Briefly, in vitro studies have suggested that deltaN-p63 may block the phosphorylation of beta-catenin, leading to its nuclear accumulation and triggering beta-catenin-responsive transcription of genes related with proliferation and oncogenic biological behaviour. To test this new mechanism, the authors evaluated the co-expression of deltaN-p63 and beta-catenin in a large cohort of human neoplasms.
Methods Two sections of TARP-4 multi-tumour tissue microarray, composed of 51 normal tissue cores and 400 human neoplasms (breast (n=75), colon (n=75), lung (n=75), prostate (n=75) and ovary (n=50) neoplasms, melanoma (n=25), and glioblastoma (n=25)) were subjected to immunohistochemistry with deltaN-p63 and beta-catenin monoclonal antibodies. p63 nuclear expression and b-catenin membranous, cytoplasmic, membranous+ cytoplasmic, and nuclear localisation were independently evaluated by three of the authors.
Results p63 expression and beta-catenin nuclear localisation were found in 92.6% and 0% of squamous cell carcinomas, 8.9% and 0% of breast carcinomas, 13.8% and 0% of lung adenocarcinomas, 1.4% and 23.2% colon adenocarcinomas, 0% and 4.8% of prostate adenocarcinomas, 11.1% and 5% of ovary carcinomas, 9.0% and 9.1% of malignant melanomas, and 12.5% and 40.0% of glioblastomas, respectively. No statistically significant association between p63 and nuclear beta-catenin expression was found for all tumours.
Conclusions At variance with squamous cell carcinoma cell lines, p63-driven nuclear accumulation of beta-catenin is an unusual phenomenon in human neoplasms. Caution should be exercised when translating the results of studies performed on cell lines to human neoplasms.

P-206

C-kit mutations in gastrointestinal stromal tumors from patients treated with STI- 571

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Aims To evaluate c- Kit mutations in Exon 11 in 23 gastro-intestinal stromal tumors (GIST) from patients treatment with STI- 571 (Gleevec).
Material and methods DNA was extracted from 23 formalin-fixed, paraffin- embedded GIST samples. Mutations in Exon 11 of c- kit were analyzed by DNA sequencing. C- kit mutational status was correlated with the clinicalpathological findings and the response to STI- 571.
Results C-kit mutations in exon 11 were identified in 18 cases (78%) (delections in 14 cases, and missense mutations in 4 cases). Four GIST had no identificable KIT sequence alteration in exon 11. Mutations in c- kit seemed to be associated with favorable response to STI- 571 ($p= 0,078$).
Conclusion Kit mutations in exon 11 are common in diseminated gastrointestinal stromal tumors and appear to be associated with response to STI-571.

P-207

The use of macro-array to study the effect of intestinal trefoil factor on gene expression in caco-2 derived enterocytes

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Intestinal trefoil factor (ITF) is a member of the trefoil family of peptides. It increases resistance to apoptosis, enhances mucosal healing and restitution in vivo and promotes migration of intestinal epithelial cells in vitro. The aim of this study was to investigate the influence of ITF on gene expression in intestinal epithelial cells. The human colon carcinoma cell line Caco-2 develops structural and functional characteristics of normal enterocytes when kept in culture. Enterocyte-like Caco-2 cells were treated with recombinant ITF during 2 hours. RNA was isolated and labelled cDNA was synthesised. The cDNA probe was hybridised to the Human Unigene Set 2 colony filters (RZPD). After several washing steps, the amount of signal was measured using a phospo-imager. Spot intensities were measured and signal quality was evaluated. Using a 2-fold increase or decrease as a cut off, comparison of the gene expression between un-stimulated Caco-2 cells and cells stimulated with ITF using the macro-array technique revealed a few hundred genes that were potentially differentially expressed. Our data show that the macro-array is a powerful technique to screen for differen-

tial gene expression as a result of trefoil administration, helping to study signalling cascades. However, it is necessary that the huge amount of data obtained is filtered through repeated hybridisations, only to retain the relevant changes and these data must be confirmed using other methods like quantitative Real-Time PCR and eventually studies at protein level.

P-208

BPC 157- related inhibition of cell growth in human melanoma cell line

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Introduction Melanoma therapy still remains without notable success of any pharmacological agent. Gastric pentadecapeptide BPC 157 is a peptide with various pharmacological effects on different organ systems including wound healing and inflammation. Its possible effect on cell growth and differentiation was not investigated on human cancer cell lines.
Materials and methods Human melanoma cells were maintained in RPMI-1840 medium supplemented with 10% FBS and antibiotics. Cells were grown to confluency in humidified incubator (95% air atmosphere, 5%.CO2) at 37°C. Upon reaching confluency, cells were maintained in chemically defined medium (CDM). BPC157 was added in the culture medium in the various concentrations (2 pg. and 2ng, respectively) for a period of 48 hours. Cell morphology was observed daily under light microscope and the photomicrographs were taken. After harvesting cells were prepared in EtOH for flow cytometry analysis.
Results Microscopy did not reveal any changes upon treatment with BPC 157 after 48h comparing to controls. Flow cytometry showed 99% of cells ($p>0.01$) were in G0-G1 phase. Control cells had total S-phase fraction of 9.11%. However, upon introduction of BPC 157, in concentration of 2pg/ml, the percentage of cells in S-phase was decreased by 20% compared to controls. Moreover, the concentration of 2 ng/ml BPC 157 lowered total S-phase fraction by 55% comparing to controls and by 30% comparing to BPC 157 in picogram values ($p>0.01$).
Conclusion We assume that gastric pentadecapeptide BPC 157 could act as a inhibitory agent during G1-S transition state of cell cycle thereby preventing DNA synthesis and cell growth.

P-209

Profile of expression of histone genes differs in radioresistant and radiosensitive human glioma cell lines

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Introduction Histones play an important role in the structural and functional properties of chromatin. Their protective role against radiation-induced damage of DNA is well established. The aim of this study was to evaluate relation between expression of histones genes and radiosensitivity of brain gliomas in vitro.
Materials and methods Five human glioma cell lines obtained from DSMZ (Germany) were irradiated with 2Gy and 10Gy doses (Co-60) and further grown for 3, 6 and 12 hrs. The expression of genes coding for histones H1, H2A, H2B, H3, H4 was measured with real-time quantitative RT-PCR assay (TaqMan). Radiosensitivity of the cell lines was assessed with cytokinesis-block micronucleus assay. Cell lines were regarded as radioresistant and radiosensitive at micronuclei per binucleate cell ratio (MN/BNC) <0.5, and > 1, respectively, as measured over a dose range of 0-10 Gy.
Results The global number of copies of histone mRNA did not differ in both groups. In radiosensitive cell lines (GAMG, 8-MG-BA) expression of H2B was highest in 2/2 cases, while in radioresistant (GOS-3, 42-MG-BA, U-138-MG) H4 predominated in 2/3 cases. In both groups significant decrease of global histone genes expression after 10Gy dose was found. Relative increase of H4 and H2B fractions after irradiation was observed.
Conclusion Overall expression of histone genes does not seem to influence radiosensitivity of glioma cells. Probably, more important is the profile of expression, e.g. differences between H4 and H2B genes. This profile may be a useful adjunctive criterion in the choice of treatment protocol for brain glioma.

P-210

Potential application of nested RT-PCR for human mammaglobin in the detection of micrometastasis in effusions

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Introduction The detection of breast cancer (BC) micrometastasis in effusions is difficult since the malignant cells are rare and spread amongst the normal population. RT-PCR for human mammaglobin (hMAM) has been described as a new, sensitive method for use in BC cell research. Some authors have proposed using this assay for screening BC effusions for BC micrometastatic disease. The aim of this study was to investigate the possible application of RT-PCR for hMAM for assessing BC cells in effusions and compare this methodology with cytological examination.
Materials and methods The study was performed on 112 fluid specimens (19 BC, 52 other types of cancer and 41 without known carcinoma). The samples were analyzed by staining with EE, Papanicolau technique, and by nested RT-PCR for hMAM.
Results 16/19 (84%) cases of BC pathology were positive using nested RT-PCR for hMAM. All 9 cytology positives were also positive by nested RT-PCR. In contrast, 7 BC cases that were positive in the molecular test were negative according to the staining assay. hMAM was also detected in 19/52 (36%) specimens of other types of cancer and only 1/41 (2.4%) samples of non-neoplastic origin.
Conclusion Nested RT-PCR for hMAM was more sensitive than cytology in determining BC micrometastasis in effusions. Positivity was not restricted to samples from BC patients as specimens from other tumors and only 2.4% of patients without cancer were also positive. This test could be helpful in investigating neoplasticity in effusions.

P-211

Hepatic ischemia reperfusion injury in patients after liver segment resection

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Introduction Hepatic ischemia-reperfusion injury results from implementation of Pringle's manoeuvre to prevent blood losses in patients undergoing liver segment resection. Reoxygenation after the ischemic period leads to failure of hepatic microcirculation, leucocyte invasion and Kupffer-cell activation. Subsequent production of inflammatory cytokines and ROS (reactive oxidative species) are triggered, thus causing oxidative stress and damage of liver parenchyma. The goal of our study is to evaluate markers associated with the severity of this particular type of oxidative stress at the gene expression level.
Materials and methods We have analyzed liver tissue biopsies at 3 different timepoints obtained from 16 patients undergoing liver segment resection. In each case, the first sample is taken before clamping, the second sample is taken after 30 minutes of ischemia, and the third sample is taken after 30 minutes of ongoing reperfusion.
Results We compared mRNA levels of several genes (SQSTM1, HSPA1A, GPX1, SOD2, HMOX) in every set of samples by quantitative RT-PCR analysis. Significant elevation of HSPA1A at timepoint 3 was detected in 10 of 16 cases. This finding suggests that HSPA1A may represent a useful marker for oxidative injury of hepatic tissue. At the same time, we show that the detection of changes in gene expression within 30 minutes of reperfusion is possible by quantitative RT-PCR from a single biopsy.
Conclusion We hypothesize that individual differences in gene expression may reflect severity of the inflicted ischemia reperfusion injury as well as patient prognosis. The clinical significance of our preliminary results is currently under investigation.

P-212

Automated tissue microarray evaluation and normalization using a DNA scanner

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Introduction Tissue microarray (TMA) immunohistochemistry is gaining importance in drug target validation. Hundreds of tissue samples on a single slide enable simultaneous immunohistochemical assessment of protein expression. Correlation of expression levels of the target antigen with clinical parameters can facilitate determination of disease relevance and therapeutic potential. The aim of this study was to extend the scope of this technology, we developed a robot for automated TMA production. We further assessed the use of a DNA microarray two color laser scanner for automated analysis of TMA immunohistochemistry and the use of an internal reference antigen for standardization to the tissue content.
Materials and methods The TMA robot was developed in cooperation with an engineering company. A breast carcinoma TMA was stained with an estrogen receptor (ER) antibody. Lung carcinomas TMAs were double stained with combinations of rabbit and mouse antibodies. Immunoreactivity was detected with fluorescently labeled secondary antibodies. The slides were evaluated using a cDNA array scanner and software.
Results and conclusions Fluorescence intensity for ER IHC on a breast carcinoma TMA correlated positively with independent assessment of the diagnostic ER immunoreactivity score, suggesting that IHC quantitation can be achieved with the DNA array scanner. Double IHC on lung TMAs demonstrated that one antigen reference can normalize tumor marker IHC signals for the cellular content of TMA cores. IHC signal normalized in this fashion correlated with clinical parameters in agreement with published results.

P-213

Manifestation of metallothionein (MT) correlates rather with cell differentiation than with intensity of proliferation. Immunocytochemical analysis of expression of metallothionein (MT) and Ki67 in cells of ductal breast carcinoma, uterine cervix planoepithelial carcinoma and non-keratinising multilayer flat epithelium

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Introduction Metallothioneins (MT) are proteins which are supposed to play role in the cell cycle. Numerous publications describe positive correlation between intensity of MT expression and proliferative potential of cells. Our study aimed at examining the relationship between MT expression and intensity of proliferation, measured using Ki67 antigen.
Materials and methods The studies were performed on samples of ductal breast carcinomas of G1, G2 or G3 grade of differentiation as well as on samples of normal epithelium and planoepithelial carcinoma of uterine cervix. Paraffin sections of the examined cases served to perform immunocytochemical reactions using mouse monoclonal antibodies to MT and Ki67. Double staining reactions were also performed using the same antibodies.
Results The performed studies showed that MT expression in breast cancers of G1 grade was significantly lower than in cancers of G2 or G3 grades ($p=0.004$). In the case of Ki67, the least intense expression characterized cancers of G1 grade and the most intense reaction was noted in cancers of G3 grade ($p=0.00001$). No relation between MT and Ki67 expressions was observed in G1 and G3 groups of breast cancers ($p>0.05$) while in the G2 group as well as in the entire group of breast cancers (G1 to G3) a positive correlation between the two expressions was noted ($p<0.05$). Analysis of reactions in the non-keratinising stratified flat epithelium and in the planoepithelial cancers of uterine cervix showed no correlation between expressions of MT and Ki67. However, MT expression was detected only in the least differentiated cells of the epithelium.
Conclusion Our showed that MT expression tends to correlate more with cell differentiation extent than with proliferative potential of the cells.

P-214

Is the activation of Akt a prognostic parameter in colorectal cancer?

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Akt (protein-kinase B, PKB) is a serine-threonine kinase that is activated mainly by insulin, IGF-1 receptor and erbB family receptor signaling in a PI3-kinase dependent manner, and is implicated in tumor proliferation in breast, ovarian, pancreatic and gastrointestinal cancers. The phosphorylation of its substrate proteins result in increased growth and decreased susceptibility to apoptosis. The activation of the PI3K-Akt pathway is downregulated by PTEN phosphatase, and mutations or a loss of PTEN activity results in a high constitutive activation of the kinase. The aim of the present study is to investigate the levels of the activation of the PI3K-Akt pathway in a series of colorectal carcinomas and to evaluate its role on tumor resistance to chemotherapy. We achieved 46 surgical colorectal carcinoma specimens at pT3 stage. All patients received at least two chemotherapy lines based on platinum and CPT-11 after surgery. Levels of activation of Akt were tested by immunohistochemistry in primary tumors using a polyclonal antibody that recognizes the phosphorylated Akt at Ser473. In the series, 33 (71.74%) showed staining for phosphoAkt in tumor cells. PhosphoAkt was detected in tumor as clusters of cells related to necrotic foci and differentiated areas and correlated with less response to chemotherapy. These results point out that 1. Activated Akt is often overexpressed in human colorectal carcinomas and 2.Activated Akt can be a parameter of chemotherapy resistance in colorectal cancer.

P-215

Mutational analyses of the *RET* proto-oncogene in Slovenian MEN2 families

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Introduction Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominantly inherited cancer syndrome that appears in different subtypes MEN2A, MEN2B and FMTC. The loci for all three subtypes have been mapped to chromosome 10q11.2 which encompasses the RET proto-oncogene, a receptor-type tyrosine kinase. The key structural features include extracellular cadherin-like and cysteine rich domains, a transmembrane domain and an intracellular domain with a tyrosine kinase function. Aims: To study familial predisposition of MTC cancer in Slovenian population, 103 MTC patients were screened for germline mutations in the *RET* proto-oncogene.
Material and methods DNA was isolated from blood of patients with MTC from Slovenian registry at Institute of Oncology. The DNA samples were screened for mutations in *RET* proto-oncogene. Exons 10, 11, 12, 13, 14, 15 and 16 of *RET* proto-oncogene were amplified in PCR and analysed by single stranded conformational analyse (SSCA), direct sequencing and/or restriction digestion with endonucleases.
Results We determined typical germline *RET* mutations in 25 patients from 13 distinct families. We identified six MEN2A families, two MEN2B families and one FMTC family. In families in which the RET mutation is known, genetic testing identifies carriers with 100% specificity and 100% sensitivity.
Conclusions These findings demonstrate that molecular genetic analysis of blood derived DNA for RET point mutations is a reliable method to detect asymptomatic gene carriers of MEN2.

P-216

Mutations in *COL4A3*, *COL4A4* and *COL4A5* genes in relation to benign familial hematuria and Alport syndrome

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Introduction Alport syndrome (AS) is a progressive inherited nephropathy, characterized by irregular thinning, thickening and splitting of the glomerular basement membrane (GBM), associated with hearing loss and ocular symptoms. AS is caused by *COL4A5* mutations in its X-linked form and by *COL4A3* and *COL4A4* mutations in its autosomal recessive form. The mutations in *COL4A3* and *COL4A4* have been reported in familial benign hematuria (BFH). Aims: We screened *COL4A3*, *COL4A4* and *COL4A5* genes in 7 slovenian families with X-linked AS and in 31 slovenian families diagnosed with BFH to determine pathogenic mutations, to correlate them with clinical features and to confirm or provide precise diagnosis.
Materials and methods Genomic DNA was isolated from blood. Screening for mutation was done by the optimised SSCA after PCR amplification of each exon of *COL4A3*, *COL4A4* and *COL4A5* and changed samples were sequenced.
Results We found six different mutations in *COL4A5* gene in AS suspected patients. One of the mutations was present also in three families with BFH. In *COL4A3* gene three and in *COL4A4* four mutations, all in heterozygous state, were identified only in patients with BFH. Eleven of the mutations are new and private. Four rare variants of unknown pathogenesis and many polymorphisms were found in the *COL4A3* and *COL4A4* genes. A mutation was detected in 86% of AS patients and in 32% patients diagnosed with BFH
Conclusions Our study broadened the spectrum of mutations in *COL4A3*, *COL4A4* and *COL4A5* and demonstrated the involvement of the *COL4A3* and *COL4A4* genes in the pathogenesis of BFH.

P-217

Screening for genetic alterations in the *PRNP* gene

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Introduction Prion diseases are a group of fatal neurodegenerative disorders. The commonest human prion disease is Creutzfeldt-Jakob disease (CJD). Sporadic CJD is genetically characterized by a lack of pathogenic mutations in *PRNP* gene but methionine/ valine polymorphism at codon 129 determines the susceptibility to

sporadic and other non-genetic forms of CJD. Homozygosity for either methionine or valine increases the risk for acquiring CJD. Aims: We searched for changes in *PRNP* gene of healthy populations and sCJD cases and compared genotypes of codon 129 in order to prove differences among populations and non-familiar origin of sCJD cases and also to confirm the influence of codon 129 homozygosity on the disease development.
Material and methods Blood samples from six populations (Slovenian, Afro-American, PIMA Indian, Cheyenne Indian, Finnish, German) and brain or blood samples from sCJD Slovenian cases were collected and DNA was extracted. PCR-SSCA was performed and changed samples were sequenced.
Results Fourteen different nucleotide changes in coding sequence of *PRNP* gene were identified in control populations, one of them was also present in a sCJD patient. Slovenian and German genotypes of codon 129 are similar, other populations differ more significantly. Pimas lack the V/V genotype and Cheyennes show an increased V/V frequence. Proportions of codon 129 genotypes in sCJD patients show a different distribution: 90% are M/M and 10% are V/V.
Conclusions Codon 129 genotype frequencies proved to be population- and location-specific. Sporadic CJD cases do not contain any mutation, specific for familial form.

P-218

Molecular pathology of the *RASSF1* gene in colorectal cancer

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Introduction Tumour suppressor gene RASSF1 encodes different isoforms, derived from alternative splicing and differential promoter usage. The major transcripts A and C are expressed in all normal tissues, but transcript A is missing all cancer tissues. Loss of expression is mainly corellated with methylation of the CpG island promoter sequences of *RASSF1A* but mutations in exons have also been found. We examined the status of promoter methylation and potential changes in the coding region of the *RASSF1A* gene in colon cancer tumours.
Materials and methods Isolated DNA from tumours of colon cancer was used for exon amplification of *RASSF1A* gene. For identification of mutations and polymorphisms SSCA was used. Abnormal patterns on SSCA plates were sequenced. For determination of methylation status of the CpG islands we used COBRA method.
Results COBRA analysis revealed the hypermethylation of CpG islands. Sequencing analysis of exons showed different nucleotide changes. We identified C>A mutation (R94S), G>C mutation (D116H), G>T mutation (A133S), G>A mutation (R200H), G>A mutation (R257Q) and C>A mutation (L305I). R94S, D116H, R200H and L305I mutations have not been published yet.
Conclusions These results indicate that hypermethylation of the promoter of *RASSF1A* gene in colon tumours and missense mutations in protein itself could be involved in tumorigenicity of colon cancer.

P-219

Expansive lesions in the pineal region: histological and immunohistochemical features of 24 cases

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Introduction Pineal lesions are rare and comprise 0,4-1% of all intracranial tumors. Various histological types of tumors arise in the pineal region. The aim of this study was to determinate histological types, immunohistochemical expression, age and sex distribution of lesions in pineal region.
Materials and methods Twenty-four expansive lesions in the pineal region, obtained between 1990 and 2002, were analyzed. Formalin-fixed, paraffin embedded surgical specimens were stained routinely and immunohistochemically for panel of antibodies; synaptophysin, neuron specific enolase, neurofilament, chromogranin A, glial fibrillary acid protein, cytokeratin, carcinoembrional antigen, β-HCG, human placental antigen, α-Fetoprotein and Ki-67.
Results Histology revealed six germinomas, seven pineocytomas, three pineoblastomas, five glial cysts and one ganglioglioma, pilocytic astrocytoma and epidermoid cyst. The study group consisted of 13 females (54,2%) and 11 males (45,8%) ranged from 4 to 66 years of age. All germinomas were in male patient while glial cysts were diagnosed in female patients. Slightly female predominance was observed in pineal parenchymal tumors. Immunoreactivity for neuron specific enolase, synaptophysin, neurofilament and for chromogranin A was positive in the pineocytomas, and partially expressed in pineoblastomas. The mean Ki-67 index in pineoblastomas and germinomas were significantly higher than in pineocytomas. Germinomas showed focal immunoreactivity for β-HCG, human placental antigen, α-Fetoprotein and low immunoreactivity for cytokeratin and carcinoembrional antigen.
Conclusion The most common tumors are pineal parenchymal tumors and germ cell tumors. Glial pineal cysts are also common. Occasionally other types of tumor are found. Immunohistochemistry is an important method in differential diagnosis of pineal tumors.

P-220

Immunohistochemical study of the metastatic brain tumors with accent of the role of thyroid-transcription factor-1

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Introduction The exact diagnosis of the metastatic brain tumors /MBT/ is challenge for the surgical pathologist especially in a case of unrecognized primary site. The aim of this study was to determine reliable immunohistochemical panel based on the histological features of the MBT.
Materials and methods Sixty five cases with solitary MBT operated in Neurosurgical department of the hospital were examined from the time period 1997- 2002; thirty nine were of unknown primary origin (60%). Immunohistochemical studies were performed using antibodies to CK-s, EMA, NSE, synaptophysin, PSA, vimentin, S-100 protein, HMB45, TTF-1 and GFAP.
Results Diagnosis of the MBT of unknown origin were as follow: squamous cell carcinoma - 5 cases from total 11, small-cell

undifferentiated - 4 from 6, large cell undifferentiated - 6 from 9, adenocarcinoma -14 from19, adenosquamous - 2 from 2, clear cell - 2 from 3, papillary carcinoma -1 from 3, tubular -1 from 7, spindle cell -1 from 1, unclassified - 3 from 3. The primary site of adenocarcinoma, undifferentiated small and large cell and clear cell carcinoma was established by using TTF-1 (positive in 27 from 41 lung cancer, 65.85%), neuroendocrine markers, CK-8 and PSA. After the investigation only three tumors were of unknown origin (7.69%). The origin of the rest unknown before studies of 36 MBT, confirmed with radiographic and endoscopic data were: lung - 27 from 41 total, breast - 2 from 7, kidney - 2 from 3, skin-2 from 4, thyroid gland - 1 from 2, stomach -1 from 1, prostate -1 from 1. The colon, testis and ovary, at one case each, were known before operation.

Conclusions Immunohistochemistry remains important and still very helpful method in determining primary origin of the MBT generally in undifferentiated neoplasmes and adenocarcinomas. Among the most reliable tumor markers should be included TTF-1, for its frequent expression in the lung cancer, predominant among MBT.

P-221

Brain calcification simulating brain tumour. A case report

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Objectives The aim of this study was to describe the plural calcifications in the brain.

Materials and methods The patient was a 32-year-old man. He had a severe headache and disorientation, he felt weakness in the left arm. The patient was mentally handicap. ST scan showed a tumour located in the deep parts of the right temporal lobe. With a working diagnosis of tumour, the patient underwent the operation. The surgical specimens were prepared by standard histological techiques.

Results Macroscopically, the lesion appeared as a stone. A histological examination demonstrated a massive deposits of calcium in the parenchyma of the brain. Calcification developed as small granules in the vessels and calcospherites of various dimensions were formed by confluence. There were zones of haemorrhage, neuronal loss, edema and proliferation of astrocytes.

Conclusions This patient is an interesting clinical case of dementia characterized pathologically by diffuse calcification.

P-222

Carcinoid tumour metastatic to the orbital muscle. A case report

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Introduction Ninety-three percent of symptomatic patients with small intestinal carcinoid tumours have metastases compared to 9% of tumours found incidentally. The most common sites of metastatic carcinoid tumours are lymph nodes and liver. Orbital metastasis from malignant carcinoid tumours have rarely been described and the majority of them involve the choroid rather than orbital structures. The aim of the study is to present an unusual case of ileal carcinoid tumor with metastasis to the orbital muscle.

Materials and methods We report a patient who developed a posterior left orbital mass eighteen months after the first operation for primary intestinal carcinoid tumour with hepatic metastases. Orbital involvement presented with proptosis, impairment of vision and reduced ocular motility on the left side. The patient was examined by CT and MR. Biopsy was taken and examined by imprint and frozen section. The tumor was removed. HE and staining for cytokeratin, chromogranin, NSE, serotonin, somatostatin and gastrin were performed.

Results CT and MR study revealed the orbital tumour mass infiltrated the inferior rectus muscle. Imprint and frozen section showed tumor consistent with metastatic carcinoid. The histological and immunohistochemical features of the tumour corresponded to those of the primary intestinal carcinoid tumour.

Conclusion Intramuscular orbital metastasis from carcinoid tumour is a rare occurrence. Diagnosis may be difficult especially where no evidence of primary carcinoid tumour is present. Although a rare event, metastatic orbital carcinoid should be suspected in patients with clinical history of carcinoid tumour who develop ocular complaints and mass lesion in the orbit.

P-223

Non-invasive photoacoustic tomography of brain: a novel functional neuroimaging with high contrast and high resolution

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Introduction Optical contrast is sensitive to monitor dynamic and functional changes, such as blood concentration, tissue oxygenation, and cellular swelling. Optical imaging has been widely employed in clinical applications including the investigations of functional neuroanatomy and neuroactivity. Due to the overwhelming scatter of light during the propagation inside biological tissues, past attempts to visualize brain architectures non-invasively with the optical contrast have been severely restricted by the unsatisfactory spatial resolution. The aim of this study was to investigate the feasibility of a novel non-invasive transcranial imaging of the structure and function of brain in vivo accomplished with laser-induced photoacoustic tomography (PAT).

Material and methods PAT visualized the tissue structures in rat/mouse brain based on the high sensitive intrinsic optical contrast while utilizing the diffraction-limited high spatial resolution of ultrasound.

Results Two-dimensional (or three-dimensional) rat (or mouse) brain structures, with and without lesions, were imaged clearly. In response to whisker stimulation, functional cerebral hemodynamic changes in cortical blood vessels around the whisker-barrel cortex were also mapped successfully. PAT imaged hyperoxia- and hypoxia-induced cerebral hemodynamic changes as well.

Conclusions This study demonstrates that (i) structural PAT of soft tissues lesions in the brain presents high intrinsic contrast, (ii)

functional PAT based on intrinsic optical signals can map the hemodynamic changes in the cerebral cortex and (iii) in contrast to other previously used optical methods, this technique enables completely non-invasive neuroimaging with high spatial resolution transcranially. This novel functional neuroimaging technique, will help to advance the research in neurophysiology, neuropathology, and neurotherapy significantly.

P-224

Microscopic disorders of cortical development of brain and the etiopathogenetic relevance of their detection in patients with temporal lobe epilepsy due to hippocampal sclerosis

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Introduction Hippocampal sclerosis (HS) represents a common structural basis of temporal lobe epilepsy (TLE). However, the etiological factors and mechanisms leading to its development still remain unexplained. The aim of this study was to identify the disturbed neuronal migration and differentiation (NMD) in the temporal lobe resected for HS and to evaluate its potential role in the individual pathogenesis of HS.
Materials and methods We present neuropathological findings in the resected hippocampus and the pole of the temporal lobe in 15 patients with hippocampal sclerosis with an attention paid to the histopathological identification of defects of NMD in the temporal lobe. The morphological findings were correlated with the results of clinical examinations and the patients´ histories.
Results 'Initial precipitating injuries' that are thought to cause the development of HS (febrile seizures in early childhood, head injury or meningoencephalitis) were present in the history of 12 patients. In the remaining 3 cases, no predisposing factors were found. Defects of NMD were observed in 7 cases; in three of these, no predisposing factors were identified in the patients´ histories. We suggest that in these cases, HS arises due to previously undetected disorders of cortical development. A latent neocortical malformation may also contribute to the development of HS in patients with an initial precipitating injury in anamnesis.
Conclusion Histopathological examination of resected epileptic brain tissue can provide insights into the individual pathogenesis of epileptic disorders, especially by the detection of microscopic disorders of cortical development.
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P-225

Cerebellar liponeurocytoma: case report

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Introduction Cerebellar liponeurocytoma is a newly recognized rare clinicopathological entity of the posterior fossa, with advanced neuronal/neurocytic and lipomatous differentiation. The term 'cerebellar liponeurocytoma' was recently adopted by the WHO group to replace several other different terms used up to now. This tumour shows many morphological similarities to medulloblastoma and neurocytoma, but according to available literature has more favorable prognosis then typical medulloblastoma. We report a case of cerebellar liponeurocytoma in a 40-years-old woman who developed typical symptoms of a posterior fossa tumor. Computed tomography (CT) scan disclosed a heterogeneous tumor mass in the left cerebellar hemisphere. The lesion was totally resected.
Methods The surgical specimen was rinsed in saline solution and fixed in 4% paraformaldehyde in phosphate buffer at pH 7.35 for 24 hours, embedded in paraffin and prepared for histological and immunostaining.
Results Microscopically, the tumor was composed of small less differenerentiated, round or oval closely packed medulloblastoma-like cells and vacuolated cells with macrovesicular lipid accumulations giving in some large areas an appearance indistinguishable from mature fat cells. Glial and neuronal differentiation was histo-pathologically and immunohistochemically (GFAP, NSE, NF) noted. These cellular components were intermixed in different proportions without distinct separation. Many tumor cells of all types showed single or multiple smaller lipid vacuoles in their cytoplasm.
Conclusion Our case provides evidence that mature adipose-like tissue in these tumors appears as result of progressive lipidization of neuroectodermal tumour cells rather than as result of adipose metaplasia.

P-226

The expression of survivin and Ki-67 in meningiomas: correlation with grade and clinical outcome

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Introduction Determination of proteins taking place in the control of proliferation in normal cells helps a better understanding of cellular transformation and proliferation mechanisms. Measure-ment of proliferative activity is important in determining tumor grade, recurrence time and malignancy. PCNA and Ki-67 are two

of the nuclear markers used to demonstrate proliferative phase of cell cycle.
Material and methods In the presented study 26 meningiomas of various histological grade, which have been diagnosed in Baskent University and Çukurova University Medical Faculty, Department of Pathology, were graded according to WHO grading system and survivin and Ki-67 monoclonal antibodies were administered by immunohistochemical method. Findings were analyzed with statistical methods; distribution of survivin and Ki-67 LI values were determined for different grade meningiomas.
Results In meningiomas, the correlation between the increasing grades with survivin and Ki-67 LI values were statistically significant. In addition, we have found correlation between clinical outcome and survivin and Ki-67 LI.
Conclusion The results suggest that survivin, alone or in conjunction with Ki-67, is a significant prognostic marker of worse outcome in meningioma.

P-227

Progesterone receptors expression in benign meningiomas

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Object Although they do not arise from a tissue normally thought to be a target tissue for progesterone, meningiomas show a number of epydemiologic and clinical features which suggest that females sex hormones can play a role in their development (higher incidence of meningiomas in woman, rapid progression of symptomas and icreasing in size during the pregnancy and during the luteal phase of the menstrual cycle, as well as significant association of meningiomas with obesity and breast carcinomas). The aim of our study was to investigate the progesterone receptor contents of 30 bening meningiomas (WHO gr. I), which is completly excised and evaluated possible relationship between receptor content and sex and age of the patients, histological tipe of meningiomas and localisation of tumor.
Material and methods Tissue samples, fixed in formalin, and embedded in paraffin were evaluated immunohistochemically for progesterone receptors using specific monoclinal antibodies.
Results and conclusion The correlation between number of positively nuclei and progesteron status was determined by the analitic statistical test Mann-Whitney. 57% of the 30 meningiomas tested were positive for progesterone receptors. Progesterone receptors in the benign meningiomas in the male patients was significantly higher when compared to the female patients ($p<0,05$).

P-228

Neonatal intractable seizures revealing diffuse and bilateral cortical dysplasia. A case report

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Cerebral cortical dysplasia is generally focal and sometimes related to tuberous sclerosis. It is believed to result from disrupted neuronal migration during cortical development. We report an unusual case of neonatal diffuse and bilateral cortical dysplasia revealed by refractory epilepsy. A male infant, born at 40 weeks' gestational age (GA) without familial history of neurologic disease, presented at birth a convulsive encephalopathy, associated with severe hypoxia with pulmonary hypertension and iterative hypoglycemia. A foetal goitre was attributed to hyperdose therapy for maternal Graves' disease between 20 and 30 GA. The patient died at 3 weeks of life from respiratory failure after intensive care. All the iso- and allocortical areas examined were normal at gross examination, but microscopically dysplastic. There was disorganization of the laminar architecture and presence of large multipolar neurones diffusely distributed over the cortex. They showed positive immunoreactivity with neurofilaments and synaptophysin, and were negative with GFAP and vimentin. Numerous ectopic neurons were found in the white matter. In addition, C shaped thickened inferior olive was noted. The cerebellum, including dentate nuclei, and other brain stem nuclei were normal. Focal cortical dysplasia lesions are localized malformation of the isocortex with positive GFAP and vimentin in dysplastic neurons. In this case, the dysplastic lesions were diffuse and astrocytes were normal. No genetic investigation was available, but the negative immunostaining of large cells by GFAP does not favour the diagnosis of tuberous sclerosis. The pathogenesis of this exceptional disease remains obscure but the responsibility of the foetal thyroid disorder cannot be excluded.

P-229

Structural genomic abnormalities of chromosomes 9 and 18 in myxopapillary ependymomas

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Myxopapillary ependymomas (MPE) are low-grade neuroepithelial tumors typically located in the cauda equina. They tend to grow slowly and may recur locally without progression. Very little is currently known about the genetic abnormalities involved in the development of MPE. In an attempt to clarify the chromosomal status of these tumors and identify commonly aberrant regions in the genome we have combined 3 molecular/cyto/genetic methods to study genomic abnormalities in 17 MPE. Comparative Genomic Hybridization analysis of 7/17 tumors identified concurrent gain on chromosomes 9 and 18 as the most frequent finding. The majority

of the 17 tumors were also studied using microsatellite analysis (14 markers for chromosome 9 and 8 markers for chromosomes 18) and interphase-FISH with centromeric probes for chromosomes 9 and 18. Our combined results were consistent with polysomy 9 and 18 in 7 cases, polysomy either 9 or 18 with partial gain in the other chromosome in 3 cases, tetrasomy or partial gain either 9 or 18 with complex rearrangement in the other chromosome in 3 cases. In the remaining 4 cases allelic imbalance alone of either chromosome 9 or 18 was identified. Other numerical abnormalities observed included gain of chromosomes 3, 4, 5, 7, 8, 11, 13, 17q, 20 and X and loss of chromosomes 10 and 22. The combination of gains of genetic material from chromosomes 9 and 18 seems to be common in MPE and may represent genomic events of importance in the development of the tumor.

P-230

Nonsymptomatic nontumor cysts of the pineal gland: frequency and morphological characteristics

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Introduction Nonsymptomatic nontumor cysts (NNC) of the pineal gland (PG) are considered as normal structures. The aim of the present study is to evaluate the frequency of NNC in random postmortem examinations and their influence upon surrounding parenchyma.
Materials and methods The study includes PG out of 101 random autopsy cases (73 men and 28 women, mean age 51.60±6.21). Gross investigation is performed prior to fixation; histological stains are applied on paraffin sections.
Results The frequency of NNC is 18.81% (17.80% in men, 21.42% in women). There isn't significant difference between genders in all age groups. The mean weight of PG with and without NNC is 175.41±49.37mg and 126.45±18.69 mg respectively. The analysis of PG dimensions reveals: mean length 9.80±1.75 mm versus 8.18±0.11mm and mean width 6.95±1.24 versus 6.13±0.59 mm in NNC versus cases without NNC. No significant difference is established for PG density (3.34±2.26 versus 3.30±1.08 kg/m3). PG parenchyma around NNC doesn't reveal particular changes.
Conclusion NNC frequency is not age and/or gender dependent; NNC presence increases PG weight and length without influence on PG width and density. These findings show that NNC eventual clinical significance may be due to comression of surrounding structures only, without effect on PG functional activity.

P-231

The effect of bcl-2 protein and Ki-67 expression on the prognosis of medulloblastomas

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Introduction Medulloblastoma is the most common primary central nervous system tumor in childhood and accounts 20-25 % of all intracranial neoplasms in children. Aim: In this study the correlation between the patients survival and prognostic factors (Ki-67 and bcl-2) and the tumor size in CT scan were evaluated.
Material and method 10 selected cases were analyzed. Immunohistochemical staining was applied as polyclonal antibody for Ki-67 (DAKO-A- 0047) and monoclonal antibody for bcl-2 (bcl-2/ 100/D5 Novo Castra) on paraffin blocks.Fisher's qhi-square, correlation and Mann-Whitney-U tests were used for the statistical analyses.
Results The ages of these patients were ranged between 1-29 (mean 8.6).The size of the tumoral mass varied between 3 to 6 centimeter. 6 patients were alive without illness.4 patients were lost due to tumor. We could not found a statistical revelance between neither immunohistochemical staining (bcl-2, Ki-67) nor tumor size on survival rates.
Conclusion Due to radiotherapy and chemotherapy following surgery, the survival rates of medulloblastoma has increased despite the agresiveness of the tumor. There are reports showing no correlation between expression of the bcl-2 with the outcome of patients. Further investigation about the correlation of the prognostic factors with the outcome is required.

P-232

Clinical analysis and histo-clinical correlations in recurrent astrocytic brain tumours

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Introduction Results of glioma treatment are still unsatisfactory. An accurate histopathological study supplemented with examination of tumour parameters, like proliferation potential and apoptosis may be invaluable in designing an individualised therapy. The aim of this study was to analyze clinical data and histo-clinical correlations in recurrent brain tumours of astrocytic origin.
Materials and methods One hundred surgically removed supratentorial gliomas were studied (mean age: 49.3yrs). Histological grade was determined according to WHO 2000 classification and the Daumas-Duport scale (St.Anne/Mayo). Proliferation activity was measured using the PCNA index and AgNOR count. The expression of BCL-2 protein was examined with immunohistochemistry.
Results and conclusion The initial symptoms of glioma were headaches and vomiting (41 %), paresis (35 %), epilepsy (33 %) and aphasia (18 %). The tumour location was predominantly in the left hemisphere, in the temporal (32 %), parietal (22 %) and frontal (20%) lobes. The PCNA index correlated with WHO grade (GII - index 4%, GIII - 10-20% and GIV - 40%). For grades I, II, III and IV progression free survival (PFS) was: 50.0, 39.0, 15.6 and 1.7 months respectively. The AgNOR count did not correlate with WHO grade, Daumas-Duport grade or PFS. BCL-2 expression was observed in single cells in GI and GII tumors, in 80 % and 40% of cells in GIII and GIV, respectively. The Daumas-Duport scale is simple and useful in prognosing. BCL-2 expression does not correlate in a linear manner with tumour grade. As gliomas are extremely heterogeneous, for better characterization of the clinico-pathologic correlations new prognostic factors are needed.

P-233

Schilder's type of multiple sclerosis in a mid-aged woman. A case report

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Introduction Schilder's type of myelinoclastic sclerosis is rare variant of multiple sclerosis in children and young adults. Usually, brain lesions occur in the white matter like solitary, or less often multifocal, acute plaques ranged in diameter more then 3 cm.
Case report The 49-year-old female patient was referred to our hospital with progressive right-sided hemiparesis, motor dysphasia and frontal lobe syndrome. Symptoms started 14 days earlier with acute headache and no febrile state was reported. MRI of the head revealed two oval lesions with hyperintensity in T2 weighted images of left frontal and parietal lobe. The first diagnostic impression was of glioblastoma multiforme. Results from standard blood and urine tests, tumor markers and cytological analysis of bone marrow were normal. Analysis of CSF and culturing CSF and serum for bacterial, viral, protozoan and fungal infections were negative. Analysis of CSF revealed increased immunoglobulins, 3OCB and increased intratecal synthesis of IgG. A stereotactic biopsy was performed one month after first symptoms. Neuropathology findings. The biopsy after neurosurgical treatment yielded five needle cores of grayish cerebral tissue. Hematoxylin and eosin-stained sections of paraffin embedded tissue showed demyelinating white cerebral tissue with relatively sharply defined margins. Lesion was infiltrated by macrophages (CD68 immunopositive cells and Luxol fast blue negative) and large reactive astrocytes with Ki67 and PCNA nuclear positivity. Around blood vessels were scattered perivascular CD4 and CD20 lymphocyte. Silver impregnation and NF immunostaining showed that the axons within the plaque are attenuated but not destroyed.
Conclusion Clinical and pathological findings suggested a demyelinating disease destroyed huge parts of white matter in before health mid-aged women, without previous infection or vaccination. Accordingly we diagnosed Schilder's type of MS.

P-234

Stereotactically guided brain biopsy: six years' experience of pathologic interpretations from Turkey

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Introduction Stereotactic biopsy is a widely used procedure in brain lesions. The aim of this study was to analyse the reasons of the discordance between the results of the intraoperative crush preparations (ICP) and final permanent paraffine preparations (FPPP) of stereotactically guided brain biopsies (SGBB) in our hospital, and find out the clinical benefits of this procedure.
Material and method Total 134 CT and/or MRI guided brain biopsies performed between 1995-2003 were analysed retrospectively. ICP and FPPP preparations were evaluated and discordant cases were detected. Then we evaluated the reasons of discrepancies.
Results The correct diagnosis was achieved by stereotactic biopsy in 91.8 % of the patients. Of them 107 were diagnosed as neoplasia and 16 were diagnosed as non-neoplastic pathology. There was discordance between ICP results and FPPP diagnosis in 8.2 % of the stereotactically diagnosed patients. The patients diagnosed as neoplasia were treated by RT and/or KT and the non-neoplastic pathologies were taken in suitable treatment and follow-up scheme.
Conclusion To improve the diagnostic accuracy of the SGBB, the following items should be taken into consideration: The quality of the ICP with giemsa staining is very important. In the biopsies from the lesions, which are suspected as neoplasia having, microscopic features such as gliosis, edema, hemorrhage must be interpreted as non-diagnostic. The number of the biopsies must be increased in the presence of necrosis. Epitheloid and pleomorphic cellular groups can mimic benign and malignant pathologies. Interpretation of the ICP and the FPPP should be done by the same pathologist.

P-235

Intraneural capillary haemangioma of spinal nerve root. Case report

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Intraneural haemangiomas of the spinal nerve roots are rare. The few cases reported in the literature are usually of the capillary and less often of the cavernous type. These lesions occur in adults, mainly males and the majority of them are located in the cauda equina. A case of intraneural capillary haemangioma involving one lumbar nerve root is reported. The patient, a 53 year old male, presented with a 1-year history of severe intermittent low back pain. Magnetic resonance imaging detected an intradural, extramedullar, space occupying mass at the level of L2-L3, causing displacement of cauda equina roots. Laminectomy and complete removal of the lesion were performed without neurological problems. On gross examination the resected specimen was a red-brown, well demarcated, lobular mass, measuring 1.8 cm in greatest diameter. Remnants of a nerve root were recognized at the periphery. Histological examination showed that the lesion was located within the endoneurium. It consisted of multiple small dilated capillaries, often showing incomplete lobular pattern, dispersed within normal nerve fascicles. The endothelial lining showed neither nuclear atypia nor mitotic activity. In the substrate mild edema and focal inflammatory lympocytic infiltrations were found. Haemangiomas of the spinal nerve roots often pose a challenging diagnostic problem, and knowledge of their existence is relevant since they can mimic other more frequent tumors of the region.

P-236

Macrocellular lung cancer with neuroendocrine differentiation associated with subacute sensorimotoric neuropathy/encephalomyelitis: an autopsy case

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Introduction Sensorimotoric polyneuropathy has rarely been reported as a presenting paraneoplastic neurological disorder associated with anti-Hu antibodies. Electrophysiological studies suggest a dysfunction of axon-Schwann cells, but the exact pathological mechanisms remain unclear.
Case report A 68-year old woman with short history of weight loss and parastesias in the legs was admitted to the hospital. Triparesis and dyspnea were found. MRI scan showed leukencephalopathy, EEG revealed diffuse encephalopathy. Sensorimotoric axonal polyneuropathy was present in EMG, and small lesion in the lung was found on the CT scan. Anti-Hu antibodies were present in the serum and in the cerebrspinal-fluid. Autopsy revealed macrocellular adenocarcinoma of the lung with neuroendocrine differentiation and sensorimotoric neuronopathy/ encephalomyelitis.
Methods The brain, spinal cord, sensory ganglia and a part of sural nerve were fixed in formalin, paraffin embedded and stained with H&E. Other part of sural nerve was prepared for immunofluorescence, semi-thin and ultra-thin sections.
Results Axonal degeneration without any inflammatory reaction and a very few signs of regeneration were found in the sural nerve. The inflammatory destruction of spinal ganglia neurons, axonal degeneration in dorsal columns and motor neurons inflammatory destruction in the spinal cord were found. Neuronal degeneration, glial proliferation, perivascular inflammatory infiltrates and some CD8+ lymphocytes surrounding single neurons were a common finding in the brain.
Conclusions This study shows that inflammatory destruction of the spinal ganglia neurons and motor neurons in the spinal cord was the main cause of clinically rapidly progressive sensorimotoric polyneuropathy in the anti-Hu positive patient with macro cellular lung carcinoma with neurendocrine differentiation.

P-237

Isolated extranodal spinal Rosai-Dorfman disease. A case report

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Introduction Sinus histiocytosis with massive lymphadenopathy was first described in 1969, by Rosai and Dorfman, as an idiopathic histiocytic disorder, which typically involves lymph nodes. Lately, involvement of many other organ systems has been documented, either alone or in association with nodal manifestations. Nowadays, Rosai-Dorfman disease is a well-recognised clinico-pathologic entity. Extranodal disease has been reported in 43% of the reported cases, occasionally representing the first and exclusive manifestation of the disease. Although central nervous system involvement has been well documented, the spinal canal is affected only exceptionally. We report a case of an isolated extranodal spinal Rosai-Dorfman disease with review of the literature.
Results The authors describe a 41 old woman with a history of spastic hemiparesis in which radiology disclosed a dural-based intramedullary spinal cord lesion at C1 level. The authors describe the histologic and immunohistologic features of the lesion and discuss the differential diagnosis.
Conclusion Isolated CNS Rosai-Dorfman disease without contemporary lymph node involvement can be a diagnostic challenge. Pathologists must be aware of this disease in order to make a correct diagnosis and avoid diagnosis of malignant histiocytic lesions.

P-238

Ezrin immunoreactivity: Is it a usefull marker for the prognosis and grading of astrocytoma?

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Introduction The actin- binding protein ezrin is supposed to increase the invasiveness of the malignant cells. In this study, the value of ezrin immunoreactivity (IR) was invastigated as a grading and prognostic parameter in astrocytomas.
Material and method Ezrin IR was studied in 13 low grade and 17 high grade astrocytomas. The mean age of the patients was 37.83 (4-75). Monoclonal ezrin antibody (MS-661-R7 Neomarkers) was applied on the sections of the paraffin-embedded tissues. Immunostaining intensity of ezrin was estimated as semi-quantitatively. When no staining was visible the score of 0, mild staining scored as 1, moderate 2, and severe staining scored as 3. Also positive stained areas were evaluated as percantage (25%, 25-50%, above 50% staining areas). Statistically Man-Whitney U, Kruskal Wallis and Spearman tests were used.
Results There was no correlation between prognosis and neither the grade nor the staining intensity of ezrin IR.
Conclusion There are controversial reports especially about the prognostic value of ezrin IR in different types of tumors. There is one report about the association of ezrin IR with the increasing malignancy of astrocytic tumors. Results of our study revealed no evidence of correlation between prognosis and ezrin IR for grading of astrocytomas. We conclude that ezrin is not a usefull factor for the prognosis and grading in astrocytomas.

P-239

Cystatin C depositon in blood vessels outside the central nervous system in hereditary cystatin C amyloid angiopathy

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Introduction Hereditary Cystatin C Amyloid Angiopathy (HCCAA), an autosomal hereditary disease uniquely found in Iceland, usually leads to early death of young adults from CNS vascular complications

following a disease progress of several years. Histopathological characteristics are cystatin C amyloid deposits in meningocerebral blood vessels, narrowing, splitting of media, segmental fibrinoid necrosis and microaneurysms of small arteries and arterioles. Associated changes: hemorrhages, infarctions and reparative reactions depending on length of the disease course. This study is a part of an investigation mapping the distribution of cystatin C immunopositive deposits outside the CNS in HCCAA.

Material and methods Formalin fixed, paraffin embedded autopsy material from 16 patients, 10 females and 6 males, was studied by conventional light microscopy (HE, Congo red; Thioflavin S) and immunohistochemistry (Avidin-Biotin-Peroxidase) with cystatin C antibodies (ABC-complex kit from Vector). For electron microscopy and specific identification of cystatin C deposits specimens were fixed in MacDowell's fixative and embedded in L.R. White resin and immunogold labelled (colloidal gold labelled protein A. Sigma).

Results and conclusion Cystatin C immunoreactive deposits were found in most organ systems outside CNS. The deposits were primarily located in the adventitia and perivascular tissue of small blood and lymphatic vessels and vascular smooth muscle; in subepithelial connective tissue of intestinal surface epithelium and glandular epithelium; in peripheral nerves. Cystatin C immunoreactive blood vessels were not obviously damaged as those in the CNS possibly reflecting environmental factors different in these two separate locations not yet identified and characterized. Details of cystatin C deposition in the vascular walls will be demonstrated.

P-240

Mononeuritis multiplex due to vasculitis in a patient with antiphospholipid syndrome

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Introduction Neurological disorders are among the most prominent clinical manifestations of antiphospholipid syndrome (APS). They are predominantly related to thrombo-occlusive vascular events in central nervous system, vasculitis playing little or no role.

Case report A 49-year-old female with a history of seven spontaneous abortions presented with sudden onset of pain and numbness in her right and later left foot followed by pruritic rash over her hands and back and livedo reticularis around knees. EMG showed signs of axson injury in multiple nerves. Laboratory tests showed lymphopenia and antiphospholipid antibodies of IgG class in medium titer at the begining and in low titer three months later. ANCA, ANA, ENA, anti-DNA were negative, platelet count, complement, lupus anticoagulant and tumor markers were normal. Sural nerve biopsy revealed active necrotizing arteritis of small epineural arteries. Intimal circular fibrinoid necrosis was accompanied by pyknosis, karyorexis and myofibroblast proliferation in media. Inflammatory infiltration by lymphocytes, macrophages, plasma cells and segmented leucocytes was present in intima, but was most prominent in adventitia. Thrombosis in organization was observed. Vasculitis was present also in arterioles and venules. Immunofluorescence showed scarce focal granular deposits of IgM, IgA class, and complement components C3 and C1q in media of small epineural arteries, together with abundant fibrin deposition.

Discussion and conclusion It has been suggested that APS can not be the cause of vasculitis and that vasculitis in APS is associated with an independent underlying disease, such as systemic lupus erythematosus. Except for suspected APS, our patient did not fulfill the criteria for any systemic autoimmune disease that could present with vasculitis.

P-241

Immunoautoradiographic characterization of serotonin transporter in human pineal gland

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The mechanism that regulates human pineal gland melatonin synthesis is not completely clear. In fact, several studies performed on animals demonstrated the possible role of serotonin and the involvement of its receptors 5-HT(2C) and 5-HT(1A) in the modulation of this process even if is not clear how it is realized. The aim of our study is to verify the presence of serotonin transporter (SERT) in the pinealocytes, a molecule involved in the reuptake of 5-HT from synaptic space, and to represent the action site of I.R.S. antidepressant drugs. We tested with immunoautoradiographic method (IARG) frozen slices conserved at –80 C^{o}, derived from twenty cadaveric epiphysis (11 men, 9 women) by utilizing a mouse monoclonal anti-SERT antibody followed by a secondary biotinilated anti-mouse IgG antibody, consecutively linked with an avidine/[3H]-biotine complex for a precise localization of the researched molecule. The tritium sensitive films were exposited to treated slices in special cassetes. After thirty days of exposure at – 80 C^{o}, the films have been developed and fixed to obtain auto radiographic images in which the radioactivity distribution followed a continuum pattern in sequential slices of each subject. It was the expression of SERT presence in epiphysis. This is the first study that demonstrate the presence of SERT in human epiphysis. We suppose that serotonin in human is not only a melatonin precursor synthesized in the pinealocytes but it is involved in the neuroendocrine regulatory mechanism of the gland.

P-242

Correlations of LOH on chromosome 10q and chosen clinico- morphological features in glioblastoma

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Loss of heterozygosity (LOH) studies allow to identify sites harbouring tumor suppressor genes involved in tumor initiation and progression. This study was performed to establish the frequency of LOH on chromosome 10q in glioblastoma. 30 cases of glioblastoma were examined (13 women,17 men; age: 33-75 years). Two cases were secondary glioblastoma. In all cases the

representative neoplastic tissue and normal brain tissue were cut out with lancet from respective paraffin blocks. DNA was isolated with proteinase K with phenol/ chloroform extraction and isopropanol precipitation. Polymorphic markers were localized close to three supressor genes PTEN (D10S1765, D10S215), LGI1 (D10S1680), DMBT1 (D10S587) which are known to be lost or mutated in glioblastoma. The fith marker was D10S607 10q22.2. Fragments were PCR-amplified and analysed using automated sequencer ABI1310. Signals from neoplastic and normal tissue were calculated. Result under 0,6 was scored as LOH. In 14 cases at least one marker from chromosome 10q was lost. 11 cases with LOH were men, both secondary glioblastomas showed deletion in 10q. The mean patients' age with and without 10qLOH was similar. 5 cases showed LOH only in PTEN locus. In 9 cases deletions were stated in two or more loci, what might suggest loss of longer part of chromosome 10. Cases with LOH in 10q revealed additional changes in different chromosomes: LOH in 1p (2 cases), 2p (2), 2q (1), 3p (2), 7q (1), 13q (1), 15q (2), 17p (1). Our results are similar to the other studies, showing changes in 10q in about 50% of glioblastoma.

P-243

Histopathological changes of the skin in systemic lupus erythematosus

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Introduction Morphologic examination of the skin is of great importance for the diagnosis of various nosologic forms of rheumatic diseases. The aim of the present study was to evaluate histopathologic changes in skin biopsies of patients with systemic lupus erythematosus (SLE).
Material and methods Skin biopsies of 30 patients (22 women and 8 men; age range from 15 to 55 years) with preliminary diagnosis of SLE, based on clinical data and laboratory analyses, were studied with the purpose to specify the differential diagnosis. Biosy tissue specimens were fixed in 4% neutral formalin, embedded in paraffin, and serial sections were stained with haematoxilin-eosin, picrofuxin, and the combined Rhitter-Olesson staining. After treatment of the specimens with 0.1 % trypsin, direct immunofluorescence technique was carried out for detection of immunoglobulins G and M at the dermo-epidermal junction (lupus band-test).
Results Our study established the presence of hyperceratosis with formation of keratic clogs, epidermal atrophy with vacuolization of basal cells, vasculitis, eosinophilic degeneration of collagen and an atrophy of appendages. Specific immunostaining for immunoglobulins G and M at the dermo-epidermal junction was observed in all except in 7 cases. We did not observe inflammatory cell infiltrates in dermis in any case.
Conclusion The pathogenesis of skin histologic lesions in SLE is most likely associated with immunological reactions and therapy carried out earlier.

P-244

Structural peculiarities of different nevuses and their prognostic value

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For the time being the causes of skin melanoma have not been clearly understood. Development of skin melanoma was related to preceding pigment formations in 18% to 85% of patients. Among a variety of investigation methods histologic one is basic to establish the final diagnostic. Complex morphologic study was carried out on 343 skin biopsies with different types of pigment nevuses and melanomas. Of them 106 biopsies were selected for the thorough pathomorphologic investigation. Light microscopy, electron microscopy and morphometric method were used. Serial sections for light microscopy were stained with hemotoxylin and eosine, picrofuchsin-fuchselin by Van-Gizon, combined staining by Riter and Oleson. The findings have shown that the rate of pigment skin neoplasms of all the biopsy material of a specialized institution came to 1,12% among neoplastic and 11,9% among non-neoplastic skin lesions. Pigment nevuses more frequently occurred in females aged 21 to 50 years applied for medical aid, while melanoma in ones above 60 years. Intradermal nevus was diagnosed in 86,9% of cases, its proliferative activity was expressed in 55% of patients. In 66,3% of patients skin melanona was assigned to 3-4th grade of invasion according Clark's classification. Each form of pigment nevus had morphometric peculiarities, significantly differing in dimensions of the cell, nucleus and their ratio. The most reliable parameter of malignization was the length of the nucleus and cell axial section circle. Of frequently occurred nevuses intraepidermal (borderline) one was more predisposed to malignization which was evident from significant increase of all morphometric parameters.

P-245

Cutaneous ciliated cyst of the lower limb

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Introduction Cutaneous ciliated cysts are a rare type of skin cyst, usually presenting themselves in women between the 2nd and 4th decades of life. Their origin is unknown and has been related to müllerian remnants based on similarities of the epithelium to the fallopian tube, but the occurrence of cases in men has given rise to the possibility of a sweat gland origin.
Case report A 31 year-old woman complained of an asymptomatic, longstanding, lesion located in the skin of the thigh. Once removed it was seen to correspond to cyst with clear fluid inside. Histopathological examination revealed a dermal cyst with ciliated cuboidal epithelium, usually double layered, but with some pseudostratified areas. Occasionally, papillary projections were seen in the lumen. There was no evidence of cellular atypia or mitotic activity. Immunohistochemical staining with oestrogen and progesterone receptors was strongly positive throughout the lesion.
Discussion and conclusions The clinical and histopathological findings fit in with the diagnosis of a typical cutaneous ciliated cyst of the lower limbs (cutaneous ciliated cyst). This is a rare cyst usually arising in the lower limbs of young women. Its origin is unknown and the demostration of sex-hormone receptors has been used to favour the müllerian origin. Nevertheless, some cases have been reported in men, which has diverted attention towards sweat glands as a possible origin of the cyst. The real incidence of the sex- hormone receptor if unknown, since it has not been clear in most published cases.

P-246

p53 and Ki-67 in non-neoplastic and neoplastic proliferative skin diseases

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Introduction p53 protein is essential for the regulation of cell proliferation and its aberrant accumulation is usually seen in malignant tumors but, also, occurs in squamous epithelium of inflammatory skin diseases characterized by hyperproliferation. The aim of this study is to elucidate the role of the p53 tumor-suppressor protein in the pathogenesis of different hyperproliferative, non-malignant and malignant skin diseases, and association between p53 overexpression and cell proliferation. We also investigated the influence of ageing on p53 and Ki-67 protein expression.
Material and methods Hundred and fifty skin specimens, consisting 30 cases of each, normal skin (NS), psoriatic skin (PS), keratoacanthomas (KA), basal cell carcinomas (BCC), squamous cell carcinomas (SCC) were examined immunohistochemicaly to assess p53 and Ki-67 protein expression.
Results p53 immunostaining of NS, PS, KA, BCC and SCC was positive in 39.0%, 46.7%, 66.7%, 80% and 86.7% cases, respectively. p53 and Ki-67 positive cells were present in basal (NS) and suprabasal layers (PS), and not only in cancer nests of KA, BCC and SCC, but also in dysplastic and even morphologically normal epidermis adjoining cancers. The positivity of p53 and Ki-67 protein differed significantly among the groups, with no differences in p53 expression between NS and PS, and in Ki-67 expression between PS and KA. Within all groups, there was a significant correlation between p53 and Ki-67 protein expression. Localisation and age were significantly related to p53 and Ki-67 expression in all groups, except for localisation in PS, and age in BCC.
Conclusions Our findings suggest that p53 overexpression occurs widely in neoplastic and non-neoplastic skin lesions. It is associated with the cell proliferation in normal, as well as, in changed epithelium. P53 accumulation is an age related process and significantly related to sun exposure, especially in NS and PS, as well as in KA and SCC. We also suggest that p53 overexpression begins in the early stage of carcinogenesis, before the appearance of malignancies in skin, and may be a useful predictor for the detection of nonmelanocytic skin cancer.

P-247

Maspin and beta catenin expression in relation to angiogenesis in basal cell carcinoma

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Background Maspin, a member of the serine proteinase inhibitor (serpin) family, has been recently pointed to as a tumor suppressor gene and angiogenic inhibitor. Objectives of this study were to investigate role of maspin and Beta-catenin in basal cell carcinoma (BCC).
Material and methods We studied immunohistochemically their expression in 30 basal cell carcinomas together with 5 trichoepitheliomas (TE) and 5 normal skin controls. Microvessel count (MVC) was also done using FVIII-related antigen. We examined the correlation of nuclear and/or cytoplasmic maspin expression with histological type, age, gender, invasion of safety margin, as well as stromal reaction (lymphocytic or fibroblastic. All primary antibodies (maspin, beta catenin and FVIII-related antigen) as well as detection Kit (ABC peroxidase/DAB Quick test), were purchased from Novocastra Labs.UK.
Results Maspin expression was strongly cytoplasmic and membranously expressed in TE and down-regulated in nodular BCC. Nuclear maspin expression was found in 43.3% of our cases. It was statistically positively correlated with invasion of safety margin ($p=0.014$) and with infiltrative type ($p=0.049$) and negatively correlated with a MVC >30 ($p= 0.046$). Cytoplasmic maspin was correlated to a strong fibroblastic stroma ($p=0.01$). Beta-catenin expression was found in both TE and BCC with the same intensity and was only correlated to younger age($p= 0.021$).
Conclusion Cytoplasmic maspin expression could differentiate between TE and nodular BCC Nuclear maspin expression could be related to aggressive behaviour of BCC and is realted to lower MVC (<30).

P-248

Trichilemmal carcinoma: report of two cases

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Introduction Trichilemmal carcinoma (TC) is rare skin tumour occurring in the sun-exposed areas (largely on the face or ears) of the elderly. Clinically, it may be mistaken for a squamous cell carcinoma, basal cell carcinoma, nodular melanoma or keratoacanthoma. Although the histological picture suggests a high-grade malignant neoplasm, TC has an indolent course, and conservative surgical excision with clear margins is curative. Aim: Histological and histochemical examination of rare cases of TC of the skin.
Materials and methods We report two patients (males) with TC on the ears, presented as slow-growing nodules. Tumour biopsy specimens were routinely fixed and processed. Deparaffinized sections were stained by HE, PAS, PAS-D.
Results Histologically tumour cells showed a solid and a lobular growth patterns in continuity with the epidermis, with foci of pilar-type keratinisation and peripheral palisading. Tumour nests were mostly composed of large atypical cells with clear cytoplasm containing PAS-positive, and diastase sensitive materials. These cases were treated only with surgical excision, and there has been no evidence since of local recurrence or metastasis.
Conclusion We believe that careful histopathological examination of pillar appendage tumours will help to classify the wide range of clinically and histologically different tumours and to establish the exact diagnosis.

P-249

HMB-45 and melan-A positive pigmented epidermotropic breast carcinoma metastasis

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Introduction Pigmented cutaneous metastases from breast carcinoma are uncommon and may simulate malignant melanoma both clinically and morphologically. We report a case of a 54-year-old female who developed multiple cutaneous pigmented metastases twelve years after radical mastectomy for left breast carcinoma.
Materials and methods Hematoxylin-eosin and immunohistochemically (streptavidin-biotin method) stained slides from three different regions of the chest skin and from the scalp plaque.
Results Numerous darkly pigmented plaques and nodules have been developed over a 8-month period on the back of the chest spreading anteriorly to the right subcostal region together with large, focally eroded scalp plaque. Histopathological examination revealed full thickness dermal infiltration and superficial subcutaneous fat involvement with cords and nests of neoplastic epithelial cells showing ductal structures and intracytoplasmic lumina in some areas. Irregular nests and single tumor cells at epidermodermal junction were accompanied with melanophages in papillary dermis. Immunohistochemically all tumor cells were cytokeratin 7 positive and cytokeratin 20 negative while epidermal and most superficial dermal nests showed HMB-45 and Melan-A reactivity as well. Scalp metastasis displayed less pronounced epidermotropism and only focal, single cell HMB-45 and Melan-A positivity.
Conclusion Epidermotropic breast carcinoma metastases may mimic malignant melanoma not just clinically and morphologically but also immunohistochemically and that might be related to the location of metastatic lesions.

P-250

Immunolocalization of lactoferrin in pigmented lesions of the skin

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Lactoferrin (Lf) expression has been immunohistochemically investigated in 55 formalin-fixed paraffin-embedded bioptic skin samples (23 nevi, 12 melanomas, 10 vulgaris or seborrhoic pigmented warts, 10 pigmented basal cell carcinoma; 10 specimens of normal skin were utilized as control. On 3µm thick sections, depigmentation and antigen retrieval procedures were performed. Lf immunoreactivity was revealed by a rabbit anti-human lactoferrin (Dako, Denmark, 1:300), incubating all sections at 4 C overnight; successively, bridging antibody and PAP complex for 30 min at room temperature (RT) were applied. For the demonstration of peroxidase activity, the sections were incubated with 3-3' diaminobenzidine tetrahydrochloride-H_2O_2 substrate solution for 10 min at RT in darkness; slides were then slightly counterstained with Mayer's haematoxylin. Quantification of Lf immunoreactivity was performed using the intensity-distribution (ID) score based on both the percentage and the staining intensity of positive neoplastic cells. An evident cytoplasmic immunoreactivity for Lf was encountered in melanocytes, either in nevi or in melanomas; no appreciable differences were found among junctional, intradermic or compound nevi as well as between spindle or epitheliod cells melanoma. Basal cell carcinomas were always unstained, even if some degree of positivity was found in areas which showed squamous differentiation; sheets and whorls of keratin also exhibited a strong immunoreactivity, when encountered in warts. If the Lf immunoexpression could represent the result of an endogenous synthesis by cells in order to have greater availability of iron for their metabolism or to modulate an unspecific inflammatory or anti-oxidant response remains to be fully understood.

P-251

Immunohistochemical expression of vascular endothelial growth factor (VEGF), c-kit and c-erb-B2 in cutaneous melanocytic lesions

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Introduction VEGF is a glycoprotein involved in angiogenesis, that promotes progression in several malignant neoplasms. The c-kit gene encodes a transmembrane receptor with tyrosine kinase activity, which plays a role in melanogenesis. Overexpression of c-erb-B2 oncoprotein has been related with poor prognosis in several malignant neoplasms. The aim of the present study has been to investigate the immunohistochemical expression of VEGF, c-kit and c-erb-B2 in cutaneous melanocytic lesions.
Material and methods We examined immunohistochemically 41 formalin-fixed, paraffin-embedded melanocytic lesions. These included 11 compound nevi, 12 dysplastic nevi and 18 melanomas. The antibodies anti-VEGF (Neomarkers), anti-Human c-kit (DAKO) and anti-c-erbB2 (DAKO) were used.
Results All compound and dysplastic nevi showed no immunoreactivity for VEGF. Strong cytoplasmic staining for VEGF was observed in 13/18 (72%) of melanomas, with higher levels in vertical growth phase. The junctional component in all compound nevi and in 8/12 (66.6%) dysplastic nevi was positive for c-kit (cytoplasmic staining with membrane accentuation). The dermal component was positive in 6/11 (54.5%) compound nevi, and in 4/12 (33.3%) dysplastic nevi. In melanomas c-kit expression was higher in radial growth phase (44%). No specific staining with c-erb-B2 antibody was noted in any of the cases examined.
Conclusion Assessment of VEGF expression might aid in the differential diagnosis between dysplastic nevi and melanomas. c-kit expression is not of diagnostic importance in melanoma, however its loss might play a role in tumor progression. c-erb-B2 overexpression is not involved in the pathogenesis of melanoma, and thus has no value as a target for specific therapy.

P-252

Apoptosis in cutaneous malignant melanomas

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Introduction Cutaneous melanomas were detected at an increasing rate worldwide. Even thought many patients are diagnosed at an early stage, the death rate continues to rise due to the increasing incidence of more advanced lesions. The aim of this

study is to detect in 30 cases of malignant melanomas.
Materials and methods Analysis were performed in 30 paraffin-embedded cases of cutaneous malignant melanomas (10 cases in situ and 20 cases invasive melanomas). Apoptotic index (AI) was determined using the in situ end-labeling technique(TUNEL), who quantify apoptotic cell death at single cell level and tissues.
Discussion and conclusion AI was high in the cases of in situ melanomas, but in invasive melanomas (nodular melanomas, superficial spreading melanomas with vertical growth phases, acral lentiginous melanomas etc) AI was decreased in tumor cells. We considered that usually apoptotic cells represent ~ 10% of tumor cells. At cases of cutaneous malignant melanomas in which AI was high (27%), frequently at in situ melanomas, the prognostic is good, when in the others cases in which apoptosis is missing or is very rare in cells (34%), the prognostic is reserved. This nonmorphologic technique to evaluate the prognosis of melanoma must be regarded as investigational and cannot yet be recommended for routine application. All necessary pathologic data, such as tumor location, size, depth, ulceration, mitotic activity and growth phase, can be obtained by examination of standard microscopic preparations of malignant melanoma as stained with hematoxylin and eosin.

P-253

Morphological and immunohistochemical findings in systemic small vessel vasculitis

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Introduction Small vessel vasculitis is a group of disorders of unknown etiology, possibly associated with connective tissue disease (CTD). The aim of this study is to find correlation between clinical, morphological and immunological changes in the skin and muscle biopsies of the patients with CTD, where small vessel vasculitis is the main finding.
Methods and results We analyzed 90 skin and muscle biopsies from patients with CTD: 20 dermatomyositis, 32 systemic sclerosis, 18 syndroma Raynaud, 5 vasculitis, 4 SLE, 1 polyarteritis nodosa. 70 were females, and 20 males. The age of the patients was between 3-78. Standard histological and histochemical stainings (HE, PAS, Van Gieson-elastica) were done. Small vessel vasculitis was found throughout skin and muscle biopsies, characterized with thickening of the basal lamina, tiny fibrinoid necrosis (if present), swollen endothelial cells and perivascular edema with lymfocytes. The muscles were necrotic with regeneration and fibrosis and reduction in the intramuscular capillaries. The changes were positive on PAS and elastic tissue staining. Direct immunofluorescence technique was done with antihuman IgG, IgM and IgA antibodies. Granular or linear immunofluorescent deposits were found at the dermo-epidermal junction zone and in the vessel walls. Antinuclear antibodies were done by indirect immunofluorescence technique using serum from same patients of which 80% were positive. Lymphoid cells around the vessels and in healthy muscle fibers were positive for markers for T cells (CD45RO and CD8).
Conclusion There is correlation between morphological and immunohistochemical changes. The presence of immunologic deposits in the vessels suggests possible autoimmune etiology.

P-254

Cutaneous angiomyolipoma

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Introduction Angiomyolipoma is a benign tumor almost exclusively seen in the kidney. Cutaneous Angiomyolipoma (AML) is a very rare benign vascular tumor that consists of a proliferation of blood vessels, smooth muscle bundles, and mature fat tissue. AML occurs more often in adult male patients and is often seen in acral locations.
Case report A 48-year-old man who had a 10-year history of a painless, solitary, noninvasive nodule on the right auricle lobule. The patient had no signs of tuberous sclerosis.
Material and methods The lesion was 3cm in diameter and clinically appeared to be a lipoma. The lesion was excised. Skin specimens was fixed in 10% buffered formalin, routinely processed, and embedded in paraffin. It was examined by routine light microscopy with histochemical and immunohistochemical stains.
Results On sections, the lesion was a well-circumscribed nodule in the dermis. There was fibrous pseudocapsule around it. The nodule was composed of an intimate mixture of mature adipose tissue, blood vessels, and bundles of smooth muscle cells. Combination of different types and sizes of vessels were particularly evident. Cellular pleomorphism, mitotic figures, and mucoid degeneration were absent. Reactivity for HMB-45 was negative. The histopathological diagnosis was angiomyolipoma.
Conclusion AMLs are very rare at extrarenal locations. In 1986, Argenyi presented the first report of cutaneous angiomyolipoma in the upper helix of the ear. The reported lesions were located at acral sites. As most cases of angiomyolipoma were long-standing lesions located at acral sites, and therefore exposed to external forces, some angiomyolipoma could be considered to be a degenerated with replacement by fatty tissues.

P-255

Primary cutaneus B-cell lymphoma. Experience in a comunity hospital

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Introduction The primary B-cell cutaneous lymphoma is a low frequent disease in a General Hospital. Its nodal equivalent has very different behaviour and prognosis. Aims: To study the incidence, behaviour and follow up of the B-cell cutaneous lymphomas in our hospital over a period of 9 years.
Material and methods In a total of 49116 cases between 1992 and 2002 we have 12 cases of B-cell cutaneous lymphoma. The skin biopsies were diagnosed acording EORTC classification. We did histological and immunohistochemistry studies. These included CD20, CD3, CD10, CD5, CD43, bcl-2 and Ki67.
Results Eith cases of 12 were SALT and 4 cases were folicular lymphomas. The ages are between 37 and 88 and is no sex predominance. SALT lymphomas were located two in the face and six in the trunk. All the cases of folicular lymphoma were in the face. The immunohistochemistry study in SALT lymphoma showed positivity for CD20 and CD43 and negative for CD3, CD5 and CD10. The expression of Ki67 was very low. The reactive foliculars centers were demostrated with the negativity of bcl-2.

The folicular lymphomas showed positivity for CD20 and CD10 and the number of positive cells for Ki67 was bigger.
Conclusions In a General Hospital in our country the incidence of B-cell cutaneous lymphoma is very low. The majority of the cases arrive with surgical excision, that is a good therapeutic approach. The general pathologist must be aware with reactive limphoid infiltrates because the majority of the cutaneous lymphomas are low grade neoplasies.

P-256

CD30 expression in inflammatory lymphoid cells in scabies

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Background CD30 expression is a distinct feature of both B and T cell activation. The presence of CD30+ large atypical lymphoma cells in a cutaneous infiltrate has been considered a characteristic feature of lymphomatoid papulosis/cutaneous CD30+. Recently, however, CD30 expression has also been described in some nonneoplastic infiltrates of de skin.
Design Twenty skin biopsy specimens from 20 patients with scabies were evaluated. Of these, the lesions had been present for more than 3 months in 12 cases. The diagnosis of scabies was based on the presence of characteristic clinical features and the demostrations of Sarcoptes scabiei mite. For each biopsy, a panel of histopathological and inmmunophenotypic features was independently assesed by two pathologists. CD3, CD20, CD8, CD30 and S-100 protein expression was evaluated in each case.
Results CD30+ cells, either in isolation or in small clusters, were seen in 12 biopsy specimens (12/20; 60%). CD30+ cell clusters were located in the papillary and superficial reticular dermis and tended to show a perivascular pattern. All 12 biopsy specimens obtained from long-standing (>3 months) scabies lesions demonstrated the presence of CD30+ cells. In contrast, no CD30 expression was observed in lesions of less than 2 months duration.
Conclusions CD30+ large cells can be detected in skin inflammatory lymphoid infiltrates associated with scabies, particularly in long-standing (>3 months) lesions. Cutaneous CD30 expresion, therefore, is not an exclusive feature of neoplastic lymphoid proliferations. Probably as the result of persistent antigenic stimulation, CD30 expression can also be detected in activated lymphoid cells of nonneoplastic infiltrates associated with insect bites, viral and bacterial infections and peculiar drug reactions.

P-257

Proliferative and apoptotic markers in cutaneous melanocytic lesions

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Introduction Malignant transformation of melanocytes is a multistep process characterized by distinct histopathological stages. Differentiation between naevi, especially dysplastic naevi and melanoma, can sometimes be difficult to assess by conventional histopathological analysis. The aim of this study was to analyse the differences in proliferative activity and apoptosis between intradermal naevi (IN), dysplastic naevi (DN) and cutaneous melanoma (CM).
Materials and methods Fifteen IN, 15 DN and 15 CM (thickness less than 1.5 mm) were immunohistochemically stained for Ki-67 which was used as a proliferative marker, whereas bcl-2 and p53 were used, respectively, as an anti-apoptotic and apoptotic marker. The number of positive cells were counted on 200 melanocytes. Statistical analysis was done using the Spearman and the Chi-square test.
Results The mean value of Ki-67 positive cells in IN, DN and CM 7 was 7.6, 8.3, and 31.7, respectively. Bcl-2 was expressed in 120.6 IN, 148.0 DN, and 178.1 CM cells while p53 overexpression was present in 14.6 IN, 151.0 DN and 85.3 CM cells. Statistical analysis showed a signifficant difference in proliferative activity and the overexpression of p53 ($p < 0.05$), while observed differences in bcl-2 expression were not statistically significant between these three groups.
Conclusion Proliferative and antiapoptotic activity showed a progressive growth from benign to malignant melanocytic lesions. The strongest expression of p53 in DN suggests that its overexpression could be the first step in the loss of cell cycle regulation and possible malignant transformation of melanocytes.

P-258

Myofibroblastic transdifferentiation of mesangial and interstitial cells as prognostic criteria of glomerulonephritis in kidney biopsy analysis

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Introduction It is now known that myofibroblastic transdifferentiation in glomerulus takes place during mesangial activation.
Material and methods We analyzed functional disorders (filtration, concentration insufficiency) and kidney biopsies of 82 patients aged from 15 to 62 years with various forms of glomerulonephritis. We used standard histological, immunohistochemical, immunofluorescence methods as well as electron microscopy in our research. For studying myofibroblastic transdifferentiation we stained tissue immunohistochemically for alpha-smooth muscle actin (alpha-SMA) and then carried out a quantitative analysis of the results.
Results Seventy patients had myofibroblasts in kidneys. We found out that level of alpha-SMA expression correlates with decrease of glomerular filtration. While disorders of concentration a lot of myofibroblasts appear in kidney interstitium. But we have not found any correlation between clinical forms of glomerulonephritis and those cell growth we had seen. And we should mention that for 12 patients without glomerular or interstitial fibrosis we didn't reveal any changes in cells phenotype.
Conclusion So we conclude that myofibroblastic transdifferentiation must be considered as an early symptom of kidney sclerosis and can be used as a prognostic criterion.

P-259

Glomerular pathology in 100 school children with abnormal urinalysis detected by mass urine screen in Taiwan

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Introduction To early detect renal and metabolic diseases, a mass urine screen in elementary and high school students was started in 1990 in Taiwan. We retrospectively reviewed the nephropathologic features to elucidate its value.
Materials and methods One hundred students, 43 boys and 57 girls, who were found to have hematuria and/or proteinuria by this urine screen program and received renal biopsy, formed the basis of this study. The glomerular pathology was divided according to disease severity into two groups.
Results Group I diseases (58%) showed minor histologic alterations, including minimal glomerular change in 40 cases and mesangial cell proliferation in 18. Among them, 42 children had electron microscopic examination, 24 (57%) had thin glomerular basement membrane (TGBM) disease. Group II diseases (42%) had more severe glomerular changes, including IgA nephropathy in 19 cases, membranous nephropathy (MN) in 12, lupus nephritis in 4, focal sclerosis in 2, chronic glomerulonephritis in 3, and type I and type II membranoproliferative glomerulonephritis in one case each. These findings confirm that glomerular diseases often progress silently. We found a high frequency (74%) of IgA nephropathy and MN in group II diseases and a close association of MN with hepatitis B virus infection.
Conclusion We conclude that mass urine screen in school children and renal biopsy are very useful for the early detection of the progressing glomerular diseases and provide great opportunity for early therapeutic intervention and long-term follow-up to better understand the natural courses of glomerular diseases, including TGBM disease.

P-260

Kidney biopsy study in patients with nephrotic syndrome

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Introduction The purpose of the study was to establish a correlation between morphometric kidney biopsy parameters and routine laboratory findings in patients with nephrotic syndrome.
Material and methods 104 kidney biopsies of 58 patients with nephrotic syndrome were studied using standard techniques of paraffin sections stained with haematoxylin and eosin, picrofuxin van Gieson and PAS reaction. Direct immunofluorescence on snap frozen sections was performed using commercially available antibodies against IgA, IgM and IgG.
Results and conclusion Of the 58 patients, 31 (53.0%) were men and 27 (47.0%) women, with an average age of 24.9±5.2 years. Biopsy study revealed different types of chronic glomerulonephritis of various incidence: membranous GN (14), mesangioproliferative GN (11), mesangiocapillary GN (4), focal segmental glomerulosclerosis (2) and terminal sclerosing GN (4). Morphometric analysis using the semi-automatic image analyser »Integral-2MT« included cross-section surface areas of the glomeruli and glomerular capillaries, the numerical density of total number of cells per glomerulus, numerical density of mesangial cells, cross-section surface area and numerical cells density of the proximal and distal tubules and cross-section surface area of the peritubular capillaries. Routine clinical laboratory analysis included the level of proteinuria, serum creatinine and creatinine clearance, erythrocyturia, leukocyturia and cylinders in urine. A correlation of clinical laboratory parameters with mathematical morphometric models was established for each type of GN associated with nephrotic syndrome.

P-261

The role of proliferation, growth arrest and apoptosis in the carcinogenesis and prognosis of renal cell carcinoma

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Introduction Nuclear grade and stage are insufficient to predict the clinical behaviour of renal cell carcinoma (RCC). Therefore additional prognostic factors are needed. In this study we investigated the role of proliferation, growth arrest and apoptosis in the carcinogenesis and prognosis of RCC using Ki67, Cyclin A, Bax, P53 and Bcl-2.
Material and methods Formalin-fixed paraffin embedded tissue blocks from 30 patients were studied. Cases were divided into low/high grade and low/high stage. Apoptotic index (AI) was evaluated as morphologically in H&E stained sections. Proliferation index (PI) was detected by using Ki67 and Cyclin A immunostaining. AI, PI, P53, Bcl-2 and Bax expressions in tumor tissue (TT) and in adjacent non-tumoral tissue (ANT) and associations with clinical and histopathological parameters were analysed statistically.
Results The mean indices for apoptosis, Ki67 and Cyclin A were: 5.10±3.86 (0-16), 15.47±17.78 (0-63) and 19.50±26.21 (0-108) in TT as well as 4.93±3.74 (0-15), 0 and 4.13±3.28 (0-12) in ANT, respectively. The number of cases in which the expression of P53, Bax and Bcl-2 were detected was: 14 (46.7%), 19 (63.3%), 13 (43,3%) in TT, and 0 (0%), 16 (53,3%), 18 (60%) in ANT, respectively. Statistically, there was a significant relationship between the values of TT and ANT for AI but not for the PI and the other parameters. Only AI and PI significantly correlated with the grade.
Conclusion In this study the role of PI as a prognostic indicator in RCC was again confirmed. Our observations suggest that apoptosis, Cyclin A, Bcl-2 and Bax may have roles in the carcinogenesis of RCC and that AI may be used as a prognostic indicator.

P-262

Immunophenotypisation of amyloid deposits in kidney biopsies - Our experience

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Introduction During the renal biopsy analyses, the presence of amorphous eosinophilic material in the mesangium, along the glomerular basal membrane and Bowman membrane, interstitial peritubular tissue, and in the walls of the arterial blood vessels is recognized as amyloid. This picture requires a panel of further stainings, both histochemical and immunohistochemical to prove the existence and possibly determine the nature of the amyloid. The reason for that is the different chemical composition of the amyloid in different underlying diseases. We reviewed a total of 7 proven cases of renal amyloidosis during the last 18 months.
Material and methods The tissue specimens went through standard histological procedure for paraffin embedded tissue. The obtained serial cuts were stained with Haematoxylin-eosin, Trichrome Goldner, Silvermethenamine Jones, PAS and Congo red. Histochemical stainings for Congo red were viewed under polarized light, and when found positive, immunohistochemical stainings for AA amyloid, beta 2 microglobulin, kappa and lambda light chains were done. We used the LSAB immunoperoxidase technique.
Results and conclusion Due to the immunophenotypisation, we classified three of the cases as primary amyloidosis (light chain positivity of the amyloid deposits) and the rest four were positive for AA amyloid staining. The history of these four patients revealed collagenoses as underlying diseases in three cases. The most interesting and still unanswered remained the fourth case where the clinical history revealed existence of two possible underlying diseases: renal cell carcinoma and inflammatory bowel disease. Both are well known to be effective stimulators of amyloid deposition.

P-263

The role of matrix metalloproteinase-11 protein in various types of glomerulonephritis

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Introduction Matrix metalloproteinases (MMPs) have been implicated in a number of developmental and pathological processes such as inflammation. In human glomeruli, the mesangial matrix turnover is controlled by a dynamic equilibrium between synthesis and degradation to which MMPs are known to contribute. MMP-11 was originally discovered as a gene whose expression was associated with tissue remodelling. The aim of this study was to investigate whether MMP-11 protein is involved in the pathogenesis of various types of glomerulonephritis (GN).
Materials and methods Using standard immunohistochemistry, we analysed MMP-11 expression (clone 5ST-4A9) in renal biopsies from 95 patients with primary GN (n=44) and secondary, either lupus-associated GN (n=21) or GN due to active Wegener's granulomatosis (n=6) or pauci-immune, ANCA-associated GN due to small vessel vasculitis (n=24). The examined cases were divided in two groups (proliferative and non-proliferative).
Results The highest incidence of MMP-11 immunopositivity (26.3%) was noticed in GNs associated with microscopic polyangiitis and Wegener's granulomatosis. Generally, MMP-11 was often expressed in segmental areas of sclerosis, microadhesions, cellular and fibrocellular crescents. Fibrotic crescents and fibrotic glomeruli were constantly MMP-11-immunonegative.
Conclusions According to the above observations, MMP-11, as an inflammatory mediator, may exert a chemotactic influence on macrophages, which aggregate in the mesangium and induce early fibrosis while it may have a parallel mitogenic effect on epithelial cells, which, in association with macrophages, form cellular crescents. MMP-11 appears to have no antifibrotic role in GN.

P-264

Investigation of diffuse mesangial proliferative glomerulonephritis in children

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Introduction Pure diffuse mesangial proliferative glomerulonephritis (MEPGN), although one of the basic light microscopic (LM) structural glomerular lesions, is morphologically highly nonspecific, and additional investigations are needed to reach the final diagnosis (dg) of disease. We studied the value of immunofluorescence (IF) and electron microscopy (EM) for dg, and which proportion of cases represents an idiopathic form of disease.
Material and methods We reviewed clinical picture, IF and EM findings in 154 children with MEPGN in LM.
Results 50 presented as nephrotic syndrome (NS), 67 as proteinuria and/or microhematuria (P/MH), 10 as recurrent hematuria (RH), 20 cases were clinically identified as purpura (P) and 7 as SLE. IF revealed 22 cases of Berger disease, while in 105 cases IF was negative or non-specific (46 NS, 55 P/MH, 4 RH). EM revealed 7 cases of thin basement membrane disease, all with MH, and 33 with GBM lesions consistent with Alport syndrome (29 P/MH, 2 RH, 2 NS). 21 patients showed EM features of resolving stage of acute GN, all with P/MH, while residual 93 showed MEPGN with or without mesangial deposits (42 NS, 22 P/MH, 2 RH, 20 P, 7 SLE).
Conclusion By means of clinical data, IF and EM, it was possible to reach definite dg in 110 (71.4 %) cases. Among residual 28,6 % with negative/non-specific IF and MEPGN in EM, there were 36 cases of NS belonging to idiopathic but clinically well-defined childhood nephrosis. So, only 8 cases represented a truly non-defined category of MEPGN (5.2 % of all cases).

P-265

Evaluation of the renal biopsy importance in isolated microhematuria in children

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Introduction Isolated microscopic hematuria (IMH) in children always raises the question of the necessity of renal biopsy. Many authors consider IMH as minor abnormality where glomerular changes are not expected, but general agreement has not yet been achieved. The aim of this study was to evaluate contribution of renal biopsy to diagnosis of glomerular disease in IMH.
Material and methods Biopsy was performed in 54 children with IMH of proven glomerular origin. Mean duration of IMH prior to biopsy was 2.8 years. Biopsy specimens were examined by light (LM), immunofluorescence (IF) and electron microscopy (EM).
Results 43 patients (79.6%) were found to have glomerular abnormalities. On LM 18 patients had normal glomeruli (NG), 22 mesangial proliferative glomerulonephritis (MEPGN), 9 focal segmental glomerulosclerosis (FGS) 3 focal glomerulonephritis (FGN) and 2 membranoproliferative glomerulonephritis (MPGN). IF revealed 10 cases as IgA nephropathy. EM discovered GBM changes consistent with Alport syndrome in 21 patients and diffuse thinning of GBM in 10. In 5 children, subepithelial hump-like deposits were found and considered as the sign of acute postinfectious glomerulonephritis in resolution. One of 2 cases of MPGN showed to be type II (DDD). On follow-up, 6 of 21 children with Alport-like GBM changes developed full clinical picture of the syndrome. Further surveillance is needed to confirm the significance of EM findings in others.
Conclusion In children with IMH renal biopsy is justified and should always be analyzed by light, immunofluorescence and especially electron microscopy, because EM was decisive for final diagnosis in about 66% of all cases.

P-266

Mesoblastic nephroma of adult. Report of a case

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Introduction Mesoblastic nephroma is a very uncommon tumor in adults, similar to the congenital mesoblastic nephroma of infancy, first described by Bolande et al. Microscopically the tumor consist in uniform spindle cells proliferations with numerous vascular spaces, nest of glomeruli, renal tubules that often cystic and dysplastic and lack of nuclear anaplasia or mitotic activity.
Case report A 69-year-old woman was admitted to the emergency services for acute abdominal pain in September 1995. She was diagnosed with acute pancreatitis. In CAT (without urologic symptoms) a solid mass in the upper pole of the right kidney was founded. With a clinical diagnosis of renal cell carcinoma a radical nefrectomy was performed. Follow-up was without event and had no recurrence 8 years afterwards. The right kidney weighed 228 grs. and was unremarkable except for a 3 cms-diameter circumscribed, yellow-tan, solid tumor in the upper pole. Microscopic examination revealed a tumor composed of spindle cells, mostly smooth-muscle cells and fibroblast, with numerous vascular structures and some areas of renal tubules with clear cytoplasm. In some places there was atrophic tubules. No mitotic figures were identified. Immunohistochemistry findings: The majority of spindle cells were positive for actin, desmin and smooth-muscle actin, and vimentin. And the tubular epithelium stain for EMA and keratins.
Conclusion A combination of histological and immunohistochemical findings is useful to distinguish mesoblastic nephroma from other tumor of the kidney with spindle cells.

P-267

Expression of cyclin D1 in secondary parathyroid hyperplasia

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Introduction Hyperparathyroidism occurs in patients with chronic renal failure and is usually associated with multiglandular parathyroid hyperplasia. Clonal analysis has suggested that in renal hyperparathyroidism glands initially grow diffusely and polyclonally. Mechanism of monoclonal proliferation observed with appearance of nodular hyperplasia is not well understood. It might be induced by certain genetic disorders such as overexpression of PRAD/cyclin D1 induced by DNA rearrangement of the parathyroid hormone gene. The aim of this study was to analyse the expression of cyclin D1 in normal and hyperplastic, diffuse and nodular parathyroid glands in order to ascertain the possible difference.
Materials and methods 140 hyperplastic parathyroid glands, from 46 uremic patients who underwent surgery during the period 1977-2003, were analysed. Immunohistochemistry for cyclin D1 protein was performed on 10 normal parathyroid glands and 47 selected hyperplastic, 14 of diffuse and 33 of nodular type. Overexpression of cyclin D1 was considered to be present if 40 % or more of the sample showed positive nuclear staining.
Results In normal glands, the mean value of nuclear positivity was 19.94%. Overexpression for cyclin D1 was present in 11/15 (78%) diffuse and 27/33 (82%) nodular hyperplastic glands. No significant difference was found.
Conclusion In comparison to normal, hyperplastic glands (nodular as well as diffuse) overexpress cyclin D1 protein. These results indicate that overexpression of cyclin D1 is not limited only to neoplastic proliferation, as the most studies showed, respectively this genetic abnormality is not the only responsible mechanism for the monoclonal proliferation.

P-268

Proteins of extracellluar matrix as predictors of glomerulonephritis outcome

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Introduction Chronic renal insufficiency develops only in those cases of glomerulonephritis in which the lumina of postglomerular

capillaries in the renal cortex are narrowed due to expansion of the extracellular matrix (ECM). Aim of the study was to compare the composition of interstitial ECM proteins with clinical data in various forms of primary glomerulonephritis (GN).

Material and methods We examined renal biopsies from 55 GN patients, while normal renal tissue was obtained from nephrectomy due to small renal tumour. Specimens fixed in B5 and/or Dubosque fixative were analysed by immunohistochemistry (APPAP-method) using a monoclonal antibodies against tenascin (TN), fibronectin (FN), collagen I, III and IV (COI, COIII, COIV) (DAKO). The same fixatives were used for control renal tissue. Evaluation of fibrin was performed on frozen sections by immunofluorescence. Clinical data were: proteinuria and serum creatinine level, obtained by standard laboratory methods, immunotherapy and ACE-genotype.

Results Fifty patients had proteinuria at time of biopsy and 33 of them continued to have it during the follow-up period. Patients with persistent proteinuria had more often interstitial fibrosis with elevated content of COI and COIV. Only 10% of patients who responded well to immunotherapy had deposits of fibrin. There were 12 patients with elevated creatinine at biopsy with similar composition of ECM as patients with proteinuria. Majority of patients with interstitial fibrosis had ACE-genotype II although this genotype wasn't the most common in the cohort.

Conclusion Our study showed that persistent proteinuria could be linked to interstitial fibrosis and fibrin deposits. Patients with II ACE-genotype are more prone to develop interstitial fibrosis.

P-269

Haemorrhagic fever with renal syndrome in Slovenia from 1983 to 2002: clinical aspects and clinical-pathological correlations

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Introduction Haemorrhagic fever with renal syndrome (HFRS) in Slovenia is caused by two hantavirus agents - Puumala and Dobrava serotypes. There is still a lack of clinical and pathological information, especially about the Dobrava virus associated disease. The aim of this study was to analyse and compare the clinical course, outcome and pathological features of HFRS caused by these two virus serotypes.

Material and methods The clinical course of 41 patients (6 women) aged 32±12 years (range 16-71) treated in the nephrological department for serologically confirmed hantavirus associated HFRS was analysed, a serological diagnosis was made using immunofluorescence test and enzyme-linked immunosorbent assay. Kidney biopsy specimens of 18 patients were analysed by light microscopy, immunofluorescence and electron microscopic techniques. A semiquantitative scale for gradation of cumulative tubulointerstitial lesions and interstitial haemorrhagic lesions was applied (scores 0-4).

Results Seventeen patients (41.5%) had Puumala and 24 (58.5%) Dobrava virus infection. All patients were febrile and had proteinuria, and other clinical features were: nausea and vomiting (85% of patients), oliguria (73%), abdominal pain (61%), headache (61%), haemorrhages (54%), myalgias (44%), diarrhea (41%), neurological and ophthalmological abnormalities (34%), hypotension (12%). Pancreatitis (12%) and elevated transaminases (7%) were observed only in patients with the Dobrava serotype. None of the patients in this study died and there was none with permanent haemodialysis dependence. Comparison of clinical and pathological features of Dobrava and Puumala diseases is given in the table.

Clinical and histopathologic features	Dobrava serotype (24 patients)	Puumala serotype (17 patients)	P
Age	34±13 years	31±10 years	0.34
Elevated creatinine at discharge	64%	47%	0.3
Haemodialysis needed	79%	29%	0.001
Oliguria	83%	59%	0.04
Hypotension	4.2%	24%	0.07
Haemorrhages	63%	41%	0.13
Proteinuria (semiquantitave)	2.6	2.5	0.86
Tubulointerstitial lesion score	2.9±0.9	3±0.5	0.78
Haemorrhagic interstitial score	2±1.4	2.5±1.2	0.44

A need for haemodialysis and the creatinine level at discharge were not associated with the age of patients. Those patients who needed haemodialysis, had clinical haemorrhages or had elevated creatinine at discharge, also had higher haemorrhagic and cumulative tubulointerstitial lesion scores, but differences were not significant. Elevated serum creatinine above basal levels was present at discharge in 22 patients (54%), range 83-554 μmol/L and some patients remained with chronic renal insufficiency.

Conclusion Our results show that Dobrava virus infection is associated not only with a significant mortality, comparable to Hantaan virus infection, but also with a worse clinical course of renal disease than Puumala. The histopathology of the kidney in Dobrava and Puumala is characterised by haemorrhagic necrotising tubulointerstitial nephritis involving particularly the outer medulla, with quantitative and not qualitative differences between these two infections.

P-270

Renal parenchymal malakoplakia after kidney-pancreas transplantation. Case report

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Introduction Malakoplakia is an unusual chronic inflammatory disease rarely involving parenchyma of the transplanted kidney. It represents an unusual host response, probably due to leucocyte bactericidal defects to an infecting agent, usually gram-negative organisms, of which Escherichia coli is the commonest. Malakoplakia in renal transplant is very rare (to date only six cases have been reported) and is associated with a high rate of graft loss and significant mortality.

Case report We report a 31-year-old female recipient of kidney-pancreas allografts immunosupressed with Tacrolimus (Prograf, 2x3mg) and Mycophenolate Mofetil (CellCept, 3x500mg). The patient had suffered from recurrent urinary tract infections. Three years after transplantation she was admitted to the hospital with decreased kidney graft function. Percutaneous kidney needle biopsy was performed and diagnosis of malakoplakia was established. Histologically, on the inflammatory background many Mi-

chaelis and Gutmann bodies were present and confirmed ultrastructurally. The patient was treated with antibiotics and simultaneously immunosuppression (Tacrolimus) was reduced. Gradually, her renal function improved (with serum creatinine of 105 umol/l). For the entire following year, the patient has been monitored regularly and her renal and pancreas function are stable and both her allografts remain in situ.

P-271

Morphometric study of transbronchial biopsies in pulmonary transplantation

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Introduction The transbronchial lung biopsy (TBB) is considered as the 'gold standard' for diagnosis of acute rejection. It has been pointed out that diagnostic utility depends mainly on the number of fragments (NF), but no other biopsy parameters have been studied. Aims: Morphometric study of TBB in frequent pulmonary transplantation pathologies, to determine differences between diverse parameters of the biopsies to reach a diagnosis.
Material and methods From 1997 to 2002, 139 lung transplantations were carried out in our institution and 158 consecutive TBB were performed for clinical indication with histologic diagnosis of: 1) acute rejection (AR) (n=65) classified according to the Working Formulation, 2) infection or highly suggestive of infection (INF) (n=49) and 3) non-diagnostic biopsy (ND) (n=44). In these biopsies we measured five parameters. We counted the number of fragments (NF), number of fragments with alveolated parenchyma (NFAP) and number of vessels (NV) on the hematoxilin-eosin slides. The microscopic images of each case were digitalized and captured to the calculated total area (TA) and the alveolated area (AA) that are expressed in mm^2.
Results See table

	NF	NFAP	NV	TA	AA
AR	3.04±1.57	2.44±1.44*	7.96±4.75**	8.76±4.20	5.75±3.92**
INF	3.24±1.66	1.73±1.22	4.91±4.74	7.29±4.73	3.36±3.47
ND	2.81±1.14	1.79±1.13	4.75±3.97	5.95±4.22**	2.63±2.77

Levels of statistical significance: * $p<0.05$, ** $p<0.005$
Conclusion In our series of TBB for transplant pathology, the AA and NV are the most important morphometric parameters of the biopsy to reach the diagnosis of AR. Conversely, the NF does not show significant statistical difference between diagnostic biopsies and ND biopsies.

P-272

The importance of glomerular deposits of von Willebrand factor in human renal allografts

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Aim To evaluate the role and prognostic importance of glomerular endothelial cell alteration in human renal allografts.
Material and methods We examined 72 patients with renal transplantation among whom 40 showed acute rejection (AR) and 32 chronic rejection (CR). Biopsies were immunostained for Von Willebrand factor (vWF), fibronectin (FN) and CD68. Glomerular staining for vWF and FN was graded in a semiquantitative manner using the 1-3+ scale.
Results In the CR group there was markedly increased expression of vWF and FN in the glomeruli, whereas only 8 of 40 AR cases showed intense (grade 3) glomerular vWF (GvWF). A significant difference was found between patients with AR and CR with regard to GvWF ($p<0.01$). A positive correlation between the degree of GvWF and early interstitial fibrosis was found in cases with AR in follow-up biopsies ($p<0.05$). The presence of transplant glomerulopathy was found significantly earlier in cases with grade 3 GvWF in AR group ($p<0.01$). We found strong correlation of both intraglomerular FN expression and macrophage infiltration with the degree of GvWF both in AR and CR groups ($p<0.05$). The outcome of grafts that showed intense (grade 3) GvWF was significantly worse than the outcome of those grafts with grade 1 and 2 glomerular vWF during the follow-up ($p<0.001$).
Conclusion Increased GvWF deposition in cases with AR is in risk of early transplant glomerulopathy, early interstitial fibrosis and early graft loss. In conclusion, it may be beneficial to use new therapeutic approaches such as anticoagulant medicine for the treatment of AR and CR in future.

P-273

The role of microvascular injury on steroid and OKT3 response in renal allograft rejection

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Aim Our aim is to understand the influence of HLA-DR positive microvascular (MV-DR) injury on the steroid and OKT3 response in renal allografts with acute rejection (AR).
Material and methods We examined 94 biopsies of 40 patients. Of 40 cases, 20 showed steroid resistant AR (group 1) and received OKT3 treatment. Remaining 20 cases showed steroid respondent AR (group 2). Biopsies were immunostained with HLA-DR, fibronectin (FN), CD-68, CD-3 and Factor VIII-RA.
Results The degree of MV-DR expression was decreased with increasing AR grade ($p<0.01$). There was a negative correlation between MV-DR expression and the degree of interstitial mononuclear cell infiltration ($p<0.001$). As the severity of MV-DR destruction increased, the response to steroid and OKT3 therapy declined ($p<0.05$). In group 2 severe MV-DR injury was found only in 15% of cases (3/20 cases), whereas severe MV-DR injury was found in 55% of cases (11/20 cases) in group 1 ($p<0.01$). After OKT3 treatment 70 % of cases in group 1 responded to therapy. Remaining 6/20 cases (30%) that had no response to OKT3 showed severe MV injury. Only 45.5% (5/11 cases) of 11 cases with severe MV-DR injury showed response to OKT3 therapy. The follow-up biopsies of all cases with severe MV-DR injury showed significant interstitial fibrosis and FN expression ($p<0.01$). The mean time between transplantation and diffuse interstitial fibrosis was found to be significantly shorter in cases with severe MV injury ($p<0.01$).
Conclusion The destruction of MV-DR could contribute to severe inflammatory reactions and subsequently to poor steroid response, early interstitial fibrosis and graft dysfunction.

P-274

Acute rejection in renal allografts: possible role for hepatocyte growth factor

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Introduction Acute renal allograft rejection is the most important threat to graft survival, and must be diagnosed and treated as early as possible. Clinical, radiological and histopathological confirmation of the diagnosis is essential. Several factors are being investigated as potential diagnostic markers for acute rejection (AR), and one of these is the hepatocyte growth factor (HGF). The aim of this study was to investigate the presence/density of HGF in core needle biopsies from renal allografts with AR using immunohistochemical staining.
Material and methods The study involved 84 cases of kidney recipients whose allografts were investigated for an initial AR episode without any other pathology. All the patients received cyclosporine-A anti-rejection treatment. Each archived slide was re-examined according to the modified Banff 97 classification, and patients were categorized as type I AR (Group 1; n=66), type II AR (Group 2; n=16) and type III AR (Group 3; n=2). Normal histopathological findings in graft biopsies were obtained as controls (Group 0; n=15). The clinical findings of patients were reviewed from archived records. The sections were stained immunohistochemically with monoclonal anti-HGF antibody. The mesenchymal cells (interstitial macrophages and glomerular endothelium) that showed cytoplasmic positivity were then counted using ocular micrometer. The results were statistically analyzed using Mann-Whitney U test.
Results On analysis of the groups' mean cell count, the only significant finding was higher concentration of HGF in the AR patient group than in the control group ($p<0.001$). Concerning all other parameters studied, the only significant differences were higher blood urea nitrogen and creatinine levels in Group 2 compared to Group 1 ($p <0.0167$).
Conclusion Our results showed that presence/density of HGF increases in favor of acute rejection and accompanies the histopathological findings. Therefore we suggest that density of HGF, excreted in renal allograft tissue, may be a useful marker for supporting AR diagnosis.

P-275

Graft biopsies after renal transplantation in children: differences between FK506 and CyA immunosuppression

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Introduction In 1997 – 2003 we performed 84 graft biopsies in 41 children aged 3 to 18 years, 7 of whom underwent repeated transplantations. Aims: Differences in the treatment of renal grafts.
Materials and methods 84 biopsies were examined. Besides light microscopy (LM) and electron microscopy (EM), IgG, IgA, IgM, fibrinogen, C3 and C4d were detected by immunofluorescence (IF). Immunohistochemistry (IHC) was performed using CD3, CD45RO, CD20, and CD68 antibodies to study the presence of different types of cells in acute rejection. Banff's classification was used to evaluate the samples.
Results Graft rejection was found in 64 (76.1%) biopsies - hyperacute rejection (HAR) 1 (1.2%), borderline changes (BCH) 9 (10.7%), acute rejection (AR) 37 (44%), and chronic rejection (CHR) 17 (20.2%). CyA and FK506 nefrotoxicity (TOX) was present in 28 (33.3%) biopsies. C4d (25 biopsies from 12 patients) showed linear positivity of peritubular and glomerular capillaries in 3 patients, with HAR and AR. These three grafts failed and the same pattern was seen in the excised grafts. In all AR cases, IHC proved the prevalence of CD45RO+ cells in rejection infiltrates, including tubulitis. The rejection infiltrate contained from 10% to 30% of monocytes-macrophages. EM showed disjunction and tubular epithelia regression in tubulitis. AR and TOX findings in patients receiving immunosuppression with FK506 were compared with a group given CyA.

Group	Number	TOX	AR
FK506	15 patients	8 (53.3%)	6 (40.0%)
FK506	23 biopsies	12 (52.2%)	6 (26.1%)
CyA	26 patients	12 (46.2%)	21 (80.8%)
CyA	61 biopsies	16 (26.2%)	31 (50.8%)

No significant differences in the rate of toxic manifestation were found between the groups of patients on FK506 and those on CyA. More frequent findings of toxicity were noted in FK506 patients´ biopsies ($p=0.024$). AR was found more frequently both in the patients ($p=0.008$), and in the biopsies ($p=0.042$) of CyA-treated subjects.
Conclusion FK506 seems to be more potent suppressant than CyA in spite of its persistent toxic effect.
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P-276

BK-polyomavirus infection in a renal transplant recipient - Diagnosis with renal biopsy, urinary cytology and electron microscopy of the urine

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Introduction BK polyomavirus causes a variety of infections in the urinary tract, involving transitional and renal tubular epithelium in immune suppressed patients, including transplant recipients. It can cause irreversible loss of graft function. We report a case of polyomavirus infection in a transplant recipient diagnosed by renal biopsy, urinary cytology and electron microscopy of the urine.
Patient and results A 19-year-old female patient underwent renal transplantation, and received immunosuppressive treatment with tacrolimus, MMF and prednisolone. Serum creatinine level increased

and hydronephrosis was observed in ultrasonography in the 3rd month. A renal biopsy was taken. No glomerular or vascular changes were observed. Some tubular epithelial cells displayed pleomorphism, hyperchromatism with chromatin irregularities and clumping, and intranuclear inclusion bodies. No immunostaining was detected with anti-CMV, HSV and EBV antibodies. Cytopathic tubular lesions in biopsy, many decoy cells in urinary cytology and intranuclear viral particles in electron microscopy of the urinary sediment proved the BK-polyomavirus infection. As the high serum creatinine levels persisted, despite alternative immunosupressive therapies, two more renal biopsies were taken, and acute rejection Banff grade Ib and IIa was diagnosed respectively. The patient is still under a novel antiviral treatment as the focal tubular cytopathic changes were present in the latter biopsy. Serum creatinine levels were still high, even though urinary cytology is normal.
Conclusion As we have seen in this case, the differential diagnosis between rejection and infection in renal transplant biopsies is critical and complicated for both clinicians and pathologists, as well as patients.

P-277

Vascular changes in donor kidneys may have an impact on late graft-dysfunction

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Introduction Morphological studies of "zero-hour" kidney donor biopsies have produced controversial results as to what type of vascular changes, if any, are associated with late graft-dysfunction. Our aim was to assess this question.
Materials and methods 94 consecutive pretransplant "zero-hour" biopsies from cadaver kidney grafts were studied. We examined the frequency and severity of arteriolar hyalinisation, intimal fibrosis, tubular atrophy, interstitial fibrosis and glomerulosclerosis semiquantitatively. The wall thickness/lumen (W/L) ratio of each vessel was determined by morphometry. Groups were then differentiated according to their blood vessel size: G1: d<79µm, G2: d=80-149µm, G3: d=150-300µm. Statistical analyses were carried out to examine the clinico-pathological correlations.
Results Non-specific morphological changes were frequently seen: arteriolar hyalinisation: 84.0%, intimal fibrosis: 76.6% (moderate 26.6%), interstitial fibrosis: 25.5%, tubular atrophy: 71.3% and the mean proportion of sclerotic glomeruli was 5.01%±7.3%. Among the vascular changes studied only the moderate intimal fibrosis ($p<0.01$), and the elevated mean W/L ratios of arteries in the G3 group ($p<0.05$) revealed a significant association with the mean elevated serum creatinine level measured at 24 months after transplantation. A significant correlation was also found between the mean W/L ratios and the degree of intimal fibrosis ($p<0.01$). As for intimal fibrosis, there was a significant correlation (a) with the non-specific morphological lesions ($p<0.001$), and (b) with the frequency of intracranial hemorrhage as the cause of donor-death ($p<0.01$).
Conclusion Our findings suggest that donor kidneys with moderate intimal fibrosis do indeed have a higher risk of late graft-dysfunction.

P-278

Importance of the immunofluorescence morphology in autoimmunity

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Introduction The analyses of serums or tissue specimens from the patients with autoimmune diseases are dispersed between microplate reader ELISA techniques and immunofluorescence (IF) microscopy techniques. The number and the complexity of IF morphological parameters are becoming greater, as well as their association with some of the clinical conditions. The quantity of the morphological signs and the notice capability exceed the possibilities of the experts who are not morphologists. The aim of this paper is to present compilation of IF morphological slides and their association with the clinical conditions, indicating the real position of this kind of diagnostics.
Material and methods More than 2000 serums and 100 tissue specimens from different locations were used as study material. The methods used were indirect IIF microscopy with commercial and own antigens, microplate reader ELISA techniques (MPR-ELISA) and direct IF microscopy.
Results The study showed that the morphology of antinuclear and anticytoplasmic antibodies, using HEp-2 kits was easily examined, wider, and associations with some clinical conditions could be easily done. The antigens for MPR-ELISA techniques derived from recombinated genetic technology, unlike those produced from extraction showed better correlation with indirect IF microscopy. The own antigens originating from the domestic animals and human cadavers, used for indirect IF microscopy in bullous diseases, had the same value as the commercial ones.
Conclusion In order to emphasize the importance of this paper, we can conclude that, undeservingly, very few papers from this area are being presented in the pathological congresses and are left to other specialities.

P-279

Expression of p53 in gastrointestinal stromal tumors predicts biological behavior and survival. A multivariable study of 49 cases

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The gastrointestinal stromal tumors (GIST) are rare tumors. There have been few factors identified that predict survival and biological behavior. This study analizes the experience in two university hospitals. Forty nine patients with GIST were identified from the tumor registry of both institutions. A retrospective review of patient clinic data, tumor and treatment variable was done. Expression of p53 and cellular proliferation antigens (ki67/PCNA) was also analyzed with overall survival as the main outcome variable. Statistical analysis was performed by log rank test and Cox regression model. Significance was defined: $P<0.05$. As results we found that median age was 53 years (range 16-82 years). Twenty-five patients were women (51%) and 24 were men (49%). Hispanics were predominant race with 22 patients (45%) followed by Caucasians with 18 patients (38%) and 9 African American

patients (1%). The stomach was the most common site of presentation (47%) followed by small bowel (37%). Mean tumor size was 8 cm (range 2-46 cm). Complete resection was performed in 38 patients (77.6%). Actuarial 3-year survival was 65.5% with a median follow-up of 35 months (4-204). Univariable analysis identified overexpresion of p53 (P=0.00001), cellular progression antigens (Ki67/PCNA) (P=0.0065), high grade (P=0.00001), tumor size bigger than 10 cm (P=0.0045), and incomplete resection (P=0.00001), as significant negative factors, Hispanic race (P=0.013) and good performance status (P=0.0002) were significantly associated with prolonged survival. On multivariable analysis, overexpression of p53 was the only independent negative factor. In conclusion, p53 is the most significant negative prognostic factor for survival in GIST.

P-280

Granulomas in gastric mucosa, with focus on microgranulomas (mg)

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Introduction Granulomatous gastritis (gg) is a poorly understood entity, reportedly identified in less than 1% of all gastric biopsies. A precise definition of granulomas has rarely been given, in particular mg, well-described in intestinal disorders, have not been focused on in gastric lesions. Aims: We summarize our experience with gg, including mg, and record prevalence as well as associated background morphology.
Materials and methods Among gastric biopsies from 6.677 patients (7.837 gastroscopies), gg was diagnosed in 20 patients. Groups of histiocytes producing conspicuous alteration of gastric architecture were classified as well-defined granuloma (wdg); subtle collections of histiocytes failing to distort surrounding glands were labelled mg. The background morphology and presence of micro-organisms were recorded.
Results The distribution of types of granulomas were as follows: mg only 7 patients, mg and wdg 4 patients, wdg only 9 patients. The background morphology of cases with only mg/wdg with/ without mg included chronic gastritis (n=2/5); chronic active gastritis (n=2/7); ulcus (n=2/2); lymphocytic gastritis (n=1/1); carcinoma (n=0/1); otherwise normal morphology (n=1/1); H. pylori (n=1/4).
Conclusion In concert with previous reports, we identified gg in less than 1% of all gastric biopsies. Since the identification of mg requires assiduous histologic examination, such subtle features may be overlooked; hence our calculated prevalence of gg is a minimun value. The functional relation between mg and wdg is uncertain. However, their coexistence in a proportion of gg could support a precursor function of mg in the development of wdg. Additionally, the background pathology of biopsies with mg and wdg were comparable.

P-281

Intravascular signet ring-like cells CD68/lysozyme positive in gastric ulcer due to chronic use of NSAID, diagnostic implications in endoscopic biopsy and surgical management of an unusual invasive cancer mimicker

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A 83-year old man treated with naproxen during two years was admitted because of hypovolemia and peritoneal irritation. A panendoscopic study was performed and an ulcer localized at the large curvature of the stomach was disclosed. The microscopic examination of the endoscopic biopsy reveled invasive intravascular and stromal neoplastic signet-ring cells. Because of the subsequent poorly differentiated carcinoma diagnosis, a gastrectomy was performed. In the specimen the ulcer showed necrosis, edema, fibrosis, chronic inflammatory infiltrate with lymphocytes and plasma cells. Additionally atypical cells with irregular and hyperchromatic nuclei or vacuolated cytoplasm were seen in the lamina propia and infiltrating the muscular layers; isolated signet-ring-like cells were also seen. There was no involvement in regional lymph nodes. Histochemical study with periodic acid-Schiff, mucicarmin, and colloidal stains revealed mucosubstances in these cells. A poorly differentiated carcinoma was initially diagnosed. However the immunohistochemical studies were positive for histiocytic markers (CD-68, S-100 protein and lysozyme) and negative for epithelial markers (cytokeratin, and EMA). The positivity mucus stains in the histiocytes could be explained in this case by phagocytosis of mucous substances released from broken hyperplastic glands in the vicinity of the ulcer. To our knowledge, atypical histiocytic infiltration in gastric ulcers has not been previously described; thus, it should be included in the group of gastric carcinoma mimickers; with this basis, the pathologist must to be careful in the assessment of vascular permeation or stromal infiltration by neoplastic cells overall in endoscopic specimens.

P-282

Ileal mucosa changes after total colectomy in patients with juvenile polyposis and familial adenomatous polyposis

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Introduction We studied follow-up ileal mucosa biopsies from 11 patients with juvenile polyposis and 4 patients with familial adenomatous polyposis who had been previously underwent total colectomy with mucosal proctoectomy with the creation of straight ileoanal anastomosis. Patients' age ranged from 9 to 36 years (mean 18.7 years). The follow-up ranged from 13 months to 15 years (mean 87.5 months). Aim of this study was to identify the long-term changes in ileal mucosa after total colectomy in patients with polyposis coli syndromes.
Methods The biopsy specimens have been taken from the posterior wall of terminal ileum. Paraffin sections were stained with H&E, van Gieson, periodic acid Schiff (PAS) and Gomori's aldehyde fuchsin - Alcian-blue (GAF-AB) stains. Six biopsies were subjected to electron microscopy analysis.
Results On histological examination ileal mucosa revealed focal shortening of villi and lengthened crypts. The villi were lined by increased number of goblet cells. Mucin histochemical studies demonstrated partial conversion of the epithelium to a colonic sulfomucin mucin profile by positive GAF-AB staining in cytoplasm of goblet cells. Only 2 out of 15 patients had increased number of mononuclear cells in lamina propria. The electron microscopy examination revealed shortening and rarification of microvilli of absorptive enterocytes.

Conclusion Follow-up biopsies show incomplete and focal neocolonic transformation of ileal mucosa. Nevertheless, in most of the cases ileal mucosa preserved its architectural and histochemical characteristics. We failed to identify evidences of chronic inflammation that made us suggest that chronic terminal ileitis is not a common long-term event in patients with polyposis coli syndromes after total colectomy.

P-283

Eosinophilic gastroenteritis with pleural outflow and ascites. A case report

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Introduction In our region eosinophilic gastroenteritis is a very rare disease. Etiology is unknown.
Case report A 39-year-old female patient was admitted to the clinic due to slight cough, right side pleural outflow and ascites, without other symptoms. Gynaecological ultrasound, ultrasound of the upper abdomen, CT refer to ascites besides normal findings of abdominal and pelvic organs. Laboratory examinations were normal except peripheral blood eosinophilia and increased values of Ca 125. Cytological findings of pleural outflow and ascites revealed a great number of eosinophilic granulocytes but without malignant cells.
Patohistological findings During explorative laparotomy resected parts of both ovaries, a lymph node, a hyperplastic part of pylorus, a knot from a small bowel designated as a tumor, and an appendix were taken out for a patohistological examimation. Both ovaries reveal normal ovarial parenhima. A regional lymph node showed reactive changes with hyperplastic follicles and prominent germinative centers. The main histological finding reveals pyloric stromal edema and a dense infiltrate of eosinophilic granulocytes throughout the muscle layer as well as eosinophils situated in perivascular spaces which is accompanied with swelling of endothelium of capillary blood vessels. Eosinophilic granulocytes were found in the smooth muscle layer of intestine as well as in the subserosal tissue. Lumen of the appendix was obliterated and the muscle layer and the subserosal tissue contained eosinophils.
Conclusion From our review it is visible that eosinophilic gastroenteritis can represent a great diagnostic problem especially when it is accompanied with pleural outflow and ascites and increasing values of tumor markers. That can possibly mislead a diagnostic procedure to the neoplastic rather than the immunological or allergic disease. Three years after surgery the patient is well.

P-284

Expression of TNF-alpha and IL-4 in patients with chronic pancreatitis

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Introduction To study the serial levels of TNF-alpha and IL-4 in patients with chronic alcoholic and chronic relapsing pancreatitis.
Methods 112 consecutive patients with chronic pancreatitis admitted to the Emergency Hospital (32 patients with alcoholic pancreatitis and 80 - with chronic relapsing pancreatitis) were studied. Serum levels of TNF-alpha and IL-4, were determined on 1 and 6 days after admission and in remission by immunoenzyme multiplied.
Results In group of the patients with chronic alcoholic pancreatitis on day one after admission the high levels of TNF-alpha (376,4 ± 91,4 pg/ml) and IL-4 (41,99±13,3 pg/ml) were marked with the subsequent consecutive decrease of these values for 6 days (TNF-alpha-18,1±5,96 pg/ml, IL-4-37,8±14,95 pg/ml) and in the period of remission (TNF-alpha-6,6±5,3 pg/ml, IL-4-9,8±1,90 pg/ml). In patients with chronic relapsing pancreatitis the low levels of cytokines on days one after admission (TNF-alpha-27,7±6,1 pg/ml, IL-4-59,85±21,2 pg/ml) were marked with the deferred peak of values on day 6 (TNF-alpha-271,6±42,2 pg/ml, IL-4-120,4±34,3 pg/ml), and decrease in the period of remission (TNF-alpha-54,5±8,4pg/ml, IL-4-19,9+-4,9 pg/ml).
Conclusion The deferred peak of cytokines levels (on 6 days) in patients with chronic relapsing pancreatitis can testify indirectly about more expressed cytokines disturbance.

P-285

Gastric glomus tumor: report of one case

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Introduction The majority of glomus tumors are benign neoplasms that occur in the dermis of extremities. However, rare case have been reported in the visceral locations, most often in the stomach.
Case report The tumor occurred in a 34-year-old male who presented ulcer-like pain. CT scan diagnosed a submucosal tumor. There was no endoscopic biopsy specimen. A wedge resection of stomach was performed with a frozen section study that conclude to a carcinoid tumor Glomus tumor was confirmed by further study of multiple sections of a surgical specimen and by immunhistochemical study.
Conclusion Correct identification of this essentially benign, very rare gastric tumor can prevent unnecessary radical operative procedures.

P-286

Composite carcinoid-adenocarcinoma of the ileum associated with transitional cell carcinoma of the urinary bladder

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It is well known that sometimes carcinoid tumor is associated with other tumors. Approximately 15% of carcinoid tumors in the small intestine are associated with non-carcinoid neoplasms, most frequently adenocarcinomas of the gastrointestinal tract. The concurrent occurrence of carcinoid and other tumors in various organs is extremely rare. Here, we present a case of a 74-year-old male who was admitted to our Hospital with a 3-month history of haematuria. On cystoscopy a large tumor of the urinary bladder has found. A total cystectomy was performed. During the operation, while exploring the abdomen, a tumor with maximum diameter 2 cm was found in the terminal ileum, from which a biopsy was taken. Microscopically, the bladder tumor was a papillary transitional cell carcinoma grade II and the tumor of the ileum fulfilled the criteria for a composite carcinoid adenocarcinoma. The carcinoid component

was positive for chromogranin and NSE and negative for EMA, CEA and CA 19-9 whereas the adenocarcinoma component was positive for EMA, CEA and CA 19-9 and negative for chromogranine and NSE. 18-months after the operation there is no metastasis radiologically and the patient is in a good condition. Due to the small number of tumors reported as composite carcinoid-adenocarcinoma there is need for larger number of similar cases as well as long term follow-up to determine their natural history and prognostic significance. We describe the first reported case of composite carcinoid-adenocarcinoma of the ileum associated with carcinoma of the urinary bladder.

P-287

Mutational analysis and expression of CDX1 homeobox gene in 20 cases of colorectal carcinomas

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Introduction CDX1 is a transcription factor specifically expressed in normal intestinal epithelium. It plays a role in the regulation of cell proliferation and differentiation. Experimental evidences suggest that it could have a pro-oncogenic effect since Cdx1 over-expression up-regulates Bcl-2 and reduces p21; it is up-regulated by ras activation and its ectopic expression has been reported in intestinal metaplasia, in carcinomas of the stomach and in Barrett's esophagus. Despite these observations the role of CDX1 in colorectal carcinogenesis is not yet fully understood. In fact it has been shown to be down-expressed in colon cancers at mRNA and protein level. The aim of the study was to investigate the presence of CDX1 gene mutations.
Materials and methods Twenty cases of sporadic colon carcinomas were collected. Total RNA and DNA from neoplastic and normal tissues were extracted. cDNA was synthesized and used as template in RT-PCR using oligonucleotide for CDX1 and â-actin. CDX1 three exons were PCR amplified and directly sequenced.
Results We found lower expression of CDX1 in tumour samples in four out of ten cases. Sequencing showed a transversion G>C (10/20 cases) of exon 1 and a T>C transition in exon 2 (4/20 cases) that, being present in the corresponding normal tissue, were regarded as polymorphisms. No base substitution was observed in exon three.
Conclusions Our results showed that CDX1 expression is reduced in colon carcinoma. However CDX1 was not found to be mutated suggesting that alterations, other than base substitution, may affect CDX1 gene in colon cancer. We are now looking at CpG islands methylation in the same cases since methylation could be a likely explanation of the low expression of CDX1.

P-288

The potential role of TGF-beta-1, TGF-beta-2 and TGF-beta-3 proteins expression in colorectal carcinomas and their possible correlation with classic histopathologic factors and patients survival

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Aim This study investigates TGFbeta1, TGFbeta2 and TGFbeta3 proteins expression in patients with colorectal carcinoma and evaluates their correlation with classic prognostic markers and patients' survival.
Design The study comprised 124 patients with colorectal carcinoma. According to Astler-Coller system, 42 tumors were of stage A, 42-B, 48-C and 20-D, whereas 106 tumors were low-grade and 18 high-grade of malignancy. On paraffin sections the streptavidin-biotin technique, using antibodies to TGFbeta1, TGFbeta2 and TGFbeta3 (SantaCruz, USA) was employed. Staining results followed morphometric analysis and were correlated with clinicopathologic parameters.
Results TGFbeta1 protein was expressed in 88/124 (71%) carcinomas whereas TGFbeta2 and TGFbeta3 proteins were detected in all tumors examined. Normal colonic mucosal epithelial cells expressed less TGFbeta2 ($p<0.01$ compared to neoplastic cells) and less TGFbeta3 ($p>0.05$ compared to neoplastic cells), but not at all TGFbeta1. Statistical analysis revealed higher expression of TGFbeta1 in low grade carcinomas ($p=0.009$) and higher TGFbeta2 presence in advanced stage tumors ($p=0.008$). TGFbeta1 expression was related with higher disease free survival and higher total survival ($p<0.05$ respectively). TGFbeta2 presence was correlated with worse prognosis ($p<0.05$). Cox analysis revealed that besides tumor grade and stage, TGFbeta1 expression constituted independent prognostic factor.
Conclusions This study shows that in cases of colon adenocarcinoma there is different expression of TGFbeta1, TGFbeta2 and TGFbeta3. TGFbeta1 may be implicated in the pathogenesis of these tumors since it is expressed only within neoplastic and not normal cells. TGFbeta1 is related with higher disease free survival, higher total survival and constitutes an independent prognostic factor. In the late stages, TGFbeta2 seems to be involved in tumor progression and it is related with worse prognosis.

P-289

CDX2 is useful in distinguishing primary colorectal adenocarcinoma form pulmonary, mammary and pancreatic adenocarcinomas metastatic to the colorectum

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Background The large bowel may be the site of metastasis from a number of tumors including pulmonary, mammary and pancreatic adenocarcinomas. The histological distinction of primary colorectal adenocarcinoma from these metastatic lesions may be difficult. The aim of this study was to evaluate the utility of the intestinal marker CDX2 in distinguishing primary colorectal adenocarcinoma from pulmonary, mammary, and pancreatic adenocarcinomas metastatic to the large bowel.
Design Routinely processed mucosal biopsies of 30 adenocarcinomas involving the colorectum [15 primary, 15 metastatic (lung 5, breast 6, pancreas 4)] and 15 control cases of primary lung, breast, and lung adenocarcinomas were immunohistochemically stained for CDX2 (Biogenex, San Ramon, CA, USA). The intensity and extent of the staining were recorded semi-quantitatively.

Results CDX2 diffussely and strongly stained the normal colorectal epithelium as well as all cases of primary colorectal adenocarcinoma. In contrast, all cases of metastatic lung, breast and pancreas adenocarcinoma as well as all control cases were CDX2 negative.
Conclusions CDX2 immunostaining is useful in discriminating primary colorectal adenocarcinomas from pulmonary, mammary and pancreatic adenocarcinomas metastatic to the large bowel. We recommend its use as a component of any antibody panel put together to discriminate between primary and secondary colorectal tumors.

P-290

Infiltrative and proliferative activity in central versus peripheral parts of colorectal cancer

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Introduction Cellular proliferation is a basic phenomenon in neoplasms. Infiltration and destruction of stroma are features of malignant tumour growth. In colorectal cancer stromal production and its invasion by neoplastic cells is encountered in peripheral and central parts of tumours. This study presents quantitative analysis of stromal infiltration and cellular proliferative activity in central and peripheral parts of primary colorectal cancer.
Materials and methods 33 cases of colorectal adenocarcinoma seated mainly in sigmoid and rectum were examined. Investigated group included 15 women and 18 men (aged 35-85 yrs). According to the TNM classification, grade II was assigned in 17 cases and grade III in 16 cases. In every case infiltrative (under x200) and proliferative activity (under x400) was assessed in 3 central and 3 peripheral tumour fields. Number of small isolated cancer cell clusters of cross section area under 1000 μm^2 was assessed as measure of stromal infiltrative activity.
Results In the studied group peripheral infiltrative activity was greater than in central parts of tumours. Median value of isolated cellular clusters was 20.3 and 6.8 per field in peripheral and central parts respectively ($p<0.01$). Proliferative activity was slightly higher in peripheral than in central areas. Mean Ki67 labelling index was 42% (+/-22%) and 37% (+/-21%) in peripheral and central parts respectively ($p=0.04$). No important correlation was found between calculated parameters ($R=0.14$).
Conclusions Stromal infiltrative activity is greater in peripheral parts of the tumour. This parameter does not correlate however with proliferative activity of neoplastic cells in examined group.

P-291

Angiogenesis in primary and metastatic foci of colorectal cancer

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Introduction Growth of primary and metastatic tumour is accompanied by formation of stroma composed of connective tissue and new blood vessels. Colorectal cancer is a malignancy characterised by marked desmoplastic reaction both in primary tumour and in its secondary foci (metastases). Presented report was aimed to describe quantitatively microvessel density in primary tumour and lymph node metastases from this neoplasm.
Materials and methods Study material included 36 cases of colorectal cancer with metastases to the regional lymph nodes. Investigated group comprised 20 women and 16 men (aged 33-85 yrs); 32 tumours were T3 and four were T4 according to TNM classification. Microvessels were highlighted using monoclonal anti-CD34 antibodies. Vessel counts were performed in five fields of each primary and metastatic focus (x200; 0.747 mm2). Numbers of blood vessels in colorectal tumour were counted in areas free from prominent necrotic foci and non-specific granulation tissue due to process of reparation. Vessel density in nodal metastases was determined in fields occupied by neoplastic cells forming their own connective tissue stroma.
Results In the primary tumour group mean vessel density was 74.5 vessel/mm2 (median value 73.3; range 44-152). In metastatic foci the same parameter was 78.1 vessel/mm2 (median value 77.5; range 41-117); no statistically significant difference was detected. Only slight correlation was found between the microvessel densities in primary and metastatic tumours ($R=0.39$; $p=0.1$).
Conclusion Presented results seem to prove the similar pattern of neovascularisation in both groups, whereas marked differences in this process can be observed between subsequent analysed cases.

P-292

Clonality analysis of mutant E-cadherin-positive, diffuse-type early gastric cancers

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Early diffuse-type gastric carcinoma, especially signet ring cell carcinoma, often shows the superficially spreading growth pattern, and one of the unsolved questions concerning the histogenesis of this type of carcinoma is whether it is monoclonal or multiclonal in origin. We have performed Human Androgen Receptor Gene Assay (HUMARA assay) based on the random inactivation phenomenon of X chromosomes in female and reported that most of this type of carcinomas were of monoclonal origin (8/9 cases). However, this method demonstrated only probability of monoclonality in each lesion. Recently, 50% of diffuse-type gastric carcinomas have revealed mutations of the E-cadherin gene at the early stage of their progression, and in-frame skipping of exon 9 has been seen frequently. Thus, we performed an immunohistochemical analysis of early signet ring cell carcinomas using E-cad delta 9-1, which is a specific antibody against a peptide spanning the fusion junction region between exons 8 and 10 of mutant E-cadherin. E-cad delta 9-1 specifically reacts with tumor cells expressing E-cadherin mRNA lacking exon 9. Seven of 44 cases were positive for this antibody. In each positive case, the paraffin blocks of the tissue slices that included the whole tumor were analyzed using E-cad delta 9-1. Although some signet ring cells with abundant mucin in the upper and/or lower layers of a layered structure did not react with this antibody, tumor cells were confirmed to express E-cadherin mRNA lacking exon-9 by reverse transcriptase-polymerase chain reaction with paraffin-embedded

formalin-fixed tissues. These findings suggest that they are of monoclonal origin (7/7 cases) and support our previous data using HUMARA assay.

P-293

Unusual types of Gastrointestinal Malignant Lymphomas (GIMLs)

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Introduction MALT lymphoma is the commonest primary GI lymphoid malignancy but for correct diagnosis and classification, immunohistochemical and molecular analysis have been proven necessary. The aim of this study was to demonstrate the value of current methodology for diagnosing GIMLs by presenting four unusual cases.
Materials Case 1. A 33 years old male with common variable immunodeficiency. An obstructive ileal tumor 3cms was resected and multiple biopsies were taken along the GI tract from nodular areas. Case 2. Female 50 years old with generalised lymphadenopathy, lung shadows, dyspepsia and duodenal nodularity. Case 3. Female age 74 underwent gastrectomy with initial diagnosis of high grade B lymphoma. Case 4. Female age 74, had gastrectomy/ splenectomy for relapsed gastric Lymhpoma which had been diagnosed as MALT type.
Methods Immunophenotypical analysis for detection of CD20, CD79a, CD10, bcl-6pr, bcl-2pr, CD5, Cyclin D1, CD23, CD43, CD45RO, CD3, Ki-67 and genotypic analysis for the clonal IgH, TCva genes (case 3) and the IgH/bcl-1 rearrangement (case 4).
Results per case: 1. Atypical Burkitt Lymphoma with diffuse lymphoid hyperplasia of the GI tract (CD10/20/79a+, bcl-6+/2-, Ki67 100%). 2. Generalised follicular B Lymphoma, Grade 1 according to the WHO classification with involvement of the Duodenum. 3. Diffuse large B cell lymphoma with aberrant CD45 RO expression. Rearrangement of the IgH genes and not of the TCva gene. 4. CD5- Mantle cell lymphoma with anaplastic foci in the spleen. Clonal rearrangements of the IgH and concomitant IgH/bcl-1 gene rearrangements involving tonsils, stomach, spleen and abdominal lymph nodes.
Conclusion All cases represent unusual types of GIMLs easily misclassified or graded without thorough immunophenotypic and molecular analysis.

P-294

Malignat gastrointestinal stromal tumor: morphological and immunohistochemical investigation

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Introduction We present two cases of a 72 years old man and 77 years old woman hospitalized for bulky abdominal mass demonstrated by clinical, echographical and CT examination.Adenopathy, liver and peritoneal metastases were present.First patient underwent the tumor ablation and the second only a biopsy.
Matherial and methods Fragments of the tumors were fixed in formaldehyde 10%, included in paraffin and the sections were stained with HE and VG.;indirect immunoperoxidase method with cytokeratine.VIM, ACT, Desmine, S100, CD34, CD 117 were performed.
Results The histological examination in both cases revealed a cellular spindle cell tumors fascicular pattern, rare mitoses and areas of necrosis(in first case)Immunohistochemical analysis:the cytokeratin profile(AE1, AE3), ACT, Desm, S100 were negative; VIM, CD 34 and CD 117 were positive.
Conclusions The diagnosis of GIST was based on morphology and on the strong diffuse positivity of the tumor cells with CD117.The classification as malignant was made on the size of the mass and the presence of metastases

P-295

Cardiac mucosa develops during pregnancy: an autopsy study in embryos, fetuses and infants

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Introduction The incidence of gastric cardiac adenocarcinoma has increased in the last decades. Gaining insight in the pathogenesis of this lesion is hampered by the limited knowledge of the origin and histology of cardiac mucosa (CM). Currently, the location, extent and even the existence of CM are controversial. Aims: We studied the development of the gastro-oesophageal junction (GOJ) in embryos, fetuses and infants to clarify whether CM is a normal structure at birth and where it is located.
Materials and methods Twenty-one autopsy cases were evaluated. Age range: 13 weeks gestational age (GA) - 7 months. The distal oesophagus and proximal part of the stomach were embedded entirely. Serial sections were stained with haematoxylin-eosin and alcian blue/periodic acid-Schiff. The following parameters were measured: length of abdominal oesophagus; length of columnar-lined oesophagus; length of CM; distance from CM to angle of His.
Results CM was present in all evaluated sections. Its mean length varied throughout gestation. A maximum value was reached at a GA of 16 weeks (1,2 mm). After term delivery, it was very short (0,3 – 0,6mm). CM was proximal to, or straddled the angle of His in all cases. During gestation, the mucin staining pattern of the CM was to a high degree similar to that of the developing pyloric mucosa.
Conclusions CM develops during pregnancy and is present at birth as a normal structure. If the angle of His is taken as a landmark for the GOJ, CM is located in the distal oesophagus.

P-296

Gastric mucosa-associated lymphoid tissue (MALT) and non-MALT lymphomas: clinicopathological study of 32 cases

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Introduction Histologic features of low-grade B-cell MALT lymphoma of the stomach have been well described in recent years, but its relationship with the more common large B-cell gastric lymphoma has not been clarified. The authors investigate clinico-pathological differences among low-grade and high-grade gastric lymphomas.

Methods Clinical and histopathological aspects of 32 consecutive gastric lymphoma cases found in gastrectomy specimens were studied: 14 cases of low-grade MALT lymphoma (LG MALT lymphoma), 10 cases of high-grade MALT lymphoma appearing in low-grade MALT lymphoma (HG/LG MALT lymphoma), 4 cases of high-grade MALT lymphoma (HG MALT lymphoma) and 4 cases of diffuse large cell lymphoma (DLCL).
Results Mean age were as follow: LG MALT lymphoma-36,5 years; HG/LG MALT lymphoma-44,5 years; HG MALT lymphoma-61,5 years; DLCL-54,3 years. There was a male predominance of MALT lymphoma patients (male to female ratio-18/10). Macroscopically, the most common pattern of LG MALT lymphomas was the superficial spreading type with or without ulceration, whereas HG MALT lymphomas and DLCL exhibited a solitary tumor-forming lesion. B-cell immunophenotype was confirmed in 31 cases, only one case of DLCL was a T-cell lymphoma.
Conclusions In our study, the prevalence of high-grade MALT and non-MALT lymphomas was 56,25%. Our results suggests that some gastric lymphomas with high malignancy can arise through blastic transformation of the LG MALT lymphomas, this process(change) seems to take about ten years at least.

P-297

MSI-L and MSI-H in colorectal serrated adenocarcinomas

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Background The recently described serrated adenocarcinoma accounts at least 5.8 % of colorectal malignancy. It develops from serrated polyps. DNA microsatellite instability (MSI) is more common in serrated adenocarcinomas than in conventional adenocarcinomas, but the frequency of MSI-low and MSI-high in these tumours is unknown.
Materials and methods An unselected series of 23 serrated adenocarcinomas were included. MSI status was evaluated using panel of five markers (BAT-25, BAT-26, D2S123, D5S346 and D17S250). A tumour was considered to present MSI-high if two or more markers showed MSI, MSI-low with one marker, and MSS when none of the markers showed MSI.
Results DNA MSI was observed in 47.8 % (11/23) of serrated adenocarcinomas, 13.0 % (3/23) being MSI-high and 34.8 % (8/23) MSI-low. MSI status showed no association with gender, age, location, size, Dukes stage, grade or mucinocity. MSI-positive serrated adenocarcinomas tended to have better prognosis (5-year survival 74.1 %) than MSS cancers (5-year survival 41.1 %), but this was not statistically significant (P=0.272, Log-Rank).
Conclusions Serrated adenocarcinomas show prevalence of MSI-low higher than that reported for colorectal cancers (7 to 15%), supporting idea that serrated carcinomas are formed through a pathway differing from other carcinonomas. Absence of any association of MSI and clinicopathological features could be due to homogeneity of these neoplasms although a more extensive material is necessary to show more conlusively absence of any association. A trend for better prognosis in MSI -positive serrated adenocarcinomas probably reflects the biological behaviour of MSI observed earlier in colorectal carcinomas in general.

P-298

Thyroid hormone receptor beta-1 expression in colorectal cancer is associated with polypoid growth type and K-RAS mutations

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Background The action of thyroid hormones is mediated via their receptors (TRa1, TRa2, TRb1 and TRb2). TRb1 expression is abnormal in cancer cells in vitro and in some malignant neoplasms, but the role TRb1 in colorectal carcinogenesis is unknown.
Materials and methods TRb1 expression pattern was evaluated by immunohistochemistry in 115 operated colorectal cancers and correlated with K-RAS mutations and clinicopathological features.
Results In normal mucosa, cytoplasmic TRb1 immunoreactivity was seen mainly in the superficial parts, while nuclear reactivity was most prominent in the cryptal epithelium. In tumours, nuclear expression was observed in 67% of cases and cytoplasmic staining in 68%, both mostly co-existing. Cytoplasmic staining for TRb1 was more common in polypoid (88%; 21/24) than in flat carcinomas (41%; 9/17; p=0.029). K-RAS mutations were more common in tumours with cytoplasmic expression of TRb1 (27.5 %; 11/40) than in those without (0%; 0/14). In lymph node metastases, occurrence of nuclear staining of TRb1 was more pronounced than in the primary lesion (P=0.003, Wilcoxon).
Conclusions Expression of TRb1 is present in normal colorectal mucosa and is mostly retained in carcinomas, suggesting that thyroid hormones and their receptors may be involved in the physiological regulation of normal colorectal epithelium and in the pathogenesis of colorectal cancer. Association of expression with K-RAS mutations indicate occurrence of functional linkage between TRb1 and ras signaling. Observations of a high expression in lymph node metastases and in polypoid cancers offer new clues to factors affecting metastatic cascade and growth pattern of colorectal cancer.

P-299

Gastric mucosal calcinosis in chronic renal failure patients

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Introduction Gastric mucosal calcinosis (GMC) is rare. Some reports indicate that GMC is associated with gastric cancer, chronic renal failure (CRF), and ingestion of sucralfate or aluminum-containing antacids (SACA) in organ transplant patients. The aim was to determine the incidence and investigate the etiology and pathogenesis of GMC in a large group of CRF patients at one transplant center.

Materials and methods A review of the records for 326 CRF patients who underwent endoscopic biopsy revealed 45 GMC (patient group) and 281 non-GMC (control group) cases. For each subject we recorded the number and sites of biopsies, and re-assessed for inflammatory activity, mucosal atrophy, intestinal metaplasia, lymphoid aggregates, Helicobacter pylori, amyloid deposition, and gastritis type. Age, sex, cause of renal failure, time from renal failure diagnosis to biopsy, type of dialysis, indications for endoscopy, SACA use, and serum levels of calcium, phosphorus, calciumXphosphorus product and parathyroid hormone were also recorded. The GMC cases were classified according to pattern of deposition (nodular, granular, mixed) and disease severity (mild, moderate, severe).
Results The incidence of GMC was 13.8%. In GMC group there was male preponderance (73%). Compared to the control group, the GMC group had a significantly higher number of corpus biopsies; a significantly lower number of antral biopsies; a significantly lower frequency of mucosal atrophy; and a significantly lower parathyroid hormone level ($p<0.05$ for all). None of the GMC patients had used SACA.
Conclusion Unlike previous reports, these results show that GMC is not linked with SACA use or female gender in CRF patients. There was also no correlation with mucosal injury. These findings suggest that GMC is probably a metastatic process, not a dystrophic one.

P-300

The value of histology in diagnosis of Inflammatory Bowel Disease (IBD)

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In patiens with colitis colorectal biopsy is helpful in the differential diagnosis between IBD and other forms of colitis and also between ulcerative colitis (UC) and Crohn's disease (CD). However the histological features in biopsy specimens are not always sufficient for a definitive diagnosis and there is need to establish more sensitive and specific criteria for the distinction between UC and CD. A retrospective blind evaluation of biopsy specimens from 102 patients with the endoscopical diagnosis of colitis was undertaken in order to assess the value of histology in the diagnosis of IBD and the degree of inter-observer agreement. Twelve experienced pathologists participated in the study. Twenty histopathological features according to a modified version of the British Society of Pathology guidelines for the evaluation of colorectal biopsies in suspected IBD were studied and the results were statistically analysed. The clinical diagnosis after follow up was UC in 57 cases, probably UC in 3 cases, IBD 2 cases, CD 3 cases and non-specific colitis 37 cases. Higher than 90% agreement (by all 12 or 11 participating pathologists) on the diagnosis of UC based only on histologic parameters was reached in 28 of the 57 cases for which clinical follow up proved the diagnosis of UC. Unanimous agreement on the diagnosis of non-specific colitis was reached in 22 of the 37 cases. Our results indicate that although detailed assessment of a combination of histopathological features and the experience of histopathologist are important for the diagnosis of IBD, the final diagnosis depends on combination of histopathology with the clinical and endoscopical evaluation.

P-301

Atypical multinucleated stromal cells in the polypoid tumor lesions of the gastrointestinal tract

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Aim The authors presented two cases of polipoid tumor lesions (gastric and anal) with pseudomalignant changes.
Methods and results Multiple sections from the stomach and anal mucosa were analysed by light microscopic and immunohisto-chemical methods. In the gastric hyperplastic polyp, there was surface ulceration with granulation tissue and acute and chronic inflammation. Within the stroma were numerous atypical cells with bizarre cytomorphology and atypical mitoses. The second case was a fibroepithelial polyp of the anus with collagenous stroma covered by squamous epithelium. Stromal cells with two or more nuclei were found and showed atypical nuclear features. In both cases, mast cells were frequently observed and sometimes intimately related to the stromal cells. Immunohistochemical study revealed that the stromal cells stained positive for vimentine and negative for actine, desmin, cytokeratin, S-100 protein and leukocyte common antigen, so considered to be fibroblastic cells. Clinical follow-up was available. None of the patients followed developed metastases or recurrences.
Conclusions Atypical stromal cells should not be misinterpreted as neoplastic cells. Mast cells may play an important role in their morphogenesis.

P-302

Prognostic value of nm 23 and c-erbB-2 expression in colorectal cancer

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Increased expression of genetic markers is observed in a variety of malignancies, including colorectal cancer (CRC). Overexpression of these markers in the primary tumor is known to predict outcome after colonic resection. In this study we aimed to evaluate the expression level of the nm 23 and c-erbB-2 proteins in CRC and its correlation to prognosis and liver metastasis. Protein expression was examined immunohistochemically in formalin-fixed, paraffin-embedded tissue from 64 colorectal patients. CRC showed a high level of nm 23 protein expression in 63% of primary tumors and 66% of liver metastasis. Expression of c-erbB-2 was observed in 59% of primary carcinomas and 57% of distant metastasis. The positive expression of protein markers in primary lesion was significantly higer in the cases with liver metastasis than in those without metastasis. No significant relationship was observed between nm 23 and c-erbB-2 expression and other clinico-pathological parameters. These results suggest that nm 23 and c-erbB-2 expression play an important role in the progression of CRC and could provide additional information for the development of liver secondaries.

P-303

Relationship between gastritis severity, Helicobacter pylori intensity and mast cell density in the antrum and corpus: An immunohistochemical study with mast cell tryptase

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Introduction Helicobacter pylori (H. pylori) is the most frequent and significant factor in the etiology of chronic active gastritis. In most persons infected with H. pylori, there is an increased chronic inflammation in the lamina propria of the stomach, including lymphocytes, monocytes, eosinophils, and plasma cells. Mast cells originate from bone marrow, which plays a role in the onset and regulation of inflammation. The aim of this study was to investigate the relationship between mast cell density and H. pylori intensity, gastritis severity in the corpus and antrum mucosa.
Material and methods 43 H. pylori- positive and 27 H. pylori-negative patients were included in the study. All cases underwent endoscopy. All biopsies were evaluated according to Sydney system and mast cell density in both corpus and antrum mucosa was analyzed by anti-human mast cell tryptase. In lamina propria and epithelial mast cells in both corpus and antrum counted one by one.
Results Both in the antrum and the corpus, epithelial and submucosal mast cell density was significantly higher in the H. pylori -positive group than in the H. pylori-negative group. The higher mast cell distribution is correlated with high scores of inflammation, activity, and H. pylori density in the antrum and corpus. No relationship was found between mast cell distribution and intestinal metaplasia or atrophy.
Conclusion Both submucosal and epithelial mast cells may be important effector cells in the pathogenesis of gastritis. They assume similar role in different regions in the development of H. pylori gastritis.

P-304

Apoptosis and activated intraepithelial cytotoxic T lymphocytes in gastric carcinomas characterized for microsatellite instability and Eepstein Barr virus infection

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Introduction Sporadic gastric carcinomas (GCs) with high level microsatellite instability (MSI+) tend to be poorly differentiated adenocarcinomas with abundant lymphocytic infiltration and are associated with a relatively good prognosis. The aim of the study was to define whether the nature and the activation status of lymphocytes infiltrating neoplastic epithelial cells (IELs)of MSI+ Gcs could be involved in an increased apoptosis of tumor cells and could be consistent with a tumor-specific immune response.
Materials and methods Tumor cells apoptosis and immunophenotype of IELs were investigated by immunohistochemistry in 35 MSI+ GCs. For comparison, a series of 31 MSI- GCs with similar histological features was investigated, including 7 cases, which were positive for Epstein Barr Virus infection (EBV+).
Results MSI+ and MSI-/EBV+ GCs displayed a significant higher mean number of cytotoxic IELs than MSI-/EBV- tumors (CD3: 30.7 and 29.8 versus 9.9; CD8: 21.7 and 22.1 versus 6.4; TIA-1: 16.7 and 15.6 versus 5.2; $p<0.01$). In addition the activated IELs were more frequent in MSI+ than in MSI-/EBV- GCs (mean granzyme B immunoreactive IELs: 7.5 versus 0.8; perforin 5.9 versus 0.9; $p<0.01$). The percentage of apoptotic tumor cells, evaluated with the M30 CytoDeath antibody, was higher in both MSI+ and MSI-/EBV+ GCs than in MSI-/EBV- GCs (5.99% and 4.31% versus 2.5%; $p<0.01$).
Conclusion The lymphoid infiltration in MSI+ GCs was significantly higher than that of MSI- GCs (except for EBV+ GCs) and this might explain the increased apoptotic index and the relatively better prognosis of these tumors.

P-305

Goblet cell carcinoid of the appendix: A clinicopathologic study and MUC1, MUC2 and MUC5A Expression

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Introduction Goblet cell carcinoid (GCC), also known as mucinous carcinoid or adenocarcinoid, is a rare tumor of appendix, which exhibits both endocrine and adenocarcinoma differentiation. Different mucin phenotypes are associated with neoplastic transformation in epithelial tissue in various organ systems. MUC2 apomucin is a mucin gene product specific to intestinal goblet cells. MUC1 is suggestive to inhibit cell-to cell adhesion. In the present study, we retrospectivelly studied the clinicopathological features and immunohistochemical expression of MUC1, MUC2 and MUC5A in GCC.
Materials and methods Three appendiceal GCC were retrieved from our pathology files from 1988 to 2002. Medical records and histologic slides were reviewed. The expression of MUC1, MUC2 and MUC5A was assessed by immunohistochemistry.
Results All three cases were males and the age ranged from 44, 52 and 85 yrs. Two patients presented with acute appendicitis. The other one had ruptured diverticulitis and renal cell carcinoma. Two patients had appendectomy, and one patient had right hemicolectomy and right radical nephrectomy. Histologic examination showed infiltrative small nests and glands of tumor cells with goblet cells and mucinous cytoplasm. Perineural invasion was noted in one case. The tumors had invaded through the appendiceal wall to subserosa in two cases and to adjacent cecum in one case. The tumor cells are positive staining for synaptophysin. There was strong and diffuse reactivity in the tumor cells with MUC1, MUC2 and MUC5A antibodies in all three cases. Follow-up ranged from 1 month, 9 months, and 13 months. All were alive and without recurrence.

Conclusions These observations suggest that there is no differential expression of MUC1, MUC2 and MUC5A in GCC and both MUC1 and MUC2 may play a role in cancer cell growth and invasion.

P-306

Gastric parietal cell carcinoma: report of three cases

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Gastric parietal cell carcinoma is an infrequent newly recognised variant of gastric carcinoma with only 16 cases published in the literature. The present study describes the findings in three cases of gastric parietal cell carcinoma consisting of parietal cells. We have used light and electron microscopy, and histochemical stains. We have studied three cases, two men and one woman, aged between 57 and 86 years. The tumour size ranged from 2,5 to 6,5 cm. The surgical specimens were stained with hematoxylin-eosin, alcian blue and PTAH. Small pieces of formalin fixed tumor tissue were collected in one case, and fresh tissue fixed in glutaraldehyde in another both for electron microscopy. Involvement of the gastric wall was observed in all tumours. The tumour pattern was characterised by an arrangement of tumour cells in sheets, elongated cords or in diffuse poorly cohesive sheets interspersed with a lymphocytic infiltrate. Tubular differentiation was only focally present in one of the cases. All the cases were composed by a large number of parietal-like cells with abundant eosinophilic cytoplasm, with a mixture of signet ring cells population in one of the cases. Alcian blue stain was negative throughout the tumour, whilst PTAH stained many tumour cells. Ultrastructurally the tumour cells were characterised by abundant mitochondria, tubulovesicles, intracellular canaliculi and intercellular lumina filled with microvilli. The three tumours presented are primary gastric carcinoma with cytological and ultraestructural patterns of parietal cell differentiation. They represent a morphologically distinct type of carcinoma, which has been said to have a favourable prognosis.

P-307

Analysis of CD8 positive cells of gastric mucosa in cases of peptic ulcer and gastritis

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Aim The aim of study is to investigate do really CD8+ cells have cytotoxic or suppressor influence upon gastric epithelium.
Material and methods We have examined biopsies from 23 patients with peptic ulcer and 22 - with gastritis only. Samples were formalin fixed, paraffin-embedded, stained with H/E, toluidineblue, Gimsa stain, PAS reaction. Comparative immunohistochemical analysis of CD 8+ lymphocytes, proliferative markers: MIB-1 and PCNA were performed. We evaluated the amount of CD8+ cells in 1 mm^2 and the proliferative activity of epithelium per 1000 nuclei. 8 biopsies were examined electronmicroscopically for the detection of cell-to-cell interactions and subcellular alterations of gastric epithelium.
Results Distribution, amount and characteristics of CD8+ cells are different in mucosa around ulcer and in gastritis (G.) cases without ulcer. In active G. around ulcer CD8+ cell are 12, 4; without ulcer - 7, 7, but in inactive G.-4, 2. In Helicobacter pylori-infected subjects CD8+ are located in addition at the lymphatic follicles. We have not proved any rise of apoptosis, exfoliation or ultrastructural injuries of epithelial cells in the areas of collections of T-lymphocytes of this subtype. In sites of increased amount of CD 8+ cells there is high proliferation activity of epithelium (19,7- 29,4%) due to the broadening of labeled nuclei zone with MIB-1 antibody.
Conclusions The amount of CD 8+cells correlates with activity of gastritis as this 34 kD glycoprotein realize molecular reactions between mediators or against H.pylori, food and other antigens in lamina propria, but it has no external direct domain upon gastric epithelium, capillaries or glandulocytes.

P-308

Prognostic value of nuclear area and microvessel density in gastric cancer

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The incidence of gastric cancer decreases worldwide. Despite declining (due to intestinal type), incidence still remains the second cause of death amongst all malignancies worldwide. Diffuse type of gastric cancer shows constant incidence. Therapy and prognosis directly correlate with extent of the disease and reliable prognostic factor is still lacking. The aim of the study is to establish a value of two morphometric methods - nuclear area and microvessel density and their influence to the survival of the patients with advanced gastric cancer. 113 patients who had undergone gastric resections for gastric cancer were analysed in this study. We estimate size of the tumor, histologic type (according to Lauren's classification), stage, nuclear area (median value of nuclear areas in 100 cancer cells measured with computerised nuclear morphometry) and microvessel density (median value of F VIII- positive microvessels in five X 200 power field) for each case. Statistical analysis was performed and p-values lower than 0,05 were considered significant. Nuclear area shows statistical significant correlation with size of the tumor, tumor stage, TNM classification and metastases in regional lymph nodes, microvessel density does not. In univariate analysis to survival, size of the tumor, tumor stage and TNM classification have $p<0,05$. Nuclear area only in intestinal type of gastric cancer correlates with survival ($p<0,05$). In multivariate analysis, size of the tumor, tumor stage and TNM classification have an influence on survival. Reliable prognostic factors in patients with gastric cancer are still stage and size of the tumor. Diffuse type of gastric cancer is still an enygma. Spreading and metastazing of intestinal type of gastric cancer could be predicted using a nuclear-area method as a support in therapy planning and prognosis. The role of microvessel density in prognosis is unreliable.

P-309

Immunoexpression of C-kit and Ki-67 in GISTs

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Introduction The kit receptor normally becomes dimerized and its tyrosine kinase phosphorylated (activated) upon the ligand binding, then enabling it to phosphorylate other proteins in the signal transduction pathway that ultimately carry the proliferation signal into the nucleus. The aim of this study was the evaluation of Ki-67 (MIB-1), proliferative cell index, in correlation to c-kit stain intensity, histological grade and immunophenotype in GISTs.
Materials and methods We studied a series of 50 GISTs (c-kit positive) in correlation to age, anatomic location, size, morphological features, mitosis and necrosis. Also we stained with vimentin, CD-34, smooth muscle actin (SMA), S-100 protein and Ki-67 with ABC method on paraffin sections.
Results The average age of the patients was 62 years (ranging from 29 to 83), included 27 male and 23 female patients. 26 were located in the stomach, 20 in the small intestine, 3 in the colon and 1 in the esophagus. Microscopically the tumors showed a fusiform cytology and 12 of them showed a mixed fusiform-epitheloid cytology. The average tumor size was 6,5±4,3cm (range 1,5-20cm). We found necrosis in tumor with size>5cm. The tumor cells expressed vim 94%, CD34 68%, SMA 37%, S-100 20% and ki-67 ranging from 0-20%. The most GISTs showed c-kit positivity in 90% of tumor cells with a small minority showed more focal staining in as few as 5% to 20% of tumor cells. The stain intenisty was mild to moderate.
Conclusion In our study, the Ki-67 expression was associated with tumor size and not with c-kit staining profile, other immunophenotypic features or histological parameters.

P-310

Expression of PCNA and Ki-67 protein in colorectal carcinomas. Correlation with tumor grade

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Introduction The prognostic significance of PCNA and Ki-67 proliferation markers in colorectal carcinomas is debatable. Therefore, we evaluated immunoreactivity of the tumor for PCNA and Ki-67 antigen in 45 colorectal carcinomas with different histological grades.
Methods The paraffin embedded tissues were immunostained for PCNA and Ki-67 antigen by employing Streptavidin-biotin(LSAB) method.
Results PCNA was positive in 96% of carcinomas and localized in the nuclei of malignant cells.Immnunoreaction for Ki-67 was demonstrated in all carcinomas and mainly and strongly expressed in the nucleoli.Positive staining for PCNA and Ki-67 correlated positively with increasing grade. The labelling index was significantly higher in the poorly differentiated carcinomas than in the well differentiated tumors.
Conclusion The expression of PCNA and Ki-67 proliferation markers clearly shows differences between colorectal carcinomas of varying differentiation grades and it is useful as a diagnostic and prognostic parametar.

P-311

Collision tumours at the gastro-oesophageal junction: do they exist?

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Introduction Collision tumours have been thought to arise from the accidental meeting and interpenetration of two independent tumours. Here we present three cases of gastroesophageal junction tumours that could fill these criteria. We employ molecular and genetic techniques to ascertain the clonality of these tumours in order to determine whether they represent true collision tumours.
Methods P53 immunohistochemistry was performed, and p53 sequence analysis was carried out on RT-PCR material derived from fresh frozen tissue. Mutations were subsequently confirmed in paraffin embedded material. Standard and laser microdissection techniques provided tumour-enriched samples from which DNA was isolated. Analysis of loss of heterozygosity was performed using the microsatellite markers D3S1478, D5S346, D9S171, D10S2491, D14S68, D16D2624, D18S64, TP53, P53Alu and Bat26.
Results p53 immunohistochemistry was positive for both tumours in each case. All three tumours possessed a p53 point mutation that was identical in both components. Polymorphic microsatellite analysis confirmed shared losses of heterozygosity with an identical pattern of retention and loss at six loci in each component. Genetic differences were also observed between tumour components.
Conclusion A comparison of immunohistochemistry, p53 sequence and the pattern of LOH for a spectrum of polymorphic microsatellite markers for three possible collision tumours suggests that both components are derived from a single precursor cell that undergoes divergent differentiation in the evolution of the tumour. The evidence presented here refutes the “collision hypothesis” and introduces a new histopathological entity of a cardia tumour with distinct diffuse signet ring cell and squamous components.

P-312

The histologic spectrum of Gastrointestinal Stromal Tumors (GISTs)

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Introduction GISTs are the most common mesenchymal tumors of the gastrointestinal tract. They are histologically heterogenous group and a practical diagnostic criteria for GISTs is c-KIT (CD117) expression by immunohistochemistry. The aim of this study was to present morphological pattern of radically operated and disseminated GISTs.
Material and methods Material included 103 GISTs treated between 1999 and 2003 in Cancer Center, Warsaw. Thirty pts. had been radically operated of gastric GISTs and 10 pts. – intestinal GISTs. Fifty three pts. had peritoneal and liver dissemination. The histopathological diagnosis based on the type of cells, mitotic activity and tumor size. The immunohistochemical expression of

CD117 (DAKO), CD34 (DAKO), SMA (DAKO) and S100P (DAKO) were performed in each of the case.
Results 1. Microscopic spectrum. Majority (70%) of cases had heterogenous pattern-spindle and epithelioid cells with high mitotic activity-more than 5 mitoses per 50 HPF. The intestinal and disseminated tumors had generally spindle cell histology with variable myxoid matrix or extracellular collagen globules reffered to as skeinoid fibers. The majority of gastric GISTs were composed of epithelioid, polygonal cells with perinuclear vacuolization. 2.The immunohistochemical analysis revealed strong and diffuse CD117 expression in 99 of cases, whereas CD34 was expressed in 65 cases. Four cases were negative for CD117 and positive for CD34. A minority of the tumors expressed smooth muscle actin which was focal.
Conclusions The heterogenous histologic pattern of GISTs CD117 (+) correlated with dissemination, diameter over 5 cm and more than 5 mitoses per 50 HPF.

P-313

Carcinosarcoma of the esophagus: two cases of the unusual neoplasm

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Introduction Esophageal carcinosarcoma comprise approximately 1-2% of all esophageal tumors. This is the first published report in Poland of the unusual malignant neoplasm consisted of both carcinomatous and sarcomatous components.
Materials and methods Two patients with esophageal tumors were presented. They were analyzed using endoscopic, histological and immunohistochemical procedures. An endoscopic examination showed polypoid tumors in the lower esophagus. Esophagogatrectomy with lymph node dissection was performed. The resected specimens were fixed in 10% formalin and paraffin embedded tissue sections were stained with routine hematoxylin and eosin. Immunohistochemical analysis for vimentin, cytokeratin, p53 and MIB1 were performed.
Results Histologically, the neoplasms consisted of two components, squamous cell carcinoma and sarcomatous components. In case1, the sarcomatous component was with no specific differentiation and in case 2 resembled malignant fibrous histiocytoma. A gradual transition between two components was found. In case 1 regional lymph nodes were involved by metastases and demonstrated both elements. The spindle cells of the tumors were positive for vimentin and negative for cytokeratin. The squamous carcinoma cells were immunoreactive for cytokeratin and negative for vimentin. The tumor cells in the transitional zone between the carcinomatous and sarcomatous elements were positive for vimentin and cytokeratin. The lymph node metastases were positive for vimentin in spindle cell component and positive for cytokeratin in carcinoma component.
Conclusion In terms of histogenesis of carcinosarcoma of the esophagus, there has been controversy over whether the sarcomatous component consist of malignant non-epithelial cells or of a morphological variation of carcinoma cells. The immunohistochemical findings suggest that spindle cells component of the tumors are epithelial in orgin.

P-314

p21/WAF1/CIP1 expression in gastric carcinomas: relationship with p53 and prognosis

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Introduction The cyclin-dependent kinase inhibitor p21 is a critical downstream effector in the p53 pathway and this protein acts as tumor suppressor in a negative cell cycle regulation. Aims: We investigated the prognostic influence of p21WAF1/CIP1 reactivity in gastric carcinomas in relation to the p53 expression.
Materials and methods The expression of p21WAF1/CIP1 and p53 protein was revealed immunohistochemically in 50 gastric carcinomas. Staining patterns were assessed semiquantitatively and correlated with clinicopathologic variables and survival times. Median follow-up time was 33 months (range 2-113 months). Chi-square and Kaplan-Meier survival analysis were performed as statistical analysis.
Results The p21WAF1/CIP1 protein was detected in 21 (42%) and p53 protein in 34 (68%) of 50 cases. p21WAF1/CIP1 expression was significantly higher in stages I and II carcinomas than in stages III and IV carcinomas (p=0.01). In tumors without lymph node metastasis, p21WAF1/CIP1 expression was significantly more frequent in tumors with lymph node metastasis (p=0.007). Low p21WAF1/CIP1 expression significantly associated with p53 protein expression (p=0.002). The survival rate of patients with p21WAF1/CIP1-positive tumors was significantly better than those without p21WAF1/CIP1 expression (p=0.02). In patients with p53 positive tumors showed significantly poorer prognosis than in patients with p53 negative tumors.
Conclusion The status of p21WAF1/CIP1 expression may have prognostic value in gastric carcinomas and evaluation of the expression of both p53 and p21WAF1/CIP1 might provide more prognostic information.

P-315

Altered distribution and decrease of interstitial cells of Cajal in patients with colonic inertia

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Introduction The normal motility of the gastrointestinal tract depends on the enteric nervous system, the smooth muscle layers, and the interstitial cells of Cajal (ICCs). The aim of this study was to investigate both the distribution of interstitial cells of Cajal and the pathohistology of the enteric nervous system in colonic inertia.
Materials and methods Large bowels were obtained from 8 patients with colonic inertia. The patients have been treated surgically with total abdominal colectomy and ileorectal anastomosis. Control large bowel specimens were collected from 4 patients with non-obstructing neoplasia. Colonic specimens were investigated with monoclonal anti-neurofilament antibody and CD34 antibody and compared with those of control patients. The analysis of ICCs included registration of the number and process length of the myenteric and muscular ICCs.

Results In the patients with colonic inertia the myenteric plexus showed moderate or severe hypoganglionosis compared with the control group. The apparently normal axon bundles in the myenteric plexus stained markedly less than normal ones with monoclonal antibody. Myenteric ICCs were evident as single cells or cell clusters closely related to the small myentric ganglia. The ICCs did not form the typical networks seen in the normal bowel. Muscular ICCs were markedly reduced in number compared to the number seen in normal bowels and were mainly expressed at the innermost layer of the circular muscle layer.
Conclusion The enteric nervous system and ICCs are altered in colonic inertia and may play a crucial role in the pathophysiology of colorectal motility disorders.

P-316

Immunohistochemical detection of the hMLH1 and hMSH2 proteins in hereditary and sporadic colon cancer tissues

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Introduction Germline mutations of hMLH1 and hMSH2 mismatch repair genes (MMR) are associated with hereditary non-polyposis colon cancer (HNPCC). Somatic impairment of these genes can be detected in sporadic colon cancers (CC). All these tumours are characterised by microsatellite instability (MSI). The aim of this study was to correlate the results of immunohisto-chemical detection of hMLH1 and hMSH2 protein with results of mutational and MSI analyses.
Patients and methods There were included: 21 CC samples from hMLH1/hMSH2 germline mutation carriers, 36 CC samples from non-carriers, and 16 CC samples from patients not subjected to mutational analysis. Immunohistochemistry was performed using mouse monoclonal antibodies (anti-hMLH1, clone G168-15; anti-hMSH2, clone G219-1129, Pharmingen, USA). MSI was analyzed at ABI Prism 310 Genetic Analyzer, mutational analysis by DNA sequencing.
Results Loss of nuclear protein expression was detected in all 21 tumours from mutation carriers, all of these exhibiting a high grade of MSI (MSI-H), and in 8 of 11 MSI-H tumours from the other patients. None of the 41 tumours with stable microsatellites or a low level of MSI exhibited a nuclear protein loss. Immunohisto-chemical analysis was able to detect MSI-H tumours with a 100% specificity and a 84% sensitivity.
Conclusion Immunohistochemical detection of hMLH1 and hMSH2 proteins is a specific method of detection of tumours with MMR gene inactivation and might be used as a screening method of HNPCC. The 3 MSI-H cases with retained nuclear protein expression might express non-functional protein or carry another MMR gene defect. Suppported by NC6741-3.

P-317

Study of mucin changes in colorectal adenoma-carcinoma sequence

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Introduction Mucins are known to have a protective role for the colorectal epithelium against extrinsic noxious factors (including carcinogens), changes of its characteristics being an early process in the adenoma-carcinoma sequence. Aims: Quantitative and qualitative evaluation of mucin changes in hyperplastic polyps (HP), adenomatous polyps (AP) with different degrees of dysplasia and adenocarcinomas (AC) of colon.
Material and methods We selected 137 colorectal endobiopsies (22 HP, 42 AP and 67 AC). Thin serial sections were stained with hematoxylin-eozin, van Gieson and Alcian blue (AB) pH1 (for strongly sulphured mucins) and pH2.5 (for acid mucins: weakly sulphured mucins, hyaluronic acid and sialomucins). Observations were made only on intracellular mucins. Intensity of the stain was analyzed and compared with non-tumoral (normal) adjacent mucosa.
Results and discussions Normal mucosa and HP showed a preponderance of sulphomucins (AB pH1) comparing with sialic acid (AB pH2.5). In AP (especially in those harboring high-grade dysplasia) we detected sialomucins in 72.96% cases and the depletion of sulphomucins (56.22%). In AC sialomucins were identified in 61.34% cases, while sulphomucins only in 36.84% cases. Significant depletion of sulphomucins occurs in AP with moderate and severe dysplasia and in AC, but it was also observed in AP with mild dysplasia. This suggests the possibility that without a proper mucin secretion consisting mainly in strongly sulphured mucins, colorectal epithelium is more vulnerable to environmental carcinogens.

P-318

Histological and immunohistochemical analysis of gastrointestinal stromal tumors (GIST)

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Introduction Gastrointestinal mesenchymal tumors are composed mostly of undifferentiated long spindle cells with fibrillar cytoplasm, with multidirectional differentiation. The term GIST was accepted.
Material and methods At the Institute of Pathology, Faculty of Medicine, Skopje, we analyzed 10 cases of GIST from 1998-2002. This study is a continuum of the analysis until 1997. We analyzed biopsy and operative materials from the stomach and small bowel. We used the following histochemical stainings: HE, Van Gieson, reticulin, as well as immunohistochemical stainings with the following antibodies: CKWS, LCA, S100, actin, desmin, NSE, chromogranin, synaptophysin, vimentin.
Results and conclusion The analyzed group of cases consists of patients of age between 21-84 years with male predominance (6:4). Four of the analyzed ten cases were located in the stomach and 6 in the small bowel. 7 cases were benign, histologically composed of bundles of spindle cells with slightly pleomorphic nuclei and eosinophilic granular cytoplasm, with hyalinised collagen between

the cells. The mitoses were either rare or absent. Immunohistochemical analyses were most frequently positive for S100, actin, desmin and synaptophysin. Three of the cases were ranked as malignant, using the following diagnostic criteria: size of the neoplasm, existence of metastases and histological appearance with anaplastic spindle cells arranged in bundles and whorls with high mitotic index (10 mitoses per 10 HPF) and marked positivity for actin, desmin and vimentin.

P-319

What is the relation of age, gender and histological types to the subsite location of colon carcinoma?

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Introduction Characteristics that determinate the anatomic site within the colon where the carcinoma is most likely to occur would be very useful in choosing optimal factories for screening of colon cancer /CC/. Aims: To study any association of gender, age or histological types with CC in the left or in the right colon.
Materials and methods Retrospectivitaly are studied cases of CC in NMTB between 1994 and 2002.The age and the gender of patients were determined. Localization, histological type and TNM staging of the cancer are identified on the histological slides.
Results 302 cases of CC at the age between 18 -92 years old are diagnosed. Gender, age and histological type were independently significantly associated with the frequency of the development of right and left colon carcinoma. The gender distribution of CC is in benefit of male independently of sub site location as they are suffered of CC in the younger age in comparison with the female. Independently age, gender and localization prevails the histological type adenocarcinoma with different morphological and functional differentiation. One CC in the left colon is developed on the background of FAP. Solitary are the cases with congenital FAP and Lynch II. In the most cases CC was diagnosed in advanced stage. Solitary is the cases with Tis and T1.
Conclusion We consider: 1/CC is more frequently in the male colon; 2/in CC predominate histological type of adenocarcinoma; 3/CC is developed in the middle and in the age advanced in years; 4/ cases with multicentrical CC with metachronic carcinoma are solitary.

P-320

Cathepsin B and L expression in colorectal carcinomas: relationship with tumor progression and clinico-pathological parameters

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Introduction Cathepsin B (CB) and L (CL) are cysteine peptidases with capacity of degrading extracellular matrix. CB expression in colorectal cancer (CRC) has been related with tumor progression. There are no data about the CL immunohistochemical expression. The aim of this study was to analyse the expression of both CB/L in CRC and to relate these findings with clinico-pathological data.
Material and methods Epithelial and stromal immunohistochemical expression of CB and CL was studied in 97 and 57 samples respectively. CB was analysed in 15 adenomas. Global and local infiltrating edge data were recorded. Clinico-pathological parameters were correlated with the immunohistochemistry.
Results CB/L expression in normal epithelium was apical, with scarce macrophages in the lamina propria. An increase of both CB/L was observed in tumoral stroma, with higher CB at the infiltrating edge. The both global and deep stromal CB increased according to the T ($p<0,005$). Neoplastic epithelial CB revealed a basal stain whereas CL was occasional. Epithelial CB tended to show high expression in T1 (like high grade dysplasia) and low expression in T2 that increased progressively in T3-4 ($p=0,058$). No differences were evidenced either as a function of N state, gender, site, differentiation or histological type.
Conclusions CB/L immunoexpression in colon have a distinctive pattern which is altered in carcinoma. Whereas increased CB stromal expression ise related with local progression, epithelial positivity seems to play an important role in the first steps of carcinoma. High stromal CL isassociated with carcinoma but CL epithelial expression seems not to be contributory.

P-321

Concomitant presence of gastric signet ring and breast ductal carcinoma

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Introduction New approach on estrogen and progesteron receptors of the gastric signet ring cell carcinoma explains its histogenesis and poor prognosis. The pointed out facts are the reason for this study.
Material and methods The material was obtained from surgically resected stomach, together with ovaries. After two months from gastrectomy right mastectomy has been done. The specimens were fixed in 10% formaldehyde and embedded in paraffin. The sections were stained with HE, PAS, HID-AB pH=2,5 methods and ABC using estrogen and progesteron antibodies (Dako).
Results Histologically, mucin-producing signet ring carcinoma that diffusely infiltrates the antral wall and the ovaries known as Krukenberg tumor, has been found. Histochemically, type B signet ring cell carcinoma was discovered: cells contained blue positive (sialo) mucin. Ductal type of breast carcinoma with lymph node metastases also was found. Immunocytochemically, estrogen and progesteron positive receptors were discovered in cancer cells of the stomach, breast and ovaries.
Conclusions 1.Concomitant presence of gastric signet ring and breast ductal carcinoma, as well as the presence of estrogen and progesteron receptors in both, signet ring cells and cancer cells of the breast, confirm the role of ovarian hormones in the pathogenesis of gastric signet ring cell carcinoma and, in the same time open the door for new adjuvant therapy. 2.Sialomucin, characteristic of B signet ring type carcinoma, is responsible for its poor prognosis.

P-322

Gastric MALT lymphoma: histological features before and after eradication therapy

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Introduction Primary gastric low-grade marginal zone B cell lymphoma of MALT type is a distinct disease entity with a characteristic histological presentation and clinical behavior. The development of gastric MALT lymphoma is dependent of H.pylori infection. Many reports have stated that the cure of H.pylori infection can induce a complete remission of low-grade gastric MALT lymphoma. We performed a prospective study to evaluate the outcome of patients with low-grade gastric MALT lymphoma and the histological characteristics before and after therapy.
Materials and methods We prospectively enrolled 20 patients with H. pylori positive low-grade gastric MALT lymphoma (Stage I 1e except one patient in Stage II 1e). Paraffin sections were stained with: HE, Giemsa, PAS and immunohistochemical LAB methods. The patients were examined by endoscopic ultrasound before and after eradication therapy.
Results Endoscopically, enlarged gastric folds, erosions or ulcerations, hyperaemia or intragastric nodularity, with preferential localization in the antrum, were described. Histologically, the neoplastic cells infiltrated between pre-existing lymphoid follicles, then colonized the lymphoid follicles. The resemblance of these cells to the centrocyte has led to the term “centrocyte-like (CCL)” cell-neoplastic component of MALT lymphoma. In some cases the cells had monocytoid appearance, but plasma cell differentiation also was prominent. Typical lesion for MALT lymphoma – “lymphoepithelial lesion”, was frequent, representing infiltration of the glandular epithelium by clusters of neoplastic lymphoid cells with associated destruction of gland architecture and morphological changes within the epithelial cells, including increased eosinophilia. After the eradication of H.pylori, the patients were regularly followed-up with endoscopic and histological assessment. 18 patients achieved complete remission within 8 weeks, one had partial gastrectomy induced by ulcer with severe dysplasia, but with regression of MALT lymphoma (within 16 weeks), but the remission of the patient with Stage II 1e was achieved within 12 months. Lamina propria appeared “empty” with gland loss. Scattered lymphocytes and plasma cells were seen within the lamina propria and there were nodular collections of small lymphocytes. Lymphoepithelial lesions were scanty or absent.
Conclusions The time taken for achieve remission of gastric MALT lymphoma in these patients varied from 2 to 12 months. The only significant prognostic variables were stage and tumor-grade.

P-323

Comparative evaluation of the prognostic value of MUC1 and MUC2 antigens in colorectal adenocarcinoma

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Introduction During the last decade, information accumulated regarding the experssion of human mucins. MUC1 belongs to the group of membrane bound mucins. It is assumed to play various roles in tumor immunology. MUC2 is marker of goblet cell or mucinous differentation of adenocarcinoma. The significance of MUC1 and MUC2 as prognostic markers in colorectal adeno-carcinoma with mucinous differentation and mucinous adeno-carcinomas was studied because previous investigations revealed inconsistent results.
Materials and methods Tissues from 20 operated patients with mucinous or mucinous differentiation of colorectal adenocarcinomas were investigated. All specimens were classified according to WHO and TNM classification, including lymph node status and distant metastases at the time of the surgical intervention. Formalin-fixed paraffin-embedded colorectal cancer tissues were cut (5μm) and deparaffinized acording to standard histological techniques. Classical HE, histochemical PAS and HID-AB pH=2,5 and immunohistochemical BSP techniques were applied. The obtained results were evaluated by the x^2 test.
Results The MUC1 immunoreactivity showed a strong correlation with tumours progression (as suggested by advancing TNM stages) and also de-differentiation (as reflected by tumorous grading). Localization and histological grading were also statistically signifficant. On the other hand, MUC2 did not show any association with the data concerning prognosis.
Conclusions According to our data, only MUC1 presents as an independent prognostic factor of colorectal adenocarcinoma. MUC1 should therefore be considered as a potential target of immunotherapeutic strategies.

P-324

Sporadic form of colon cancer of a 12-year-old boy

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Introduction Sporadic form of colon cancer is one of the most frequent cancers in adults, but very rare in children; thus we report this case.
Case report We describe the case of a 12-year-old boy who had the agressive sporadic coecal cancer, surrounded by nodular lymphoid hyperplasia.The boy underwent to partial colectomy cum omentectomy during the night with clinical diagnosis “acute abdomen”. The surgical-pathological stage was: Stage III (T3NlMo). The patient received postoperative therapy, but he died with liver metastases eight months from surgery.
Materials and methods Formalin-fixed paraffin-embedded coecal cancer and surrounding nodular tissue were cut and deparaffinized according to standard histological techniques. HE, PAS, HID-AB pH 2,5 and immunohistochemical LAB techniques were applied.
Results Fungous advanced carcinoma, 28 mm in diameter, induced stenosis and clinical symptoms of occlusion. Histologically, it was poorly differentiated adenocarcinoma with high mitotic index(high activity of Ki-67) and neuroendocrine differentiation; cancer cells were diffusely positive for p53 protein. Immunological reaction to MUC2, MUC5AB and MUC6, as well as to B lymphocytes, was negative. Microscopic examination of surrounding MALT showed

large, confluent lymphoid follicles without germinal centres within lamina propria, causing nodular appearance of the overlying mucosa. B lymphocytes were markedly depressed in this nonhereditary form of colon cancer.
Conclusions The authors have suggested that antibody deficiency, environmental, endocrine and genetic factors cooperated in the development of this nonhereditary coecal carcinoma.

P-325

The histological findings in the gastroesophageal junction of fetuses

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Introduction The histopathological concept of the gastroesophageal junction has been changed by American pathologists providing the evidence that the gastric cardia is not a physiological structure. Instead, it develops through glandular metaplasia of the squamous epithelium of the distal esophagus due to gastroesophageal reflux.
Methods, results and conclusion The development of the esophageal and gastric mucosa in the gastroesophageal junction was studied in 61 fetuses of 13 – 41 weeks of the gestational age. During the 13th –15th week, the esophageal multilayered epithelium was covered by a continuous layer of columnar mucous ciliated cells which were present only focally till the 25th week and disappeared later. Before the 15th week, the gastric mucosa was formed by pits only. The glands started as proliferating tubules in the basal parts of the pits in the 15th week. Further, they differentiated into oxyntic glands. The mucosa of the corpus was fully developed in the 27th week. The cardiac mucosa was absent in all the 10 fetuses examined between the 27th and 41st week of gestation. This supports the view that the gastric cardiac mucosa is not a physiological structure but that it results from glandular metaplasia of the distal esophageal mucosa due to gastroesophageal reflux.

P-326

ABO and Rh blood groups distribution and correlation with pathologic stage of colon adenocarcinoma

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Introduction There is not enough studies about relationship between colon cancer and ABO and Rh blood groups in compare to gastric cancer. Therefore in this study we will examine ABO and Rh blood groups distribution and correlation of them with pathologic stage of colon adenocarcinoma.
Methods Data on age, sex, ABO and Rh blood types and pathologic stage (wall thickness involvement and involvement of lymph nodes) of the 232 patients with colon adenocarcinoma; were under surgical operation, were collected from three Tehran hospitals. And also data on ABO and Rh blood groups from 1500 healthy persons from above hospitals collected by randomized selecting as control group.
Results The distribution of ABO blood groups in patients was different from control group; However, blood group A in patients was 5.3% higher than the corresponding control. There was no significant association between ABO blood groups and pathologic stage of colon adenocarcinoma. The distribution of Rh in patients was likely equal with control group; Although, there was a significant relationship between Rh (+) and wall thickness involvement ($P<0.001$) and involvement of lymph nodes ($P<0.021$).
Conclusion 1- ABO blood group A is more common in patients with colon adenocarcinoma. 2- Rh(+) has correlation with poor prognosis in colon adenocarcinoma.

P-327

Vascular endothelial growth factor, microvessels count and overexpression of p53 protein in colorectal carcinomas

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Introduction Vascular endothelial growth factor (VEGF) is a cytokine involved in tumor angiogenesis. Compelling data implicate that deregulation of p53 protein function may be associated with increased neovascularization and aggressive tumor growth. The aim was to investigate the prognostic significance of VEGF overexpression and microvessel count (MVC) at the deepest invasive site in colorectal carcinoma tissue as well as their relationship with p53 protein expression in tumor tissue.
Material and methods Surgical specimens of 81 operated colorectal carcinomas were studied by immunohistochemical methods for detection of VEGF and p53 protein in tumor tissue and CD31 positive microvessels. Median folow up was 31.4 month (range, 2-66 months).
Results VEGF expression and nuclear p53 protein overexpression were found in 66,7% and 58% of colorectal carcinoma. VEGF and p53 protein positive status was identical in 75.9% of tumors. The Dukes stage C and high MVC were significantly more frequent in VEGF positive tumors (53.7% and 64.8% respectively) comparing with VEGF negative tumors (25.9% for both Dukes stage C and high MVC). Hypervascularity significantly correlated with advanced Dukes stages, but not with the p53 protein expression. Survival analysis showed that VEGF and p53 protein expression as well as hypervascularity of colorectal carcinomas correlated with poor survival ($p= 0.01$).
Conclusion VEGF is an important angiogenic factor in colorectal carcinoma, and its expression being dependent on p53 protein expression. Our data suggest that combined analysis of VEGF, p53 protein and MVC may be useful for predicting the more aggressive biological behaviour of colorectal carcinoma.

P-328

Occult involvement of lymph nodes in the splenic hilus and along the splenic artery in patients with proximal gastric carcinoma

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Introduction The aim of this study was to find out the incidence of occult metastatic involvement in lymph nodes in the splenic hilus (lymph node group 10 according to JRSGC) and along the splenic artery (lymph node group 11 according to JRSGC) as well as to identify pathological features that might be prognostic factors associated with involvement of these lymph nodes in the patients with proximal gastric carcinoma.
Material and methods Total of 24 patients with advanced proximal gastric carcinoma and up to 6 positive perigastric lymph nodes (T2/T3 N1 category) have been studied in the period between 1996 and 1998. Histopathological features (tumor size, macroscopic and histologic type, grade of differentiation, depth of penetration, invasion of intramural lymphatics and veins) as well as lymph node involvement have been routinely examined according to TNM and JRSGC systems. Detection of microcarcinosis (occult lymph node involvement) in lymph nodes groups 10 and 11 has been performed by multiple serial sectioning and using immuno-histochemistry (monoclonal antibodies against cytokeratins 8 and 18, LSAB+ technique) and classified according to modified Kikuchi criteria.
Results Occult lymph node involvement in lymph node group 10 has been observed in 7 patients (29,2%). Three of them (12,5%) had only single cell micrometastases (G1 type of microcarcinosis) and 4 patients (16,7%) had micrometastatic deposits greater than 200 microns (G3 type of microcarcinosis). Occult lymph node involvement in lymph node group 11 has been observed in 2 patients (8,4%) expressing single cell micrometastases in one case and micrometastatic cell clustering (up to 200 microns in diameter) in another case.
Conclusion Occult involvement of lymph node groups 10 and 11 could be expected in lymph node positive patients with more than three positive perigastric lymph nodes. In the patients with advanced proximal gastric carcinoma examined pathological tumor characteristics could not be associated with the involvement of lymph node groups 10 and 11.

P-329

Lymph node micrometastases in patients with gastric carcinoma: immunohistochemical evaluation

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Introduction For patients who underwent curative resection for gastric carcinoma, lymph node status seems to be a major prognostic factor of disease recurrence. Contrary to histologically overt lymph node metastasis, the biological significance and prognostic relevance of micrometastatic lymph node involvement are still controversial.
Material and methods We examined frequency and distribution of micrometastases in 883 lymph nodes obtained from 27 patients with advanced gastric carcinoma (32,7 lymph nodes per patient) without primarily histologically overt lymph node metastases. Identification of lymph node micrometases was performed using multiple serial sectioning and immunohistochemical detection of cytokeratin 8 and 18 positive cells. The results were correlated to tumoral histological type, growth pattern, depth of penetration and vascular invasion.
Results The overall detection rate of micrometastases showed their occurence in more than half of examined patients (15/27 or 55,55%) and in 10,87% of examined lymph nodes (96/883). Most of them were so-called single cell micrometastases (6,34%), followed by cluster-type of micrometastases up to 200 microns (4,07%) and few greater than 200 microns (0,45%). The incidence of micrometastases was 59,09% (13/22) in Lauren`s diffuse type of carcinoma contrary to 40% (2/5) in Lauren`s intestinal type of carcinoma. In addition, there was no significant difference in occurence of micrometastases in cases with and without penetration of muscular layer, contrary to cases with serosal tumoral involvement and obvious vascular invasion.
Conclusion Detection of micrometastases might influence tumour stage migration to TNM stage III in further 10% of examined patients with primarily stage II determined diseases. The frequency of micrometastases seems to be closely correlated to poorly differentiated carcinomas, especially Lauren`s diffuse type of carcinoma and higher pT stages, including serosal involvement. However, possible independent prognostic significance is still to be elucidated after survival analysis of larger series of these patients` subgroups.

P-330

Gastrointestinal stromal tumors of the stomach

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Introduction Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract with the stomach as the most common site. The term 'GIST' is preferentially used for the tumors that express CD34 and CD117/c-KIT and the latter has been suggested as a specific marker for GISTs. As GISTs constitute a group of phenotypically heterogenous mesenchymal neoplasms with uncertain biological behaviour, we reevaluated their histogenetic differentiation and proliferative status.
Material and methods We reexamined clinically, histologically and immunohistochemically 33 cases of paraffin-embedded GISTs noted in the Tumor Registry of Clinical Centre of Serbia between 1997 and 2001, and especially in regard to their proliferation, measured by mitotic index and immunohistochemically by Ki-67 and PCNA expression scores. In addition, evaluation of malignancy of GISTs was based on mitotic count greater than 5/50 HPF and/or tumor size greater than 10 cm and extra-gastrointestinal spread.
Results Most of gastric GISTs were composed of spindle cells (73%), epithelioid cells (20%) or both cell types (7%), and nearly 30% showed obvious clinical malignant behavior. Immunohisto-chemical examination showed strong CD117 expression in 93%, CD34 in 66,7% (two of cases exlusively CD34 positive) in non-myogenic and non-neurogenic tumors. In addition, in GISTs with CD117 expression, focal expression of S-100 was found in 26,6%,

focal SMA in 33% and desmin in 6,7% of cases. Ki-67 and PCNA indices were correlated to mitotic index and other criteria of malignancy.
Conclusion Phenotypic differentiation of GISTs toward smooth muscle were found to be correlated with proliferative indicators of poor prognosis more consistently than those toward partial/focal neurogenic differentitation or uncommited type.

P-331

Deep topographic compartments of colorectal adenocarcinomas: Down-regulation of mlh1/msh2 expression results in lack of physiologic cell kinetic balance

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Background Topographic tumor cell selection is not well characterized in colo-rectal adenocarcinomas (CRC). The contribution of mlh-1/msh-2 expression to cell kinetics by topographic compartments remains unknown in sporadic CRC.
Design We selected 90 (73 low-grade, 17 high-grade) consecutive sporadic CRC, including 48 stage I, 18 stage II, and 24 stage III. Mitotic figure counting, Ki-67 index, G2+M phase fraction, in situ end labeling (ISEL) of DNA fragments, and mlh-1/msh-2 immuno-expression were scored by topographic tumor compartment (above muscularis propria vs. muscularis propria). Statistical correlation between proliferation and apoptosis variables was performed in each topographic compartment. Variables were studied by topographic compartments and considered statistically significant if $P<0.05$.
Results Results by topographic compartments appear in the table.
Kinetic results by topographic compartments:

	Superficial	Deep	Significance
MF/50 HPF	117.08±84.92	67.62±56.10	0.0001
Ki-67 Index (%)	29.88±11.02	21.31±12.03	0.0001
ISEL Index (‰)	9.57±4.85	4.14±2.61	0.0001
G2+M (%)	11.67±7.54	10.98±6.43	NS
mlh1 (%)	18.04±15.55	6.95±8.12	0.0001
msh2 (%)	13.47±16.71	4.92±8.58	0.0001

Physiologic correlations were preserved only in the superficial compartment for mitotic figures, Ki-67 expression, ISEL index, and mlh1/msh2 expression. G2+M phase fraction correlated with mlh-1 expression only in superficial compartments ($P=0.002$) and msh-2 expression only in deep compartments ($P=0.047$).
Conclusions Superficial compartments of sporadic CRC are characterized by high cellular turnover and maintained cell kinetic balance. mlh-1 and msh-2 expression in sporadic CRC is inefficient (no G2+M phase fraction differences by topographic compart-ments) and is dissociated (only one gene product correlates with G2+M), eventually resulting in mutation accumulation and cell progression.

P-332

Gastro-Intestinal Stromal Tumors (GISTs) classifications and their predictive value

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The biologic behaviour of GISTs has been difficult to predict based on the original criteria proposed in the early 1990s. Many isolated histological features, such as site, size, extent of bowel-wall involvement, necrosis, cellularity, mitotic counts, atypia, etc., had to be evaluated and integrated into a single and reproducible result that had also to be as accurately predictive as possible. Therefore numerous studies have been performed in order to reach the ideal combination of factors that may predict these tumors correctly. 55 GISTs were diagnosed either originally or retrospectively after reviewing diagnoses of GIT spindle cell tumors, examined in our Pathology Department between the years 1988-2002. All tumors were defined as GISTs only when displaying c-KIT (CD117) immunopositivity. Additional immunohistochemistry was performed to corroborate the diagnosis. We reclassified the tumors according to different proposed classifications and correlated the results with the clinical followup available for these patients. The followup periods ranged between 2-100 months for gastric GISTs (mean-32.2 months, median-24.5 months) and 6-62 months for patients with small-bowel GISTs (mean-32.3 months, median-30 months). While the median disease-free survival was 27.6 months for patients with malignant gastric GISTs, it was only 5 months for patients with small-bowel malignant GISTs. The results of the study show that all classifications stratified the patients in a very similar manner although some were more accurate than the others and more user-friendly and therefore probably also more reproducible. The relative high rate of deaths occuring in the small-bowel GIST group (45%) as compared to the 15.4% death rate in the gastric GIST patients, strengthens the supporters of different criteria to be applied to the GISTs arising in these different sites. Another important issue that has imerged in our study was the much higher incidence (80%) of malignant GISTs developing in the younger age group (20-49years), compared to 26.3% malignant GISTs in the age group of above 70 years, making this feature to be probably of major significance. It should therefore be considered whether age should be a built-in criterion when evaluating the biological potential of a GIST.

P-333

Epithelial metaplasias circle gastric ulcer disease

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Introduction Gastric ulcer disease is the most important disease of the digestive tract becouse this illnes have big frequecy and circle gastric ulcus presents many histologic features characteristics which may be precancerous lesions. Gastritis is lesion which is always associated with gastric ulcer. Epithelial metaplasias are transformation of one kind of epithelium to other type epithelium and circle gastric ulcus it is very frequency changes of epithelium. The aim of this study was to to find frequency of appearance epithelial metaplasias circle gastric ulcus, types of metaplasias and grade of gastritis. Also we searched changes of epithelial mucins. We had citochemistry examination endocrine G, EC and D cells and we wanted to describe immunohistochemicaly feature characteristics in different kinds of epithelial metaplasias.
Material and methods For examination we use biopsy material:surgery and endoscopic of 100 patients with gastric peptic ulcer. We use standard HE method for identification micromorpholic changes; AB-PAS (pH 2,5) and HID-AB method for illustration biochemistry alteration about mucins;Grimelius (1968) and Masson citochemistry methods; immunocytochemistry Avidin-biotin

peroxidasa (ABC) with antiserotonin, antigastrin, antisomatostatin.
Results We found 16 cases completly mature intestinal metaplasias (IM), 5 cases incompletly IM;1 case imature IM and 1 case imature IM with displasia. Also we detected composition completely and incompletly IM in 2 cases, composition incompletly and pyloric metaplasia in 1 cases.We find pyloric metaplasias in 9 cases, colons metaplasia in 2 cases. Composition colons and incompletly mature IM were detected in 2 cases;composition pyloric and incompletly mature IM in 1 case.Summary the bigest frequency of appearance metaplastic alteration were in older patients.Histologicaly claracteristics metaplasias were imitation some segments of digestive tract. Metaplasias include many biochemicaly alterations and it can be present with changes in secretion of mucins.Gastric metaplasia is dynamic process with changes both in epithelial and endocrine cells.
Conclusion Metaplasias in gastric mucosa circle gastric ulcer is sign very important alteration.Standardisation of research this alteration may be very important guider about prognosis for patients with gaster ulcer disease.

P-334

Choice of immunohistochemical method for detection of defect mismatch repair system

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Introduction Defects in the Mismatch Repair (MMR) system is involved in the pathogenesis of Hereditary Non Polyposis Colorectal Cancer (HNPCC) and in 15% of sporadic colorectal cancers, and in the latter the defect is associated with a better prognosis but posssibly also a higher risk for metachronous cancer and a different response to adjuvant chemotherapy. Screening for such defects can be done by testing for microsatellite instability, but immunohistochemistry gives similar results with less costs. The aim of this study was to select the optimal antibodies and methods for immunhistochemical detection of proteins involved in the MMR system.
Material and methods Twelve antibodies (4 against hMLH1, 5 against hMSH2, 2 against hMSH6 and 1 against PMS2) were tested using different dilutions and HIER-techniques on a material consisting of paraffin embedded tumours from 21 patients with well defined mutations and from 7 controls. The results were evaluated semiquantitatively.
Results The following clones, dilutions and pretreatment regimens were selected:

MLH1 (G168-15, BD Pharmigen)	1:50	EDTA 1mM, pH 8
MSH2 (27, Transduction Lab.)	1:400	EDTA 1mM, pH 8
MSH6 (44, Transduction Lab.)	1:200	EDTA 1mM, pH 8
PMS2 (A16-4, BD Pharmigen)	1:100	EDTA 1mM, pH 8

Conclusion All chosen antibodies gave a specific and reproducible staining. There were no false positive or negative results. The panel can be recommended for screening for defects in the MMR-system.

P-335

Immunoexpression of MUC1 and MUC2 mucins in advanced gastric cancer

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Introduction Mucins are a group of glycoproteins having a central polypeptidic structure and O-linked carbohydrates. To date, 12 MUC genes have been described that encode secreted and/or membrane associated epithelial mucins. In the normal gastric mucosa, MUC1 has been detected in the superficial foveolar epithelium, while MUC2, a marker of intestinal differentiation, has been reported as absent. In gastric carcinomas, the presence of MUC1 as well as de novo expression of MUC2 has been described. Aim: The relationship between the immunoexpression of MUC1 and MUC2 and histopathologic subtypes of gastric carcinomas was investigated.
Materials and methods According to Lauren's classification, a series of 45 cases of gastric carcinomas (30 of intestinal type and 15 of diffuse type) was studied. The percentage of stained neoplastic cells was graded: (-) 0-5%; (+) 5-50%; (++) >50%. MUC1 and MUC2 were demonstrated using monoclonal antibodies (NCL-MUC1, clone Ma695, 1:100; NCL-MUC2, clone Ccp58, 1:200; Novocastra).
Results Staining for MUC1 was present in 25 of 45 cases (55.5%) gastric carcinomas. In addition, the expression of MUC1 was significantly higher in the intestinal type (21/30) than in the diffuse type (4/15) ($P<0.01$). On the contrary, MUC2, expressed in 20 of 45 (44.4%) gastric carcinomas, showed a significantly higher positivity in diffuse type (12/15) than in the intestinal type (8/30) ($P<0.01$). Moreover, the co-expression of MUC1 and MUC2 was observed in eight cases of the intestinal type gastric carcinoma.
Conclusions Our results indicate that diffuse gastric carcinomas have features consistent with intestinal differentiation as documented by MUC2 expression.

P-336

Colon's metaplasia of gastric mucosa circle ulcer disease

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Introduction Colon's metaplasia may be variant of intestinal metaplasia in the gastric mucosa circle gastric ulcer.It is important precancerous stadium because 80 % colon's metaplasias of gastric mucous membrane transverse in gastric carcinoma. Aims: We want to find frequency of appearance colon's metaplasia in gaster mucosa with ulcer disease.Also we want to describe histologicaly, histochemicaly and immunohistochemicaly feature characteristics colon's metaplasia in gastric mucosa.
Materials and methods For examination we use biopsy's matherial:surgery's and endoscopic of 100 patients with gastric peptic ulcer. We use standard HE method for identification micromorfologic changes; AB-PAS (pH 2,5) for illustration biochemistry alteration about mucins;Grimelius citochemistry method;Imunocytochemistry Avidin-biotin peroxydasa with antiserotonin, antigastrin and antistomatostatin.
Results We find two cases so-called colon's metaplasia gastric mucous membrane. It was males in VI decade of life.Both of them were detected near parts with intestinal metaplasia and atrophic gastritis. Histology's design metaplastic epithelium appear big changes than normal gastric mucosa watched on cellular and tissue level;it was alike epithelium of colon. There was winding and arborescent cryptas, and wasn't find villosity. Cells in cryptas were voluminous and they group. Predominant componentof cells were

goblet cells. Cylindric cells also was alike goblet cells. We didn't see Paneth's cells. Histochemycaly we detected sufficiently sulphomucins and less sialomucins. G cells and ECL cells were insufficient; EC cells were less multiplied.
Conclusions Colon's metaplasia is sign of big intracellular alteration and we can detect this with changes in secretion of mucin. Colon's metaplasia gastric epithelium is dynamic process with changes both in epithel and endocrine cells.

P-337

Upregulated expression of RELP, a REG-like protein, in inflammatory, metaplastic and neoplastic gastrointestinal mucosa

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Material and methods We screened expressed sequence tag (EST) databases to detect genes specifically expressed in the gastrointestinal tract.
Results We identified a gene, called RELP (regenerating protein (REG) family-related protein). The RELP cDNA encodes a 158-amino-acid preprotein with a 22-amino-acid signal peptide. The amino acid sequence of RELP is 30-40% identical and 40-46% similar to the previously characterized members of the REG protein family. The REG proteins, which all belong to the family of C-type lectins, have been implicated in cell proliferation, migration and differentiation in the gastro-intestinal tissues. The RELP gene spans 17.5 kilobases and maps to chromosome 1p12-13.1, whereas the genes of the previously known REG proteins cluster on chromosome 2. Dot-blot hybridisation revealed expression of RELP mRNA mainly in the gastrointestinal tract, but also in prostate, and testes. Immunohistochemical staining demonstrated expression of RELP in mucosal neuroendocrine cells of in the small intestine and in the parietal cells of normal gastric mucosa. Regenerating borders of gastric ulcera, the goblet cells of inflammatory mucosa in ulcerative colitis and Crohns disesase, showed a strongly upregulated expression of RELP. Areas of intestinal metaplasia in the stomach and esophagus also displayed a robust content of RELP. More-over, RELP was strongly expressed in mucocellular and mucinous tumors of the gastrointestinal tract. The orthologous gene for mouse RELP was also cloned.
Conclusions RELP represents a novel marker of inflammatory, metaplastic and neoplastic changes in the GI-tract.

P-338

Current approach to the biopsy diagnosis of gastrointestinal stromal tumors (GISTs) - is it possible to predict clinical behaviour?

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Introduction GISTs are the most common GI mesenchymal tumors showing CD117 positivity. Although according to accepted criteria none of them are 'benign', their metastatic risk depends on the tumor size (TS) and mitotic count (MC). Aim: Reclassification of a series of GISTs using morphological and immunohisto-chemical analysis of paraffin-embedded material and evaluation of the relevant clinical data.
Material and methods 32 patients with GISTs fulfilled the criteria of Fletcher et al. (2002). In addition to histological stainings, primary antibodies against vimentin, desmin, actins, S-100 protein and CD117 (all DakoCytomation) and CD 34 (Immunotech) were used for the immunoanalysis.
Results Majority of the tumors was localized in the stomach (n=18) and small intestine (n=11), one in the colon and two were extragastrointestinal. In relation to the accepted MC and TS criterias, 16 of 32 cases were of low and one of very low risk category. 8 tumors belonged to intermediate risk category, mostly due to TS in contrast to low MC. The inclusion of 7 cases into high risk category was based predominantly on the MC (>5/50HPF and TS 5-10 cm /n=5/ and >10 cm /n=1/; in the last one it was >6-10/50HPF in contrast to TS <5 cm).
Conclusion A prediction of clinical behaviour of GISTs requires a correct histopatological analysis related to initial staging data. Although the categorisation of the GISTs into different risk categories uses both criteria of TS and MC, the significance of the mitotic activity evaluation in our set of cases seems to be higher.

P-339

Multiple gastric composite carcinomas (adenocarcinoma and endocrine carcinoma) with adenosquamous differentiation

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A case of multiple gastric composite carcinomas composed of endocrine carcinoma and adenocarcinoma with adenosquamous differentiation is presented. Tumours showed a composite architecture both within the site of origin and in lymph node metastasis. A 44-year-old woman had a diagnostic upper gastrointestinal system endoscopic evaluation for her long term dyspeptic complaints. After determination of two concurrent tumours in the stomach she underwent total gastrectomy operation. Gross examination of the stomach revealed a polypoid lesion in the anterior wall and an ulcerous lesion in the posterior wall of the corpus. Also there were multiple minute nodular lesions both in the corpus and fundus. Histopathological examination of the polypoid tumour revealed a well-differentiated tubular adenocarcinoma admixed with an endocrine carcinoma proved immunohisto-chemically. Ulcerous lesion was composed of a poorly-differentiated adenocarcinoma showing adenosquamous differentiation admixed with an endocrine carcinoma. Other nodular lesions were composed of endocrine tumours having different levels of invasion in the wall of stomach. Non-tumourous mucosa harboured atrophic gastritis. This is a unique case of multiple composite carcinomas devoloped in the setting of atrophic gastritis worth to discuss.

P-340

Primary retroperitoneal malignant fibrous histiocytoma metastasing to the stomach

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Introduction One of the most common malignant soft tissue tumours in adults is malignant fibrous histiocytoma. These tumours are accepted as an aggressive tumour and mainly treated surgically. Primary and metastatic malignant fibrous histiocytomas of the gastrointestinal tract are rarily described.
Patients and results Our case is a 55-year-old woman who had a retroperitoneal malignant fibrous histiocytoma that had metastased to the lung after 10 months following an adequate initial treatment. Four months later, because of having recurrent attacks of vomiting and nausea, gastroscopic examination was performed. Multiple nodular lesions in her stomach were seen and microscopic examination of these lesions revealed spindle–shaped, pleomorphic tumour cells. The positive immunoreactivity for anti–vimentin and anti-CD 68 justified the diagnosis of malignant fibrous histiocytoma's metastases. Total gastrectomy was performed following that diagnosis and she died after 1 month due to renal failure.
Conclusions This case is presented because of the rarity of distant metastases of malignant fibrous histiocytoma to the stomach and to discuss the entities that should be considered in the differential diagnosis of these tumours.

P-341

Role of p16INK4a in gastrointestinal stromal tumours (GISTs)

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Introduction The classification based on c-KIT/CD117 expression reordered the formerly rather confuse category of GIST. p16INK4a (p16) is a tumour suppressor gene of the cyclin D-Cdk4, 6/INK4/Rb/E2F pathway, altered in >80% human neoplasms. It is located on chromosome 9p21, a region consistently involved in DNA losses found in malignant GISTs. Aims: We studied the role of p16 in GISTs.
Materials and methods CD117-immunoreactive mesenchymal tumours with morphological and clinical features consistent with GIST were studied, excluding smooth muscle (desmin+, CD117-) and schwannian (S-100+, CD117-) neoplasms. 21 cases (17 gastric, 3 small intestinal and 1 colorectal; 10 males, 11 females; age at diagnosis: 51-87 years; follow-up: 6-193 –mean: 60.3- months) were considered. Immunohistochemistry, RNA extraction with semiquantitative RT-PCR and methylation specific PCR (MSP) were used. Kaplan-Meier method followed by log-rank test, and univariate and multivariate analyses with a proportional hazards model were performed (malignancy criteria adopted for uncensored events: recurrence, invasion of adjacent organs, peritoneal dissemination, metastases and death of disease).
Results A tumoral cell fraction with low/absent p16 immunoreactivity >20% was associated with malignancy; semiquantitative RT-PCR confirmed this result. By MSP, 7 cases (all underexpressing p16, six malignant) showed p16 promoter methylation. Low/absent p16 immunoreactivity, together with size, cellularity, mitotic count and presence of coagulative necrosis resulted associated with malignancy by univariate analysis, with the former factor being the only prognostic one selected by multivariate analysis.
Conclusions p16 downregulation is implied in GIST progression; p16 promoter methylation is one of its causes; p16 assessment opens possible perspectives in GIST prognostication.

P-342

Investigation of glandular epithelium cells proliferative activity in chronic superficial and deep inflammations of gastric mucosa

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Introduction Regenerative abilities of gastric mucosa glandular epithelium cells are the main mechanism which condition its right function both within the range of gastric mucosa integrity and normal activity of its epithelial cellular elements. The aim of the work was to evaluate dependence between proliferative activity of gastric mucosa glandular epithelium and sex, age, chronic inflammation type and Helicobacter colonization.
Material and methods The examinations were conducted on paraffin blocks from gastric oligobiopsies taken from 35 women (of average age-52) and 34 men (of average age-54).To the examinations were rated only cases where oligobiopsies were taken from at least two gastric prepyloric parts (antrum) and corpus with recognized unspecific chronic gastric mucosa inflammation. Chronic gastritis was graded according to modified Whitehead's classification. Helicobacter pylori (Hp) was evaluated according to the Sydney system. Examination of the cells with PCNA proliferative antigen expression were conducted separately for foveolar area glands and for glands below gastric mucosa foveolar area. All the measurements were performed separately for gastric mucosa prepyloric part and gastric corpus. In tissue sections, in which were carried out proper immunocytochemical reactions, measurements of the PCNA positive cells number in circular, oblique short, oblique long and oblong gastric glands sections were conducted five times. The statistic analysis also included age, presence of Hp colonization, gastric mucosa inflammation type and the area where an oligobiopsy section was collected.
Results No significant differences were found in proliferative activity depending on the place of collecting a section, age and Hp colonization, while a higher proliferative activity was found in superficial chronic gastritis, particularly in gastric mucosa prepyloric part.
Conclusions 1. Chronic superficial inflammation coexists with significantly higher proliferative activity of gastric mucosa glandular epithelium, particularly with connection to its prepyloric part. 2. Changes in gastric mucosa glandular epithelium proliferative activity do not depend on its age and histotopography and Hp colonization.

P-343

Chromogranin-A-plus and D cells in superficial and deep gastric mucosa inflammation

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Introduction Chromogranin-A-plus (chA) and D cells exist almost in the whole alimentary canal and in pancreas. These cells have an influence on secretory functions and normal gastric mucosa histostructure. Up to now investigations confirmed participation of these cells in e.g HCl and gastrin secretion regulation and in some of the proliferative states of gastric mucosa. The aim of the study was to estimate participation of chA and D cells in microscopic superficial (gchs) and deep (gch) gastric mucosa inflammation's indices.
Material and methods Paraffin blocks from gastric oligobiopsies was the material which was collected from 35 women (of average age-52) and 34 men (of average age-54). To the examinations were classified only the cases where oligobiopsies were taken from at least two areas of gastric prepyloric part (antrum) and corpus, with diagnosed unspecific chronic gastric mucosa inflammation. Chronic inflammations were graded according to modified Whitehead's classification. Helicobacter pylori (Hp)was evaluated according to the Sydney system. Neuroendocrine cells of gastric pylori were counted after proper immunohistochemical identification in circular, oblique short, oblique long and oblong gastric glands sections. All measurements were conducted separately for gastric mucosa prepyloric part and gastric corpus. The results of quantitative researches were analysed with the use of statistic methods.
Results In gastric mucosa antrum there were twice more chA and D cells in comparison with corpus. Gastric mucosa inflammation type did not have a significant influence on cells localization in gastric mucosa. The number of chA cells diminished in gchs gastric antrum among patients who were over 50 years old, whereas the same number increased in corpus inflammations of this type. The increase of chA cells number was found in gchs gastric antrum with Hp colonization. In these cases D cells number diminished.
Conclusions 1. In gchs gastric antrum with Hp colonization chA cells number significantly increases while D cells number decreases. 2. Quantitative changes of chA and D cells run differently in gchs and gch both in prepyloric part and in gastric corpus.

P-344

Response to preoperative chemoradiation on rectal adenocarcinoma: histopathological effects and relationship with level of p53 and PCNA expression

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Introduction Adjuvant chemoradiotherapy delivered before surgery has been used to improve control and to reduce the likelihood of local recurrence in selected patients with rectal cancer. Although the status of p53 gene and the expression of PCNA has been widely studied in these tumors, little is known about their value to predict the responsiveness of rectal cancer to chemoradiation. The aim of this study was to To assess effects of preoperative chemoradiation in advanced rectal adenocarcinoma.
Materials and methods We examined the histopathologic effects of chemoradiotherapy on 73 rectal tumors and correlated the efficacy of treatment with the level of p53 and PCNA proteins expression in pretreatment biopsies.
Results Three patients showed no primary tumor in the resection specimen. Another eight carcinomas showed a good response to chemoradiation with only small nests of viable tumor cells. Grouping these two categories, we considered that the tumor response had been total or subtotal in 11 cases (15%). Nuclear accumulation of p53 protein was detected in 53 (72%) specimens. Thirty five tumors showed a high PCNA index (48%). No obvious relationship seemed to exist between pretreatment, p53 immunostaining and response to chemoradiotherapy. Tumors with high PCNA index were more likely to respond to chemoradiation: 8/35 (72%) versus 3/38 (43%)(p=0.07).
Conclusion Knowledge of proliferative activity of rectal cancer, as determined by PCNA immunostaining, should be useful in predicting the likelihood of response to preoperative chemoradiation.

P-345

Value of the mitosis-related PHH3 antibody in the prognosis of gastrointestinal stromal tumors

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Introduction Mitotic count and tumor size are the main predictors of outcome in gastrointestinal stromal tumors (GIST). However, they are not always useful in individual cases. The aim of the present study has been to assess whether mitotic counts performed using phosphorylated histone 3 (PHH3) antibody, expressed from the early steps of mitosis, could improve the prediction of prognosis in these tumors.
Materials and methods Sections from 30 GIST were stained with PHH3 antibody (Upstate Biotechnology, Lake Placid, NY; 1:1,000 dilution). Mitoses were counted per 50 high power fields (40X, Olympus BX). In addition, the H&E-based mitotic counts and tumor size were obtained from the pathology reports. Patient outcome was recorded as the endpoint. Statistical analysis: non-parametric tests (Mann-Whitney, Wilcoxon, Spearman), Kappa and Cox regression.
Results There were 17 gastric and 13 intestinal tumors. Correlation coefficient with mitotic counts performed by both techniques was 0.459 (p=0.006), indicating a moderate correlation. The cut-off was 2 mit/50 HPF for H&E, with an odds ratio (OR) of 3.39 (0.83;13.82; p=0.08); 4 mit/50 HPF with PHH3 antibody counts, with an O.R of 6.16 (1.39;27.22; p=0.016), and 7 cm for tumor diameter, with an O.R. of 4.29 (0.86;21.31; p=0.07).
Conclusion In the present series, PHH3-based mitotic counts allow a better prediction of prognosis than H&E-based counts or tumor size. Thus, PHH3 antibody could be applied in the pathological classification and grading of these tumors, and in the more precise definition of cases with higher risk of progression.

P-346

Immunohistochemical expression of cyclooxygenase-2 (COX-2) in human colorectal cancer

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Introduction Recent studies have implicated metabolites of arachidonic acid, such as prostaglandin E(2) in colorectal carcinogenesis. COX-2, a key enzyme in arachidonic acid metabolism, is present in the majority of colorectal cancer (CRC). Its overexpression seems to be associated with worse prognosis. The aim of this study was to examine the relationship between the expression of COX-2 in human CRC and the clinicopathological characteristics of the tumor.
Materials and methods Paraffin embedded tissue samples from 35 patients (M:19, F:16, Median Age: 63 yrs) with CRC (TNM staging: I - IV), who underwent radical surgical treatment in Thessaloniki Cancer Hospital "Theagenio" from 1/1/1997 until 1/8/97, were evaluated. Tumor sections were stained for COX-2 using a rabbit polyclonal antibody against human COX-2 and the staining was graded on a scale 1 – 4, using mononuclear cells as reference.
Results COX-2 expression was found in tumor epithelial cells in 85.7% of CRC (30/35 cases) with diffuse mainly, cytoplasmic staining and in inflammatory cells, vascular endothelium and fibroblasts. Low COX-2 expression (grade 1-2) was found in 54.3%, high COX-2 expression (grade 3-4) in 31.4% of cases and the remaining were negative. There was no correlation between clinicopathological characteristics of patients and intensity of COX-2 expression, although there was a slightly better overall survival rate in low expression group of patients (p:NS).
Conclusions This small study shows that COX-2 protein is overexpressed in most cancer cells in colorectal carcinomas and may be related to overall survival.

P-347

The expression of Bcl 2 family proteins in B-chronic lymphocytic leukemia

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Introduction B-chronic lymphocytic leukemia (B-CLL) is a neoplastic disease caused primarily by defects in the apoptosis mechanism. Apoptosis is the process of programmed cell death or cell suicide. The Bcl-2 proteins are a family of proteins involved in the response to apoptosis. Some of these proteins (such as Bcl-2 and Bcl-Xl) are anti-apoptotic, while others (such as Bad or Bax) are pro-apoptotic.
Materials and methods Our study includes peripheral blood specimens from 20 patients with B-CLL and 20 healthy individuals (as a control group). Using Western blot analysis, we examined the levels of Bcl-2, Bcl-Xl, Bax and Bad protein expression.
Results The level of Bcl 2 (p= 3,68 x 10-10), Bax (p= 0,019) and Bad (p= 0,073) proteins were significantly increased in all analyzed patients while the level of Bcl Xl protein (p=0,75) showed no difference between patients and healthy individuals. Differences between the levels of analysed protein expression in group of patients had no statistical significance.
Conclusion Increased level of expression Bcl 2, Bax and Bad protein represents the most striking feature of B – CLL cells. The variations in expression of only one protein of the Bcl 2 family can not represent the prognostic factorr in B-CLL.

P-348

Recurrent Kikuchi-Fujimoto desease during pregnancy

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Introduction Kikuchi - Fujimoto disease (KFD) is a distinctive type of necrotizing lymphadenitis with unknown cause, has a self-limited clinical course and affects usually the cervical lymph nodes of young women. Although KFD occurs most often in young women, only two cases have been reported during pregnancy. In one of these cases KFD developed initially 6 years before.
Case report We report a case of a 37-year-old pregnant woman on her 20th week of gestation who was admitted with a history of swelling in the right side of the neck and fever of 20 days duration. On physical examination a 2 cm cervical lymph node was palpable. Microscopically, the excised lymph node showed large areas of necrosis and extensive karyorrhexis. No neutrophils or plasma cells or eosinophils were identified. It was found an admixture of non-phagocytic histiocytes and T-lymphocytes in the necrotizing area. There was a predominance of CD8+ cells over CD4+ cells. The patient received no treatment and 2 months later had no symptoms. Later she delivered a healthy boy. Five years ago the patient presented similar clinical history and histological diagnosis of the excised cervical lymph node. We present the second reported case of recurrent KFD during pregnancy and the third case of KFD developing during pregnancy.
Conclusion From our case and the other two reported cases it seems that if KFD occurs in a pregnant woman there is no need to terminate the pregnancy as there was no effect on both mother and fetus.

P-349

Monitoring of patients with follicular lymphoma carrying t(14;18)(q32;q21) by real time quantitative PCR

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Introduction Follicular lymphomas (FL) are characterized by t(14;18)(q32;q21) in a majority of patients. The identification of the translocation is utilized to monitor the disease behavior, and,

specifically, to detect minimal residual disease (MRD). Qualitative PCR is routinely used but without specification of the tumor cell quantity. Real time quantitative PCR (RQ-PCR) may be employed to address this issue. Aims: RQ-PCR was introduced to monitor MRD in patients with FL bearing the t(14;18)(q32;q21).
Materials and methods Samples of primary tumors, bone marrow and peripheral blood (BM/PB) specimens were taken in the course of the disease and were used for the t(14;18)(q32;q21) assessment. For translocation screening, FISH on interphasic nuclei was applied. Qualitative PCR was used to detect the break in the major breakpoint region (mbr) of the BCL-2 gene (18q21). Relative quantity of cells bearing mbr t(14;18)(q32;q21) was performed by the RQ-PCR. Fifteen samples of primary tumors and BM/PB in duplicates were investigated with appropriate controls.
Results and conclusion The relative quantity of cells with t(14;18)(q32;q21) was significantly higher in the primary tumors than in the BM/PB samples. The changes of the BM/PB infiltration were found during the course of the disease and included shifts toward the tumor progression or disappearance of the tumor cells. RQ-PCR is a technique suitable for monitoring the disease response to the therapy or the disease progression and it is superior to morphology and immunohistochemistry. Supported by: IGA-MZCR-NC/6296-3, Research Project FN-Motol/6073.

P-350

Phosphatase activity dependent apoptotic effect of transforming growth factor beta1 in HT58 human lymphoma cells

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Introduction Transforming growth factor beta1 (TGFb1), a multifunctional cytokine, has antiproliferative and/or proapoptotic effect on lymphoid cells. The continuously proliferating lymphoma cells could be resistant to the endogenously produced TGFb1. Aims: To study the responsiveness of lymphoma cells using exogenously activated TGFb1.
Materials and methods The apoptotic effect and depolarization of the mitochondria was detected by flow cytometry, the different gene expression was studied by RT-PCR. The amount, the activity (Western blot) and the localization (confocal microscopy) of the signal elements (Smads, phoshpo-Smad2, Erk1/2, JNK, p38MAPK) and other proapoptotic and survival proteins (Bcl-2, Blc-xL, Bid, Bax, Bad, NFkB, phospho-IkB) were also studied. The role of PP2A phosphatase, MEK1 kinase and caspase activity was estimated by different inhibitors (endhothal, okadaic acid, PD980559, Z-VAD-fmk, Z-IETD-fmk, Z-LEHD-fmk) in vitro.
Results HT58 B cell lymphoma cells lost their sensitivity to the antiproliferative action of exogenous TGFb1, but the cells were killed by apoptosis. Smad2 phosphorylation, nuclear localization and the increased expression of TGFb1 induced early gene (TIEG) proved the rapid activation of Smad signal. The role of PP2A phosphatase activation followed by decreased expression of active phospho-JNK and phospho-Erk1/2 was supported by the antiapoptotic effect of PP2A inhibitors and the proapoptotic effect of specific MEK1 inhibitor. The Apoptosis was induced primarily through the mitochondrial pathway. It seems that both main iniciator caspases (caspase 8 and 9) took part in the apoptotic response.
Conclusion These data suggest that exogenous TGFb1 had double but interrelated actions on HT58 cells: a) suppression the survival signals produced by protein kinases, which b) allowed the Smads to induce apoptosis. Therefore, it is possible that the lost sensitivity of malignant lymphoid cells to proapoptotic regulators (such as TGFb1) could be reactivated especially by lowering the survival threshold. The work was supported by OTKA (T034892), ETT (192/2000,193/2000), FKFP (0150/2001) and Békésy foundation (118/2001).

P-351

Clinicopathological study of chromosome 7 abnormality related hematological disorders

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Introduction Cytogenetic abnormalities sometimes determine the subtype of hematological disorders and are common in myelodysplastic syndromes (MDS). Although chromosome 7 abnormality is frequently observed in MDS, the specific characters of this type are not determined. In this study, we examined the clinicopathological characters of hematological disorders associated with chromosome 7 abnormality. Aim of this study is to assess whether these disorders are classified in special subtype or not.
Materials and methods We selected 54 hematological disorder cases with chromosome 7 abnormality from the bone marrow files of the Department of Pathology, Nagoya University Hospital. Clot sections of aspirated bone marrow were examined histopathologically. Immunohistochemical study was performed using paraffin embedded tissues.
Results 27 patients, 40 males and 14 femals respectively, with monosomy 7, 11 with der (1;7) and 16 with other abnormalities were studied.The median age of the patients was 65.5 years (range, 16-82 years). Pancytopenia was observed in 28, bicytopenia in 7 and monocytopenia in 11 cases. Past history of aplastic anemia was in 7 cases. Histopathologically, they were 40 cases of MDS, 7 of AML, 2 in ALL and 5 of others. Hypoplastic MDS (<40% cellularity) were found in18 cases, 2 of RA, 9 of RAEB, 6 of RAEB-t and 1 case of MDS with fibrosis. All cases of acute leukemia had dysplastic multi-lineage features other than blasts. Histopathological characteristics were weak erythroid dysplasia, increased micromegakaryocytes and hypocellular tendency. These characteristcs were common in patients with monosomy 7 and other abnormalities.
Concusion Hematological disorders with chromosome 7 abnormalities share similar clinicopathological characteristics.

P-352

Expression of topoisomerase I and topoisomerase II alpha as an independent prognostic factor and evaluation of the overall survival in NHL

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Introduction Topoisomerase II Alpha and Topoisomerase I (Topo I and II alpha) are the key targets for some anti-cancerous drugs that inhibit these enzymes. The purpose of this paper is to examine the expression of Topo I and Topo II alpha in different types and stages of NHL's the response of treatment with the overall survival.
Materials and methods Immunohistochemistry techniques with anti-topo I and topo II alpha were used. The results were evaluated with a semi-quantitative method.
Results and conclusion 30 cases were studied with topo I and topo II alpha. We include 21% of follicular lymphomas, 61% diffuse, 16% follicular and diffuse and high grade 2%. The stage of these tumors were stage I, 16%; stage II, 8%; stage III 18%; stage IV, 22%. The results show a higher intensity expression of the topo II alpha, and topo I indices, in the higher grade NHL. There appears to be a similar expression between the intermedium and indolent NHL. The survival indicates that the large cell NHL's show a higher response rate and better survival than the mixed or small cell type. The expression of these two enzymes may be useful for selecting the appropriate chemotherapy in NHL. Our data, although small, suggests an excellent correlation in response to treatment and longer survival in the NHL that have a high expression of topo I and topo II alpha.

P-353

Mantle cell lymphoma (MCL): Ki-67 antigen and chromosome 12 ploidy in an estimate of the tumor progression

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Introduction MCL belongs to prognostically unfavorable lymphomas. However, the behavior may vary and it is difficult to predict it. A more aggressive course tends to be associated with blastic and anaplastic subtypes. Numerical chromosomal changes and proliferative activity may be of importance to improve the prediction. Aims: Risk of the tumor progression with the assessment of Ki-67 antigen-positive cell count and ploidy status of chromosome 12.
Materials and methods 44 patients with MCL characterized by t(11;14)(q13;q32) and by overexpression of cyclin D1. The proliferative activity was evaluated immunohistochemically (IHC) by double staining for Ki-67 antigen and CD20 (to rule out counting admixed Ki-67 positive T-lymphocytes). Ploidy status of chromosome 12 was evaluated by interphasic FISH.
Results and conclusion Trisomy and polysomy of chromosome 12 (9 patients) was associated with blastic/anaplastic forms of MCL with the same frequency as with the common variant. A finding of >30% Ki-67 and CD20 positive cells was associated with the blastic variant of the disease – 7 patients. Of them 5 died of the disease progression, one of unrelated cause and one is alive with the disease. Of the remaining 23 patients in whom the follow up was available, 2 died of the tumor, and the proliferative activity, measured by counting the Ki-67 positive cells was <15%. The assessment of proliferative activity with immunohistochemical demonstration of Ki-67 antigen seems to be useful in prediction of clinical behavior of MCL
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P-354

Morphological criteria for differentiation of lymphocyte predominance Hodgkin's lymphoma from lymphocyte-rich classical Hodgkin's lymphoma

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Aims To reveal morphological features of differential diagnosis between lymphocyte rich classical Hodgkin's lymphoma (LRCHL) and nodular lymphoid predominance (NLPHL).
Materials and methods Retrospective analysis of 20 lymph node biopsies (age 3-69) with histological diagnosis of "nodular paragranuloma" or "Hodgkin's disease, lymphocytic and histiocytic variant". Immunological data were available in all cases. All patients presented with the early stages of disease. We used the following morphological criteria: growth patterns (nodular, nodular-diffuse or diffuse), distribution and cytomorphology of tumor cells ("popcorn", lacunar, classical, mummified cells), cellular composition of reactive background, presence or absence of secondary and atrophic follicles. The results of morphological algorithm were compared with immunology data in every case.
Results Among 20 biopsies,4 cases (20%) were classified as NLPHL. 16 cases were attributed to LRCHL, including 3 cases which were characterized by interfollicular sheets of cells resembling lacunar cells ("cellular phase of nodular sclerosis"), 3 cases had rare classical cells within expanded mantle zone ("follicular variant" of Hodgkin's disease). All 16 cases LRCHL exhibited secondary follicles. There were no cases of LRCHL with "pure" diffuse growth type; both NLPHL and LRCHL had nodular or nodular and diffuse pattern. 20 cases corresponded morphologically to NLPHL or LRCHL and this division was confirmed with immunological analysis..
Conclusion We proposed the algorithm of morphological criteria for differentiation of NLPHL from LRCHL

P-355

Bone marrow sinusoids in acute lymphoblastic leukemia (ALL)

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Introduction Bone marrow sinusoids regulate the development of hemopoietic precursors and migration of stem cells and mature blood cells in blood stream. The purpose of the investigation was to study endothelium of bone marrow sinusoids in disordered hemopoiesis in ALL.

Materials and methods Pretreatment bone marrow trephine biopsy sections from 30 patients with ALL were studied using morphometric and ultrastructural methods.
Results Development of myelosuppression in ALL was accompanied by considerable changes in the morpho-functional status of sinusoid endothelium. Most vessels became thinned, endothelial cells were flattened. In such vessels cells of endothelium came apart with formation of gaps between cells. The basement membrane was interrupted. However, blast migration continued to be transendothelial. There were vessels in which the structure of endothelial cells was identical to that of blast cells. There were no signs of endo- and exocytosis in such cells. Intranuclear virus-like inclusions were found in endothelial cells of sinusoids of patients with ALL.
Conclusion The above data suggest the involvement of endothelial cells in the disordered haemopoiesis in ALL. Defects of the bone marrow sinusoids in leukemia can be the cause of the early release of blasts into circulation. Blast transformation of endothelial cells does not contradict the suggestion about existence of a common cell precursors of the endothelial and hemopoietic cells.

P-356

Complex diagnosis of follicular lymphomas

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Introduction The second most common type of non-Hodgkin lymphomas is follicular lymphoma (FL). Moreover, follicular lymphomas are the most common type of the low grade lymphomas. In Hungary they comprise about 15-20% of all the lymphomas but its occurrence shows increasing tendency. Our aim was to survey and revise FLs diagnosed in the last 10 years in the National Institute of Oncology. We studied the diagnostic relevance of histology, immunohistochemistry and the detection of immunoglobulin heavy chain (IgH) and bcl-2 gene rearrangements.
Materials and methods We surveyed 52 cases which were diagnosed as follicular or centrocytic, centroblastic lymphoma before. After the histological re-examination immunohistochemistry (CD20, CD3, bcl-2, CD10, bcl-6, CD5, p53, cyclin D1 and Ki-67) was performed on each case. We studied the IgH and bcl-2 (MBR) gene rearrangement both with conventional and real-time PCR methods from the paraffin embedded blocks. The classification was made according to the new WHO classification.
Results After the revision of the 52 cases we found 36 follicular, 11 diffuse large B-cell, 1 mantle cell and 1 marginal zone lymphoma, 2 paragranulomas and 2 follicular hyperplasias. The grade of the FLs showed relation to the expression of different antigens. CD10 and bcl-2 expression decreased with the grade. p53 expression was very rare and occurred only in grade 3 cases. The proliferation index with Ki-67 showed good correlation with the grade of FLs. The IgH showed 3 types of pattern: monoclonal, polyclonal and a sharp line in a polyclonal background. Grade 3 and grade 1-2 FLs were monoclonal almost in the same ratio. We could detect bcl-2 gene rearrangement in 55% of the FLs (72% grade1-2, 30% grade3). All the grade 3 cases which showed bcl-2 gene rearrangement belonged to grade 3a group while all the grade 3b cases were negative.
Conclusion In agreement with others we found that grade 1, 2 and 3a FLs showed similar morphological, immunohistochemical and genetical features while gr3b FLs are more similar to diffuse large B-cell lymphomas. Considering the diagnostic relevance of the different methods we can conclude that histology alone is not sufficient. 84% of our cases were solvable with the help of immunohistochemistry and in 10% of the cases the diagnosis based on the molecular pathological results. In the remaining 6 % even with the help of all diagnostic tools no unambiguous diagnosis could be reached.

P-357

Prognostic significance of angiogenesis, apoptosis and proliferation in B cell lymphomas

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Introduction The bcl-2 and p53 oncoproteins play important roles in carcinogenesis. Mutation of the p53 gene is considered the most common genetic aberration in many cancers including hematologic malignancies. Ki-67, a proliferation antigen, evaluates the proliferative activity of a lesion. Angiogenesis is also critical for tumor proliferation and has a prognostic value. The vascular density of tumor directly correlates with poor outcome in many malignancies. The aim of this study was to investigate correlation between Ki-67, p53 and bcl-2 immunostaining in B cell lymphomas and to identify any relation between these markers and microvessel density (mvd) and histopathological parameters including stage and tumor grade.
Materials and methods 19 low grade and 35 high grade non-Hodgkin lymphomas (NHL) were immunohistocemically stained with p53, Ki-67, bcl-2 and Factor VIII. The results were analysed statistically.
Results There was no correlation between stage and Ki-67 LI, mvd, p53 and bcl-2 expression. The cases which showed immunoreactivity for bcl-2 had decreased mvd counts compared with the cases negative for bcl-2. p53 immunoreactivity was correlated with mvd. Microvessel counts were significantly higher in patients with high grade NHLs. Statistical analysis showed association between Ki-67 LI, p53, bcl-2 expression, mvd and histological grade.
Conclusions The levels of Ki-67, bcl-2 and p53 expression and microvessel counts were closely related with the grade of NHLs. Our findings suggest that apoptosis, angiogenesis and proliferative activity can be used as prognostic indicators in NHLs.

P-358

Diffuse large B-cell lymphoma of the maxillary sinus complicated with Blastomycotic leptomeningitis

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Introduction Sometimes a Non-Hodgkin's lymphoma presents as an isolated mass in the fossa canina, just anterior to the maxillary

sinus; however such a lesion is more likely to arise from an infraorbital lymph node, part of the facial lymph node group.
Case presentation We present a patient with diagnosed diffuse large B cell lymphoma of the maxillary sinus as well as involvement of the infraorbital region, who suffered from infective complications. 39-years old male was admitted at the Clinic of hematology because of tumor mass in nasal cavity. CT-scan showed tumor mass filling right maxillary sinus. Surgical biopsy was done. Histochemical and immunohisto-chemical findings suggested a diagnosis of diffuse large B cell lymphoma. Other clinical investigations showed I-E clinical stage. Treatment with four cycles of CHOP and Mab-Thera followed. After that adjuvant radiotherapy was delivered. Because of increased body temperature and meningeal symptoms, this therapy was interrupted. Microbiological investigations showed positive hemoculture for Escherichia coli and Staphylococcus aureus, and positive finding of Blastomyces in cerebrospinal fluid, while serological analyses revealed positivity for CMV and EBV. Beside severe antibiotic, antimycotic and antiviral treatment, the patient suffered from sepsis with main features of suppurative leptomeningitis and acute hepatitis.
Conclusion This case implies the conclusion that the clinicians should have in mind the possible presence of immune disregulation in patients with NHL, that could complicate the administered chemotherapy and radiotherapy.

P-359

Non-Hodgkin's lymphomas of head and neck in Macedonia classified accordingly to the WHO classification of lymphoid neoplasms

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Aim The aim of this study is to determine the most frequent pattern of involvement by NHL of head and neck and to correlate it with clinical outcome of the disease.
Material and methods A retrospective review was performed on 190 patients with NHL during five years period. Paraffin sections stained with standard histochemical and immunohistochemical stainings were histologically analyzed for classification of the NHL accordingly to the WHO classification of lymphoid neoplasms. Clinical histories were used for clinical follow-up.
Results In fifty cases out of 190 patients with NHL the disease was primary localized in the head and neck region. Male-female ratio was 1,5. Twenty four cases were primarily nodal, 22 primarily extranodal and 4 had extranodal/nodal expression of the disease. Diffuse large B cell lymphoma comprised 40% of the group: 8 nodal, 11 extranodal and 1 extranodal/nodal. Three of these cases were CD30+ and ALK-1 -. Six cases (12%) with B-CLL NHL had nodal manifestation. MALT-zone lymphoma (12%) showed extranodal involvement in 4 cases and extranodal/nodal in 2 cases. B-lymphoblastic lymphoma (12%) showed 4 cases with nodal, one extranodal and one extranodal/nodal involvement. Two cases with Burkitt's lymphoma (4) had typical mandibular localization. Follicular lymphoma comprised 8%, with two nodal and two extranodal involvement. T cell NHL-s comprised 10% of the group with three nodal, one extranodal and one extranodal/nodal involvement.
Conclusion There was no significant predilection for clinical expression of the NHL of head and neck, and no difference between the mean survival of the groups for these parameter.

P-360

Expression of the bcl-2 family proteins, caspase 3 (CPP32) and poly adp-ribose polymerase (PARP-1) in multiple myelomas

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Introduction Multiple myeloma (MM) is a malignancy of the bone marrow characterized by the clonal expansion of plasma cells with low proliferative activity. Aberrant expression of genes regulating apoptosis/survival seems to be essential in the development of multiple myeloma. The aim of this study was to investigate the immunohistochemical expression apoptosis-regulating proteins in bone marrow of patients with initially presented multiple myeloma.
Methods The expression of bcl-2, mcl-1, bak, bax, CPP32 and PARP was examined on paraffin sections of 36 bone marrow biopsies obtained from equal number of patients with newly diagnosed MM, using commercially available monoclonal and polyclonal antibodies and LSAB technique. Specimens containing >10% immunostained tumor cells were considered positive.
Results Bcl-2, bak and bax positive immunostaining was detected in 94%, 100% and 94% of cases respectively. Most positive tumors showed high percentage of tumor cells immunoreactive for the above proteins: >50% for bcl-2, >80% for bak and >60% for bax. The mean value for bcl-2/bax ratio was 1.66, and the mean bcl-2/bak ratio was 0.95. Mcl-1 expression was detected in a small percentage of tumors (17%). Cytoplasmic expression of CPP32 was observed in 8,3% of cases and the number of positive cells was low. Nuclear expression of PARP was seen in 44,5% of cases and no association was found with the other apoptosis-related antigens, bcl-2/bax or bcl-2/bak ratio.
Conclusion The above data demonstrate an overexpression of bcl-2, bax and bak in myeloma plasma cells and an increased bcl-2/bax ratio. CPP32 does not seem to mediate any apoptotic event in MMs. The role of overexpression of PARP remains to be elucidated.

P-361

Immunoexpression of bcl-2 family members, p53, caspase 3 (CPP32), and poly adp-ribose polymerase (PARP-1) in diffuse large B-cell lymphomas

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Introduction Diffuse large B-cell lymphomas (DLBCL) represent a biologically and clinically heterogeneous group. Although these tumors are curable with chemotherapy, their response to treatment varies. Apoptosis-related proteins may play an important role in the

chemosensitivity or chemoresistance of tumors. The aim of this study was to investigate if the expression of apoptosis-regulating proteins in DLBCL could be indicative of tumor chemosensitivity.
Methods Formalin-fixed, paraffin-embedded tissue from 58 cases of DLBCL [29 nodal, 27 extranodal, 2 splenic (42 centroblastic, 2 immunoblastic, 2 Burkitt's-like, 2 anaplastic, 1 TCRBCL, 9 primary mediastinal)] were immunostained with monoclonal [bcl-2 (DAKO), mcl-1, CPP32 (Neomarkers), p53 (Biogenex)] and polyclonal [Bax, Bak (DAKO), PARP (Neomarkers)] antibodies using LSAB method. Specimens containing >10% immunostained tumor cells were considered positive. Clinical data were available for 44 cases (30 chemosensitive, 14 chemoresistant).
Results Bcl-2, mcl-1, bax, bak, p53, CPP32 and PARP positive immunostaining was detected in 68.9%, 85.9%, 84.2%, 100%, 75.8%, 57.8% and 89.4% of cases, respectively. Mcl-1, bax, bak and PARP were overexpressed in >80% of tumor cells in most cases. There was a significant association between the expression of bcl-2 and mcl-1 ($p = 0.004$). A higher immunoexpression of bcl-2 (78.5% vs 63.3%), mcl-1 (92.8% vs 80%), bax (92.8% vs 80%), p53 (92.8% vs 70%) and CPP32 (69.2% vs 60%) was observed in chemoresistant cases. PARR expression was higher in chemosensitive cases (93.3% vs 84.6%). In the p53-negative group of cases (14 cases) those with a strong (3+) PARP expression showed a significant better response to treatment ($p = 0.0016$). A significant chemosensitivity was also detected in the group (8 cases) with strong (3+) bax and weak (+) or negative (-) p53 immunostaining ($p = 0.028$).
Conclusion Apoptosis-related proteins, though overexpressed in DLBCL, were not associated with response to treatment. A p53(-)/PARP(3+) or p53(+ or -)/bax(3+) tumor status may reflect sensitivity to chemotherapy.

P-362

Bcl-2 expression and apoptosis in non Hodgkin lymphomas

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Introduction Non-Hodgkin lymphomas (NHL) are a heterogenous group of lymphoid malignancies classified according to their morphological, immunophenotypical and genotypical characteristics as well as their clinical behaviour. However, the progression of these diseases may be influenced by various others factors such as apoptosis, proliferation and angiogenesis. Aim: A possible correlation between Bcl-2 expression, apoptosis, disease outcome and overall survival in patients with NHL was analysed.
Material and methods 72 NHL devided in low- (N=29) and high-grade (N=43) were immunohistochemicaly analysed for Bcl-2 expression, while 12 low- and 12 high-grade NHL were procesed for terminate TdT mediated dUTP nick end labeling (TUNEL) method in order to determine the apoptotic index of tumor cells. Results were obtained by image analyser and statistically processed.
Results The expression of Bcl-2 protein, as an antiapoptotic marker was significantly higher in low- compared to high-grade NHL, $p=0.024$, whereas the apoptotic index obtained with the TUNEL method demonstrated inverse results, $p<0.001$. In high-grade NHL, partial remission was observed in patients whose tumors expressed significantly higher values of Bcl-2, ($p=0.03$), while complete remission was observed in a group with a lower expression of Bcl-2. Furthermore, patients with lower Bcl-2 expression showed longer overall survival $p=0.02$, whereas patients with a higher apoptotic index showed shorter survival, $p<0.001$. In both low and high-grade groups of NHL, Bcl-2 has been found as an independent predictor of patients disease relaps, $p< 0.05$.
Conclusion The expression of Bcl-2 is a strong independent predictor of disease progression in NHL patients.

P-363

The heterogeneity of anaplastic large cell lymphomas (ALCL)

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Introduction The aim of this study was to investigate the expression of CD30 and the distribution of the reticular network on the ALCL.
Material and methods Twelve cases of lymphomas were analyzed using morphologic and immunophenotypic features.
Results The patients (7 male and 5 female) were from 5 to 50 years. Five patients presented with generalized lymphadenopathy and seven with involvement of extranodal sites. ALCL was composed of large cells that grow in a diffuse pattern and involve the lymph node sinuses. The tumor cells have an abundant eosinophilic or pale cytoplasm. The nuclei were irregular with indentation, multilobated, round to oval. Multinucleated cells with wreath-like configurated nuclei were commonly seen. ALCL cells may closely resemble cells of malignant histiocytosis. Additional features include Reed–Sternberg-like cells, reactive infiltrate, partial lymph node involvement with localization of tumor cells in the paracortical region resembling Hodgkin's diseases. Tumor cells are CD30 +. The regular expression of CD30 antigen in both carcinoma and ALCL (both are anaplastic large cell tumors) has important implication for the differential diagnosis of these tumors. Leukocyte common antigen (CD45) positivity excludes an embryonal or any other non-hematopoietic tumor, but its absence does not rule out ALCL (a significant portion of ALCL are CD45 negative). Antibodies to cytokeratin label embryonal carcinoma but not ALCL. Thus, if cytokeratin is expressed in an anaplastic large cell tumor simultaneously with CD30 antigen, it is most likely to be an embryonal carcinoma
Conclusion This study confirms the view that ALCL are heterogeneous group of lymphomas and the CD30 is a useful marker for the diagnosis.

P-364

Leiomyosarcoma of the Meckel's diverticulum. Synchronous occurence with B-chronic lymphocytic leukemia. A case report

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We describe a 66-years-old male developing a well differentiated leiomyosarcoma of the Meckel's diverticulum synchronously with B-CLL. The patient suffering disuria and malaise was admitted at the Clinic for Surgery. Clinical examinations showed leucocytosis (14.1x109/L) with lymphocytosis (56%), anemia (Hgb = 113 g/L) and thrombocytosis (473x109/L). CT-scan found a tumor mass near the urinary bladder and enlarged spleen. The FNAB from the tumor mass revealed neoplastic tissue composed of ribbons of spindle-shaped cells with hyperchromic and pleomorphic nuclei and variable amount of cytoplasm, surrounded by a fine reticulin network. Mitoses were rare (4-5 on HPMF). Immunohistochemistry showed positivity for SM-actin and desmin. S-100, CD31 and CD34 were negative. A leiomyosarcoma was diagnosed. Three weeks after the biopsy a large tumor mass (880g) from the vicinity of the urinary bladder was extracted, together with incidental Meckel's diverticulum. At the same time a splenectomy was performed because of splenomegaly (500g). Pathohistologic examination of the tumor mass confirmed the previous diagnosis, and the PCNA proliferative index was estimated to 35-40%. The analysis of the Meckel's diverticulum revealed a disturbance of the muscular layer structure caused by a tumor proliferation with the same histological features, infiltrating into the serosa. The pathohistologic examination of the extracted spleen showed partially presence of B-CLL infiltration (monoclonal positivity for lambda-light chains, CD20, CD79, CD5, CD43). Follow-up shows that the patient is in Binet A stage, and still in a good shape without any further treatment. It remains unclear which of the neoplasms developed first.

P-365

Composite low grade B cell lymphomas: Report of three cases

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Introduction Synchronous composite low grade B-cell lymphomas involving the same site is rare and few cases have been reported. We describe three cases of composite lymphoma.
Material and methods Histological, immunohistochemical and molecular study.
Results The three neoplasms showed different morphological and phenotypic areas: Case 1. 64 years-old female with large splenomegaly. The splenectomy showed a blastoid mantle cell lymphoma (MCL) with nodular pattern, positive to CD20, CD79a, CD5, CD43 and cyclin D1, and a splenic marginal zone B-cell lymphoma CD20, CD79a and IgD. The MCL progressed, with liver and bone marrow involvement, treatment failure and death 8 months after diagnosis. Case 2. 75 years-old female with cervical and inguinal lymphadenopathy. A nodular MCL CD20, CD5 and cyclin-D1 positive, p27 negative, was observed. The internodular areas demonstrated a small lymphocytic lymphoma (SLL) expressing CD20, CD79a, CD5, CD23, and p27. Progression to peripheral blood involvement of SLL, and gastric and nodal relapses of MCL two and four years after diagnosis. Case 3. 51 years-old female with nodal follicular lymphoma expressing CD20, CD10, Bcl-2, IgD-kappa restriction. The internodular areas showed a lymphocytic lymphoma with plasmacelular differentiation, CD20, CD79a, CD23, IgD, and bcl-2 positive. IgH showed a biallelic arrangement, and bcl-2/JH rearrangement was obtained, suggesting the presence of two different clones. Alive with disease after first chemotherapy cycle.
Conclusion Morphology and immunophenotypic profiles may allow the identification of each component in composite lymphoma. Molecular studies can recognize different clones originating the neoplastic compartments.

P-366

Malignant B-cell lymphomas non-associated with Epstein-Barr virus (EBV) infection in angioimmunoblastic T-cell lymphoma (AITL)

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Introduction AITL accounts for about 20% of peripheral T-cell lymphomas (PTCL), presents with advanced stages at diagnosis and follows aggressive clinical course. In 10-40% of cases oligoclonal or monoclonal B-cell populations are detected, usually associated with EBV infection. We describe three cases of AITL with clonal B-cell lymphoma non-related to EBV infection.
Material and methods Histological, immunophenotypical, including EBV (LMP-1, EBERs), and molecular study.
Results Three cases of AITL in three female, 66, 69 and 77 year-old patients were diagnosed. All of them showed atypical lymphocytes with aberrant T-cell phenotype (CD3/CD5+ with loss of CD7), associated with vascular and dendritic proliferation, the latter showing T-cells CD10+. In addition, a population of B-cells with plasmacelular differentiation and immunoglobulin light chain restriction (1 kappa/2 lambda) was identified. EBV was not demonstrated in these populations. TCRgamma and IgH clonality was observed in two cases. The first patient presented isolated nodal AITL involvement, and she is in complete remission after chemotherapy six months after diagnosis. The second patient presented nodal AITL and developed skin involvement two months after the diagnosis. The third patient presented with an atypical ganglionar T-cell proliferation, developing cutaneous PTCL with associated clonal B-cell proliferation one year later and ganglionar PTCL with AILT features two years after the first biopsy. The three biopsies showed identical TCRgamma gene rearrangements in this case.
Conclusion AITL can show different morphological patterns. Alternative mechanisms non-related to EBV infection may be involved in the development of B-cell lymphomas in AITL.

P-367

Pregranulomatous phase of sarcoidosis: immunohistochemical diagnosis?

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Introduction Histopathological confirmation of clinical suspicion of sarcoidosis is based on finding of non-caseating granulomas in biopsy material, usually in prescalene lymph nodes. Reactive sinus histiocytosis seen in relation to various inflammatory and non-inflammatory diseases can mimic pregranulomatous phase of sarcoidosis. Differentiation of sinus histiocytosis based on histopathological features alone can be impossible. Aim of this study is immunohistochemical determination of lymph node cellular

response in granulomatous sarcoidosis, pregranulomatous phase of sarcoidosis and reactive sinus histiocytosis using panel of antibodies.
Materials and methods Patient groups under study, each contained 25 patients include those with clinical and histopathological picture of sarcoidosis; the group of patients with clinical picture of sarcoidosis and histologically detectable sinus histiocytosis without granuloma formation and finally, the group free of clinical suspicion of sarcoidosis with 'reactive' sinus histiocytois in lymph nodes. Lymph node biopsy tissue processed routinely and stained immunohistochemically using triple-layer APAAP protocol with purified antibodies SP 70 and SP90 from Mycobacterium tuberculosis and monoclonal antibodies CD22, CD4, CD8, CD56, CD68. Intensity of immunostaining assessed semiquantitatively by two independent observers using the scale from 0 to 4+
Results An increased CD4:CD8 ratio, moderate increase of immunopositivity of CD68 and slight decrease of immunopositivity of CD20, CD56, and SP90 noted in PSH when compared with RSH. The most notable difference between studied groups is the intensity of immunostaining with SP70 and CD4 antibodies.
Conclusion Both immunostainings were more intense in lymph nodes with sarcoid granulomas and pregranulomatous sinus histiocytosis and can be used in differentiation of these lesions.

P-368

Haemophagocytic syndrome followed by a large B-cell lymphoma showing only bone marrow involvement: Case report

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Haemophagocytic syndrome (HPS) is a rare disease characterized by generalized histiocytic proliferation showing phagocytosis of hematopoietic cells. It occurs mainly in adults associated either with an acute viral infection, or with non-Hodgkin's lymphomas. The latter are usually T- or NK-cell lymphomas. The present case concerns a patient with HPS followed by a large B-cell lymphoma showing only bone marrow involvement. This constellation is extremely rare in Western countries and has been reported mainly in Asia. The patient was a 60 year old male admitted for persistent fever. Peripheral blood examination showed pancytopenia. Bone marrow biopsy showed haemophagocytosis by proliferating macrophages often situated into dilated marrow sinusoids. No viral infection was detected. The patient was treated with haemopoietic growth factors and showed improvement. After 6 months his blood cell count began to fall again and a second bone marrow biopsy showed, apart from haemophagocytosis, a focal, mainly intra-sinusoidal, infiltration by large B-cell lymphoma cells immunopositive for CD20 and CD45RA. Epstein-Barr virus RNA was not detected. Dissemination of lymphoma to other sites was not apparent. The patient was treated with 6 cycles of CHOP and Mab-Thera and showed remission. Some authors believe that these rare cases represent a peculiar "Asian" variant of intravascular lymphoma characterized by HPS and early involvement of bone marrow without dissemination to other organs. This variant has a poor prognosis and might constitute a distinct biological and clinical disease entity which merits separate consideration because of the problems posed in the initial diagnosis and therapeutic approaches.

P-369

Nodular type of mycosis fungoides. A case report

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Introduction Mycosis fungoides is a mature T-cell lymphoma presenting in the skin and characterised by epidermal and dermal infiltration of small to medium size T-cell with cerebriform nuclei. It is a rare disease wich accounts for no more than 0,5% of all non Hodgkin lymphomas.
Case report A 82 year-old man was refered to the Clinic of hematology because of the nodular red dermal tumours on the trunk. These lesions persisted for one year. The blood and bone marrow had not been involved. Lymph nodes were normal. The patient had the so called d' emblee lesions, presenting with skin tumours to 2 cm of diameter. The initial diagnostic lesions were rare limited patches and plaques. Extracutaneous dissemination did not occur. Formaldehyde fixed and paraffin embedded histological sections, taken from 4 surgically resected skin tumours, were stained with HE, Giemsa, trichrome and immunohistochemical stains. Morphology: Epidermotropic infiltrates consisted of medium size cells with cerebriform nuclei. A minority of larger cells with similar nuceli and with many mitotic figures also were present. So called Pautrier microabscesses consisted of aggregates of cerebriform cells in the epidermis (highly caracteristic for Mycosis fungoides) were numerous. In the dermis, infiltrates were band like and diffuse, associated with inflammatory infiltrate consisting of small lymphocytes and eosinophils.
Conclusions In spite of the presence of bad prognostic factors (very old patients, extent of disease and tumorous form) he had a very indolent course of the disease. The role of the observed inflammatory small lymphocytes and eosinophils is probably very important for the excellent prognosis of mycosis fungoides in this patient.

P-370

Low expression of p27 protein combined with altered p53 and Rb/p16 expression status is associated with increased expression of cyclin A and cyclin B1 in diffuse large B-cell lymphomas

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Introduction Reduced p27 expression combined with alterations in Rb or p53 status, may have cooperative effects resulting in uncontrolled tumor cell proliferation and aggressive malignancies in experimental mouse models and in humans. The aim of this study was to investigate p27 expression in relation to a) the expression status of p53, Rb, p16, and b) proliferation profile (Ki67, cyclin A, cyclin B1).

Materials and method Expression of cell cycle regulating proteins was investigated immunohistichemically, in 80 cases of de novo Diffuse Large B-cell Lymphomas (DLBCL).
Results P27 expression was low/null in large tumor cells in 58/80 and intermediate/high in 22/80 cases. Increased expression of p53 was observed in 39/80 cases. Decreased expression of Rb/p16 was found in 5/80 and 14/80 cases, respectively. Low/null p27 expression was correlated to increased p53 expression (p=0.018). There was a correlation between increased p53 and decreased Rb/p16 expression levels (p=0.050). These findings suggest a tendency for concurrent alterations of cell cycle regulators p27, p53 and Rb/p16 in DLBCL, which might result in impaired tumor growth control. Indeed, combined p27/p53/Rb/p16 expression status analysis, with respect to proliferation profile showed that: 1) three alterations in the combined p27/p53/Rb/p16 status were correlated with increased expression of cyclin B1 (p=0.005) and 2) two or three alterations were correlated with increased expression of cyclin A (p=0.014).
Conclusions These findings suggest combined impairment of a complex cell-cycle control network involving the CDK inhibitor p27, as well as P53 and Rb1 pathways, which exerts a cooperative effect resulting in enhanced tumor cell proliferation.

P-371

Expression of cyclin D3 and cyclin E and identification of distinct clusters of proliferation and apoptosis in diffuse large B-cell lymphomas

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Introduction The aim of this study was to examine a) cyclins D3, E and D1 expression in relation to other proliferative features (Ki67, cyclin A and cyclin B1), apoptosis status and p53, Rb, p16, p27 expression and b) whether distinct clusters of proliferation and apoptosis could be identified in DLBCL.
Materials and method Immunohistochemical expression of cell cycle regulating proteins were studed in seventy-nine cases of de novo Diffuse Large B-cell Lymphomas (DLBCL).
Results Overexpression of cyclins D3, E was found in 35/79 (43%) and 18/79 (22%) cases, respectively, whereas cyclin D1 was not detected. In 39/46 cases, overexpression of cyclins D3 and E was mutually exclusive, proposing different underlying pathways that induce deregulated expression of these cyclins. In 29/35 cases, cyclin D3 overexpression was equally exclusive with Rb/p16 aberrant expression status, supporting an oncogenic role for cyclin D3 and suggesting that pathogenetic effect of cyclin D3 overexpression occurs through perturbation of Rb1 pathway. Combined alterations of P53 and Rb/p16/cyclin D3 expression profile were significantly correlated to higher mean values of cyclins A (p=0.023) and B1 (p=0.033) indicating that concurrent impairment of p53 and Rb1 pathways induces increased tumor cell proliferation. Cluster analysis of apoptosis and proliferation status permitted separation of DLBCL into distinct groups with low and high apoptotic activity, as well as into groups with low, intermediate and high proliferative activity.
Conclusions In the present study, identification of separate groups with respect to proliferation and apoptosis profile indicates that groups with discrete cellular kinetic properties can be defined in the DLBCL population.

P-372

Immunohistochemical expression of p53, p21/waf1, Rb, p16, cyclin D1, p27, Ki67, cyclin A, cyclin B1, bcl2, bax and bak proteins and apoptotic index in normal thymus

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Introduction Immunotopographical distribution of protein networks involved in cell-cycle and/or apoptosis regulation is useful in thymic histophysiology understanding. The aim of this study was to examine a) p53 and Rb1 growth control pathways, b) proliferation, c) apoptosis in relation with age.
Materials and methods P53, p21, Rb, p16, Ki67, p27, bcl2, bax, bak, cyclin D1, cyclin A, and cyclin B1 expression, and apoptotic index (AI) were investigated in 8 adult, 3 adolescent, 5 infant and 4 newborn normal thymuses, with immunohistochemistry.
Results and conclusions Rb, Ki67, cyclins A and B1 expression was elevated in cortex with a decreased tendency toward medulla. Apoptotic cells were mainly detected in cortex/corticomedullary junction, but rarely in Hassall's corpuscles. Mean values of Ki67 and cyclins A, B1 immunoreactivity were 77.2 %, 32.2%, 21.4% (newborns), 62.4%, 33.7%, 18.5% (infants), 56.9%, 23.4%, 18.9% (adolescents), 38.7%, 21.7%, 14.6% (adults), respectively. Mean values of AI were 1.4 %, 2.9%, 2.7%, 3.8 %, respectively. Most of Hassall's corpuscles were p16-positive. P16 expression paralleled aging, suggesting a role of p16 in cellular senescence. P27 was detectable within medulla, but not in subcapsular thymocytes Expressions of Ki67, cyclin A and cyclin B1 were inversely related to p27. P21 and p53 showed primarily subcapsular cortical epithelial cell localization. Our findings suggest: a) thymocyte apoptosis is mainly p53-independent, b) p21 function as negative cell cycle regulator seems redundant to other negative regulators (p16/27), and c) p21 expression is chiefly p53-independent. Hassall's corpuscles were p21-positive. Bcl2 was principally detected in medullary thymocytes. Expression of Bax/bak was widely distributed, suggesting an advanced role in thymic apoptosis, compared to bcl2.

P-373

Blastic MDS and AML - bone marrow biopsy findings versus clinical manifestation

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Introduction MDS represents a group of clonal hematopoietic stem cell disorders, which may progress to AML. According to WHO classification, the treshold of 20% blasts is recommended for the distinction of AML from MDS. The aim of this study was analysis of biopsy cases showing blastic proliferation and MDS patterns diagnosed in 1999-2002 to verify the percentage of blasts and the disease´s course.

Material and methods Paraffin-embedded bone marrow biopsies of 107 patients were examined by a standard panel approach and evaluated semiquantitatively. The course of the disease was recognized by evaluation of instrumentalized questionnaires.
Results a/ MDS with less than 20% of blasts (n=32) /either of myelo- (n=14), megaloblastic (n=9) or bilinear profile (n=9)/ - AML evolution appeared in 7% of patients, b/ MDS with more than 20% of myeloblasts (n=75) - AML diagnosis was established in 47% of cases.
Conclusion Although the therapeutical AML managment requires 'clinical verification', the identification of the MDS blastic proliferation higher than 20% is of a great clinical importance. However, the clinical manifestation of AML may be influenced by a delay of the increase of blasts in the peripheral blood.

P-374

Increased expression of the bcl6 and CD10 proteins is associated with increased apoptosis and proliferation in diffuse large B-cell lymphomas

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Introduction There is increasing evidence that bcl6 and CD10 expression may be related to apoptosis and cell cycle progression.
Materials and methods To examine immunohistochemically the expression profile of bcl6 and CD10 in 79 cases of de novo diffuse large B-cell lymphomas, in relation to a) the apoptotic index (AI) b) the proliferation-associated proteins Ki67, cyclins A and B1 c) the expression of bcl2, p53, Rb, p16, p27.
Results and conclusions Expression of bcl6, CD10 and bcl2 was detected in 54/79 (68%), 28/79 (35%) and 47/79 (63%) cases, respectively. The bcl6/CD10 patterns were as follows: bcl6+/CD10+ (26 cases, 32%), bcl6+/CD10- (28 cases, 33%), bcl6-/CD10- (23 cases, 31%) and bcl6-/CD10+ (2 cases, 4%). Significantly positive correlations were found between bcl6/Ki67 (r=0.328, p=0.003), bcl6/cyclin A (r=0.265, p=0.018), bcl6/AI (r=0.327, p=0.010), CD10/Ki67 (r=0.296, p=0.008) and CD10/AI (r=0.397, p=0.001). High expression of bcl6 was correlated with null/low bcl2 expression (Hi-square-test, p=0.002). These findings indicate that increased expression of bcl6 and CD10 is associated with increased apoptosis and proliferation in diffuse large B-cell lymphomas. Association between increased bcl6 expression and enhanced apoptosis might be due to null/low bcl2 immunoreactivity, since previous in vitro data revealed that bcl6 overexpression induces apoptosis accompanied by bcl2 and bcl-xl downregulation. Significant correlation was found between increased AI and bcl6+/CD10+ pattern (T-test: p=0.014, Mann-Whitney test: p=0.046). This result combined with positive correlation between AI and bcl6 and CD10 immunostaining may be in accordance to previous studies showing that expression of these proteins has favorable effects on the clinical outcome of these neoplasms.

P-375

Primary non-Hodgkin lymphomas of bone: morphologycal and immunohistocehmical aspects

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Introduction Primary non-Hodgkin lymphoma (NHL) of bone (PLB) is a rare clinic-pathologic entity that accounts for approximately 3% of all malignant bone tumors and 4-5% of all extranodal NHL. We analyzed morphological and immunohistocemical aspects of 8 PLB presented as solitary localized bone tumor.
Methods Paraffin embedded biopsy specimens of all 8 patients were diagnosed by the histomorphology and by immunohistochemistry using a wide panel of monoclonal antibodies (EMA, LCA, NSE, TdT, CD34, HLA-DR, CD79a, CD20, CD10, surface and cytoplasmic Ig, CD3, CD5, CD43, CD99, CD30, CD30, CD138, BCL2, BCL6 and Ki-67).
Results The median age of our pts. was 40 (from 14 to 65) with slight female predominance. Various sites were involved: 4 tumors were in tubular long bones, 2 in pelvic bones and 2 in spine with infiltration of soft tissue and without bone marrow involvement. All 8 cases were classified as diffuse large B-cell lymphoma (DLBCL) according to morphology and immunophenotype. Patohistological analyses showed markedly polymorphous cellular infiltrate with large cells with complex nuclear contours and multilobated nuclei, together with centroblasts-like cells and immunoblasts. All tumors were associated with some degree of fibrosis. Immunologic studies showed strong expression of pan-B markers and HLA-DR, weak expression of CD10 and cIg (mostly IgM) in half of tumors. BCL2 was positive in all cases as well as BCL6. The plasmacytic differentiation was observed in 1/3 of cases. The proliferative fraction was high, >50% cells in all patients.
Conclusion Our analysis revealed that all PLB were of DLBCL type with characteristic morphology and immunophenotype. Further study will show the outcome of patients with PLB, as well as the validity of various prognostic parameters.

P-376

Peripheral T-cell lymphoma with perifollicular growth pattern expressing CD10

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Peripheral T-cell lymphomas (PTCLs) may reveal a variety of morphologic patterns and usually grow in a diffuse pattern with

inflammatory cells. PTCLs with nodular or perifollicular growth patterns are very rare. Only 18 cases have been reported in the literature. We report an unusual case of PTCL showing perifollicular growth pattern with expression of CD10 and bcl-6. The patient was a 46-year-old male who presented with a generalized lymphadenopathy, fever, and weight loss. Hepatosplenomegaly and polyclonal gammopathy were not identified. Cervical lymph node biopsy was performed. The histology showed nodular effacement of nodal architecture with proliferation of atypical lymphoid cells which mainly infiltrate perifollicular area. Interfollicular area also showed proliferation of atypical lymphoid cells and high endothelial venules with a few immunoblasts and eosinophils. Neoplastic cells were medium-sized, with irregular nuclei and clear cytoplasm. Immunohistochemically, the neoplastic cells were CD3+CD4+CD5+CD8-CD10+CD30-CD57-bcl-2+bcl-6+, demonstrating helper/inducer T-cell origin. The CD10 was strongly expressed mainly in the perifollicular tumor cells, whereas the normal germinal center (GC) cells showed down-regulation of CD10. The distribution of CD10+ perifollicular tumor cells was closely related with CD21+ follicular dentritic cells (FDCs), suggesting CD10 up-regulation might be induced by the proliferating FDCs. TCR-gamma gene rearrangement using PCR-SSCP was monoclonal. This case could be classified as PTCL, unspecified, and should be discriminated from other lymphomas, including marginal zone B-cell lymphoma and follicular lymphoma. Further studies are necessary to clarify the distinction of this tumor from angioimmunoblastic T-cell lymphoma.

P-377

Primary bone CD30+ anaplastic large cell lymphoma. Clinico-pathologic analysis of 5 cases

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Introduction CD30+ anaplastic large cell lymphomas (ALCLs) frequently involve extranodal sites, mainly skin and gastrointestinal tract, but primary bone ALCLs are exceedingly rare. The aim of this study was to detail the morphofunctional features and outcome of 5 cases of primary bone CD30+ ALCLs.
Materials and methods all the patients (M:F 3:2, median age 17 years, range: 6-50), but 1 (with 2 simultaneous occurring skin lesions) presented with bone confined lymphoma (osteolytic lesions of jaw, rib, sternum, iliac and pelvic bones). Biopsies from all cases were analysed.
Results and conclusion according to the WHO lymphoma classification, all cases were CD30+ ALCLs (4/5 common type, 1/5 small cell predominant variant). Lymphoma population showed a T (2/5) or "null" (3/5) phenotype, expressed cytotoxic associated molecules (TIA1, granzyme B and/or perforin); CD246/ALK was positive in 4/5 cases, whereas 1/5 expressed CD56. TCR-gamma clonal rearrangement was detected in 3/5 cases. All patients received polychemotherapy; at last follow up they all are alive, in complete remission (mean: 35 months, range: 5-132). In our cases, the morphofunctional features of the lymphoma population were similar to their nodal counterparts, including CD246 expression in the younger patients. On such bases, the hypothesis must be considered that primary bone CD30+ ALCLs represent the first extranodal site of presentation of a systemic disease. In contrast with similar reported series, our patients run a favourable prognosis and in the single positive case, CD56 expression does not negatively influence outcome. However, larger series of similar cases must be analysed before definitive conclusions can be drawn.

P-378

Immunohistochemical detection of follicular dendritic cells in B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma

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Introduction Follicular dendritic cells (FDC) form a meshwork in the lymphoid follicle and take part in the regulation of the germinal centre reactions. FDC also interact with the neoplastic lymphoid proliferations. In B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma (B-CLL/SLL), FDC structures are reduced. The aim of this study was to evaluate the presence and patterns of follicular dendritic cells B-CLL/SLL using several anti-FDC antibodies.
Materials and methods 50 formalin-fixed paraffin-embedded lymph nodes diagnosed as B-CLL/SLL according to the WHO criteria were studied using CD21, CD23, and CNA.42 antibodies after microwave oven antigen retrieval
Results In 17 cases (34%) FDC meshworks were identified in none of the reactions. In 15 specimens (30%), FDC meshworks were exceptionally rare as follicle remnants at the lymph node periphery or as small irregular clusters not reminding of the normal follicle meshwork. In 18 cases (36%) FDC formed either frequent small irregular clusters, or rings at the follicle periphery, or regular follicular meshworks. Reticular structures in a given case were most frequent in CNA.42 stain, scarcer in anti-CD21, and anti-CD23 stains. The majority of cases showing FDC was obtained by CNA.42 (33 cases), CD21 (22 cases) and CD23 (16 cases).
Conclusion FDC structures were absent or scarce in up to 64% of B-CLL/SLL cases which can be used as an auxilliary diagnostic criterion. The positive cases showed small irregular clusters, rings at the follicle periphery, or normal follicular meshworks. Best results were obtained with CNA.42 antibody. This may be due to better antigen preservation or to non-specifity.

P-379

Usefulness of CD34 immunoreactivity in classification of myelodysplastic syndromes

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Introduction Myelodysplastic syndromes (MDS) are clonal haematopoietic stem diseases characterised by dysplasia and ineffective haematopoiesis. The dysplasia may be often associated with an increased number of myeloblasts in peripheral blood and

bone marrow, which represents an important prognostic factor. Immunohistochemical detection of blast cells in bone marrow histological sections using CD34 antigen and its correlation with smear blast count and MDS classification are not well established. We report a quantitative study of CD34 blast cells in MDS and acute myeloid leukaemia with multilineage dysplasia (AMLMD), and analyse the relationship with both cytologic smear and clinical diagnosis.
Materials and methods We have studied 15 bone marrow biopsies diagnosed of MDS (4 with refractory cytopenia with multilineage dysplasia (RCMD), 3 with refractory anemia with excess blast (RAEB) type I and 8 with RAEB type II) and 5 with AMLMD. We added 6 controls and calculated the CD34 blast cells mean per high power field (hpf). Cytologic smears were reviewed.
Results The results were: controls showed 4-20 blasts/10hpf, RCMD 6-38/10hpf, RAEB I 21-46/10hpf, RAEB II 44-440/10hpf and AMLMD 358-950/10hpf.
Conclusions -CD34+ blast cells density is a good parameter in the histological diagnosis of MDS and has a close correlation ($p<0,01$) with smear count
Results -CD34 does not distinguish between RCMD and RAEB I. -More than 50 blast cells/ 10 hpf raise the diagnosis of RAEB II. More than 50 blast cells/ 1 hpf raise the diagnosis of AMLMD. The extrapolation of these features to "dry tap" aspiration remains to be established.

P-380

Primary intestinal lymphoma: clinical and pathologic features of 16 patients

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Introduction Although the gastrointestinal tract is the most common site of extranodal lymphoma, primary intestinal lymphomas (PIL) are rare. We report a retrospective analysis of 16 patients with PIL, presenting clinical and pathologic features.
Patients and methods PIL was defined according to the criteria of Dawson. Sixteen patients (11 males and 5 females) with age range of 4-74 years were studied. The formalin-fixed and paraffin-embedded tissue was used for routine microscopic examination and immunohistochemical stainings.
Results The most frequent clinical presentations were ileus (7/16) and intestinal bleeding (6/16). Painful abdominal tumor (2/16) and perforation (1/16) were rare. The most frequent locations were ileocecal region (6/16) and ileum (5/16); 3/16 were located in duodenum and 2/16 involved colorectum. All children showed involvement of ileocecal valve. Three patients had associated other diseases: AIDS (1/16), Crohn's disease (1/16) and celiac disease (1/16). Intestinal T-cell lymphoma (associated with celiac disease) was diagnosed in one case. Among B-cell NHL there were 8/16 aggressive NHL (4 Burkitt's lymphomas, 3 diffuse large B-cell lymphomas and one Burkitt-like lymphoma) which were localized as follows: 5 in ileocecal region, 2 in ileum and one in duodenum. Among indolent lymphomas there were 5/16 MALT-omas and 2/16 follicular lymphomas with predilection to duodenum (2/16) and colon (2/16).
Conclusion PIL are rare malignancies with distinct clinical features, but with ileus as the most frequent clinical presentation and ileocecal region as the most frequent site of involvement. Burkitt's lymphoma was predominant histological type. Indolent and aggressive type of NHL were almost equally presented.

P-381

Bone marrow angiogenesis in hairy cell leukaemia - a morphometric study

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Inroduction Bone marrow angiogenesis has been recently implicated in the pathophysiology and course of various haematological malignancies. Little is known, however, about the significance of this phenomenon in hairy cell leukaemia (HCL).
Methods We evaluated various morphometric characteristics of microvessels, highlighted by means of anti-CD34 immunohisto-chemistry, in the bone marrow of 44 patients with typical HCL, before and after treatment with interferon-α. Overall 104 bone marrows from HCL patients and twenty control bone marrows were examined. Microvessel density (MVD) and several size- and shape-related parameters were quantitated in the region of most intense vascularization using image analysis.
Results MVD, size-related parameters and the percentage of branching microvessels were higher in HCL than in controls and in partial/non-responders in comparison to complete responders. Acvievement of complete response was accompanied by smaller calibre microvessels. Interferon-α induced a decrease in MVD and branching values in cases with diffuse marrow involvement. In univariate analysis, progression-free survival was adversely affected by MVD and branching. Multivariate analysis indicated that factors related to the size of the microvessels and MVD/ branching independently affected progression-free survival. MVD/ branching was also related to the likelihood of complete response.
Conclusions Our data suggest that the generation of bone marrow microvessels connotes an increased risk for progression and interferon-α treatment failure in HCL. Furthermore the prognostic significance of angiogenesis requires the concomitant assessment of MVD, the size of microvessels and the complexity of the microvascular network. Key words: Hairy cell leukaemia, bone marrow, angiogenesis, morphometry

P-382

Inflammatory pseudotumor of the spleen and its relationship to Epstein-Barr virus (EBV): Report of a case

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Introduction Inflammatory pseudotumors are uncommon and enigmatic benign lesions which may develop at several anatomical sites and recent studies demonstrated the frequent presence of

Epstein-Barr virus (EBV) in them. The occurence of inflammatory pseudotumor in the spleen is rare. A case of inflammatory pseudotumor of the spleen is presented and its relationship to Epstein-Barr virus (EBV) is examined.
Material and method The case was a 62-year-old woman in whom a solitary splenic mass was discovered as part of the workup of idiopathic thrombocytopenic purpura. Splenectomy was performed.
Results Grossly, the removed spleen, weighing 140 g, contained a tan-white, circumscribed mass, measuring 4.5X4X4 cm. Microscopically, the mass was composed of a heterogeneous cellular population of acute and chronic inflammatory cells. Plasma cells predominated, but lymphocytes, neutrophils, eosinophils, interlacing fibroblast-like spindle cells and rare multinuclear giant cells were found. Amorphous, acidophilic material was present focally with the plasma cell proliferation. Plasma cells presented light chains polyclonality. Lymphoid cells were mature and mainly of T-lineage. Spindle cells exhibited histiocyte phenotype (CD68+++). Immunohistochemical stain for EBV was negative.
Conclusions Inflammatory pseudotumor of the spleen is a benign tumorous lesion that has been described in only a few cases in the world literature (<75 cases). Its etiology and pathogenesis is unknown and association EBV is not always present. Recognition of this rare entity is important, as the clinical manifestations and imaging features could be indistinguishable from a lympho-proliferative disorder or another malignancy of the spleen.

P-383

Histopathological and immunohistochemical patterns of nodal non-Hodgkin's lymphoma in Montenegro

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Introduction The diagnosis of nodal non-Hodgkin's lymphoma (NHL) in Montenegro has been done only in Clinical Center of Montenegro - incidence of diagnosed NHL can be considered as the incidence in epidemiological sense.The use of monoclonal antibodies since 1997, has improved our ability to diagnose and to treat NHL. Aims: By revision of immunohistologically diagnosed cases of NHL between 1997-2003, we wanted to estimate (a) the frequency of particular clinico-pathological types of NHL, (b) hypothesis about geo-socio-economic influences on patterns of NHL, and (c) the influence of immunophenotypisation of NHL on speciffic chemo- and immunotherapy.
Materials and methods NHLs were diagnosed with the use of HE, Giemsa, MGP, PAS, Gomori, LCA, bcl2, TdT, CD3, CD43, CD20, CD79a, CD15, CD30, and EMA stained slides and classified according to the Working Formulation criteria.
Results Between '97 and '03 we diagnosed 116 NHL. Low, intermediate and high grade malignancy comprised 33,6 %, 50,9 % and 15,5 % respectively. The highest incidence was estimated in 1999:1,5/100000. The NHLs of FCC origin constitute 60,3% of all our NHLs. In 80% of patients (25) low grade malignant NHLs were highly CD20+. Nine patients recieved Mabthera+CHOP/or +COP therapy during the last three years.All patients have been in remision.
Conclusion The estimated pattern of NHL - the most frequent NHL derivated from FCC- is comparable with geographic and socio-economic description of Montenegro as "underdeveloped, ecological, farming European country".The high frequency of CD20+ and initial therapeutical results indicate future prospective patho-clinical studies of this issue.

P-384

Lack of CD 20 expression in herpesvirus 8 (HHV8) infected cells in multicentric Castleman's disease (MCD)

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Background The multicentric Castleman's disease (MCD) is a lymphoproliferative disease with increased risk for non-Hodgkin lymphoma. In AIDS patients, MCD is usually associated with human herpesvirus 8 (HHV8), the infectious agent of Kaposi's sarcoma but in contrast to Kaposi's sarcoma, HHV8 infected cells in MCD show evidence of lytic viral infection. Similar to other immunosuppression-associated lymphoproliferative diseases it has been suggested that anti-CD20 antibodies may be useful in the therapy of these lesions. Aim of the present study was the analysis of CD 20 expression in HHV8 associated MCD and their association with lytic viral infection.
Material and methods Formalin fixed, paraffin embeded lymph nodes were analyzed by a double immunofluorescence assay using antibodies against viral proteins and CD20. HHV8 proteins which were analyzed include the latent viral protein LANA (ORF 73) and the viral interleukin 6 (vIL-6) and the ORF74, both associated with lytic HHV8 infection.
Results Eleven lymph nodes of seven HIV-infected patients with MCD were analyzed. In the mantle zone of the lymphoid follicle, 8,4%, 2,8% and 1% of cells show positivity for LANA, vIL-6 and ORF74, respectively ($p < 0,02$). By double immunofluorescence, 91% of HHV8 infected cells showed co-expression of LANA/vIL-6 and LANA/vORF74 and 96% showed co-expression of LANA/vIL-6, respectively. In addition, there was statistical significant correlation between vIL-6 and LANA as well as vIL-6 and vORF74 ($p < 0,01$). Of the HHV8 infected cells, 6,5% showed CD20 expression and no difference between the three viral proteins analyzed were observed (5,5%, 7,4%, 6,6% of LANA, vIL-6 and vORF 74, respectively).
Conclusion The present data support the concept that HHV8 runs, in contrast to Kaposi's sarcoma, through a lytic viral infection in lymph nodes of patients with MCD. However, only a small subset of infected cells show co-expression of CD20, independent of the state of viral replication. This small number of infected cells expressing CD20 indicate little effect of anti-CD20 therapy. Based on the evidence of lytic HHV8 infection, however, anti-viral drugs inhibiting HHV8 replication may be more efficient.

P-385

Anaplastic large cell lymphoma - pathological and immunohistochemicals aspects

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The WHO Classification of NonHodgkin Lymphoma (NHL) included also the cathegory of T and null Anaplastic Large-Cell Lymphoma (ALCL). ALCL is a very rare NHL (about 5%), with the systemic-aggressive variant, and the cutaneous variant. The presence of the t(2;5) and the formation of ALK protein identified one subgroup of ALCL with a good prognosis. The aim of our

study is to analyze the histopathological and immunohistochemical characters of the ALCL diagnosed in our department in the last 2 years. 15 cases of T and null ALCL (M/F=12/3, more half of them younger of 30 years) were clinical and immunohistochemical analyzed (by ABC indirect methods, with a large panel of monoclonal antibodies). The pattern of lymph node infiltration was diffuse (9 cases), parafollicular (5 cases) and only with sinus infiltration - 1 case. The pathological subtypes were common (7 cases), lymphohistiocytic (4 cases), small cell variant (1 case), sarcomatous (1 case), eosinophils-rich (1 case) and neutrophil-rich (1 case). Immunohistochemically, the malignant cells presented with T-fenotype (7 cases) and null (7 cases); 1 case (M, 17 y) presents histiocyte markers (CD68+CD30+EMA+ALK+). All cases of ALCL were CD30+, but only 13 cases were EMA+ (2 cases were CD45RO+CD30+ALK-EMA-). 2/3 of cases were ALK+ (5-T and 5-null), with a nuclear and a membranous pattern. 5 cases were BNH9+ (4 cases ALK+). Only 1 case presented CD15 expression(CD30+EMA+ALK+BNH9+). 2 of the cases with T fenotype expressed CD4 markers (both were CD4+CD8-). The PCNA and Ki67 proliferation markers were positive in a very large percent of the malignant cells (between 30 and 80%). Numerous reactive T- lymphocytes presented a helper fenotype (CD4>CD8), and Leu-7+ cells were very rare. The null and T-cell ALCL pose numerous problems of differential diagnosis and immunohisto-chemicals methods are very useful for diagnosis and prognosis of these NHL.

P-386

Myeloid sarcoma -9 cases analysis

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Myeloid (granulocytic) Sarcoma (MS) – also known as “Chloroma”, is a localized tumor of primitive myeloid cells, that can present before, simultaneously with, or after the diagnosis of acute myeloid leukaemia (AML). Rarely, MS may represent the first sign of blastic transformation of myelodysplastic syndrome (MDS) or chronic myeloproliferative disorders (CMPD). The aim of this study is to present our experience in the diagnosis of MS. In the last 7 years, we diagnosed 9 cases of MS (M/F=4/5, between 18 and 66 year-old, with 50,2 years median). A large panel of monoclonal antibodies (lymphoid and myeloid cell markers) was used for the diagnosis of MS and for the differential diagnosis with NonHodgkin Lymphomas (NHL). The tumor localizations were: lymph nodes (5 cases, including a bulky inguinal tumor), lymph node and skin (1 case), paravertebral (2 cases), testis (1 case). In our cases, 2 patients presented with MS before the AML diagnosis (F, 26y, paravertebral MS, 10 months before AML-M3, and M, 57y, 15 years after NHL diagnosis, presented with an inguinal lymph node MS, 6 months before AML-M4). In 3 cases, MS was diagnosed simultaneously with AML (AML-M2; AML-M3; AML-M4), and in 2 cases after AML (m, 34y, MS with testis localization, 3 years after AML-M3; F, 66y, lymph node and skin MS, 6 years afterAML-M4). In one case (F, 66y), 6 years after successful treatment of an acute lymphoblastic leukaemia (ALL), the patient presented with a lymph node MS, considered as a blastic transformation of a chronic myelo-monocytic leukaemia (CMML). The diagnosis of MS may be very difficult if this tumor precedes the myeloid proliferative disorders, and the differential diagnosis with NHL may pose a lot of problems.

P-387

B-cell prolymphocytic “leukemia” (B-PLL): a heterogeneous disorder with a broad clinical and genetic spectrum

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Introduction B-PLL is an infrequent disease, considered by the WHO lymphoma classification as an aggressive condition and characterized by the presence of B symptoms, massive splenomegaly without significant lymphadenopaty and peripheral lymphocytosis with more than 55% prolymphocytes. Herein we report 2 cases of B-PLL to show the wide clinical and genotypic spectrum of this disease.
Case reports Patient 1. A 51-year old male with a history of petechiae and ecchymoses of 1 year evolution. A CBC showed significant lymphocytosis with >55% prolymphocytes. Physical examination revealed an enlarged cervical lymph node and splenomegaly. Lymph node biopsy was diagnosed as infiltration by B-PLL (CD20+, CD79a+, IgM+, CD5-, CD23-, CD43- and cyclin D1-). A bone marrow biopsy showed infiltration by B-PLL. Cytogenetic analysis of the bone marrow showed a Burkitt’s variant translocation t(2;8) (p12;q24) Molecular analysis of the lymph node showed IgH rearrangement and germline bcl-2 and bcl-1 by PCR. He was treated with four cycles of fludarabine (25 mg/m2) and rituximab (500 mg/m2) and is in clinical and cytogenetic remission at 1 year of follow-up. Patient 2. A 54-year old female with a 2 month history of multiple cervical lymph node enlargement and fever. Physical examination was normal and she had no lymphocytosis on the CBC. Lymph node biopsy showed infiltration by a non-Hodgkin’s lymphoma with a prolymphocytic morphology (CD20+, CD79a+, IgM+, CD5-, CD23-, CD43- and cyclin D1-). Bone marrow biopsy showed focal infiltration by B-PLL. Cytogenetic analysis of the bone marrow revealed a normal female cariotype and normal FISH patterns were found for the following probes: chormosome 8 centromere, 8q24 (c-myc), chromosome 12 centromere, 13q14 (D13S319), 14q32.33 (IgH) and 17p13.1 (p53). She was initially treated with CHOP and then switched to fludarabine having achieved a good clinical response after one cycle each. Discussion. The association between B-PLL and translocation t(2;8) (p12;q24) has been reported in very few cases, however direct involvement of c-myc alterations affecting specifically 8q24 has been proposed as an important factor in the pathogenesis of B-PLL. In patient 1, the observed genetic alterations could be of significance to define the genetic spectrum of B-PLL. Case 2 corresponds to a B-cell prolymphocytic lymphoproliferation with isolated lymph node involvement and no leukemic component. To our knowledge this presentation has not been reported previously and it is not considered in WHO classification. Morphological and immunohistochemical characteristics of this case are fully compatible with a B-PLL. Even though it has been considered that some cases of B-PLL could in fact represent a morphological variant of mantle cell lymphoma, in this case the negativity for CD5 and cyclin D1 partially rule out this possibility. B-cell prolymphocytic lymphoproliferations constitute an heterogeneous group of disorders in which c-myc rearrengement can be present. In this group of neoplasia therefore, the nodal involvement can be either predominant or the only clinical manifestation, thus we

propose to consider the term B-cell prolymphocytic lymphoma/ leukemia instead of B-PLL to denote these disorders.

P-388

EBV-positive anaplastic large cel lymphoma (ALCL) in Mexican patients

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Background ALCL is a particular type of T-cell non-Hodgkin's lymphoma originally characterized by a peculiar morphology and the consistent expression of CD30. This entity is frequently associated with the translocation t(2;5) (p23;q35) or some of its variants resulting in the overexpression of the chimeric protein NPM/ALK (ALK+), even though in the current classification ALK-negative cases are also considered within this group. EBV is classically and constantly associated with certain specific types of lymphoma but its association with ALCL is not considered a characteristic feature of this disease. The aim of this study was to investigate the frequency of the ALCL-EBV association in a population of adult mexican patients.
Results Eighteen patients with a morphological and immunohistochemical diagnosis of ALCL with nodal involvement were included. Immunohistochemical and ISH analysis was performed for CD3, CD43, CD45RO, CD45RB, CD20, CD30, CD15, EMA, LMP-1, ALK.and EBER1. Mean age was 45 years and the male-to-female ratio was 1.2:1. Immunohistochemistry and in situ hybridization results are summarized in the next table. All the so considered EBV positive cases expressed LMP-1 and EBER 1.
Conclusion In contrast to the information available in the literature we found a high incidence of the ALCL-EBV association 10 /18 (55%). The high incidence of ALK-/EBV+ cases 9/18 (50%), with morphological and immunophenotypical characteristics of ALCL gives rise to the possibility that this is a different subset of T-cell lymphomas with anaplastic morphology in which EBV plays a pivotal roll in the oncogenesis and evolution. These disorders have been considered so far to be ALCL /ALK- lymphomas. The high incidence of EBV infection in Mexico leads to the development of peculiar lymphoid proliferations as well as associations (EBV/ALCL) different from those considered to be frequent in world literature.

P-389

CDK9/Cyclin T expression in reactive lymph nodes and malignant lymphomas

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CDK9 is a member of the CDC2-like family of kinases. Its cyclin partners are members of the family of Cyclin T (T1, T2a and T2b) and cyclin K. CDK9/Cyclin T1 complex is very important in the differentiation program of several cell types, controlling specific differentiative pathways. Limited data are available regarding the expression of CDK9/Cyclin T1 in haematopoietic and lymphoid tissues. The aim of this paper is to analyze the expression of CDK9/Cyclin T1 complex in lymphoid tissue, in order to assess its role in B and T cell differentiation and lymphomagenesis. In reactive lymph nodes CDK9/Cyclin T1 expression was found by immunohistochemistry in germinal center and in B and T cell blasts in interfollicular areas, suggesting a role for CDK9 in the activation and differentiation of B and T cells. The staining of CDK9 and cyclinT1 complex in malignant lymphomas seems to reflect their normal counterpart, as it is highly expressed in lymphomas derived from germinal center cells, such as follicular lymphomas and classical Hodgkin's lymphomas and from activated T cells, (i.e. anaplastic large cell lymphomas). Diffuse large B-cell and Burkitt's lymphomas showed a wide range of values. No expression of CDK9 and Cyclin T1 was detected in marginal zone and mantle cells lymphomas. However, at RNA level we found an imbalance in CDK9/Cyclin T1 ratio in follicular lymphoma and diffuse large B cell lymphomas with germinal center phenotype, and in the cell lines of classical Hodgkin's lymphomas, Burkitt's lymphomas and anaplastic large cell lymphoma, in comparison with reactive lymph nodes.
Here we report CDK9/Cyclin T1 complex deregulation in neoplastic conditions for the first time, representing a step towards better understanding of through which molecular mechanism CDK9/Cyclin T1 complex acts on the activation and differentiation program of lymphoid cells and the role of its deregulation in malignant transformation.

P-390

The value of tartrate resistant acid phosphatase (TRAP) immunoreactivity in diagnosis of hairy cell leukaemia

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Introduction Hairy cell leukaemia (HCL) is a chronic lymphoproliferative disease characterized by the presence of neoplastic B cells with hairy cytoplasmic projections in spleen, bone marrow and peripheral blood. Complete or near-complete remission can be achieved by recent chemotherapy protocols. These treatment methods are quite different from the chemotherapy protocols used for the treatment of other lymphoproliferative diseases. An absolute and correct diagnosis, i.e. detection of HCL cells by specific methods, is necessary for the achievement of complete or near-complete remission by special chemotherapy programs. Detecting intracytoplasmic tartrate resistant acid phosphatase (TRAP) enzyme activity in the peripheral blood and bone marrow aspirates is a classical, simple, sensitive and quite specific method for the detection of HCL cells and in the diagnosis of HCL. However, this method is insufficient in some cases. For example, the most of the HCL patients are leukopenic and there are a few number of neoplastic cells in their peripheric blood. An adequate number of neoplastic cells cannot be obtained by bone marrow aspiration because of the increase in reticulin fibbers of the bone marrow. In such cases, bone marrow trephine biopsy is almost the only and the most important method in diagnosis and follow-up of this disease. Specific detection of TRAP enzyme activity in bone marrow trephine biopsy materials is not possible because of the effect of tissue fixation and processing on these materials. In conclusion, a new method for detecting TRAP enzyme activity in bone marrow trephine biopsy

materials and for conforming the diagnosis of HCL is needed. A recently developed anti-TRAP antibody has been started to be used as an immunohistechemical marker in the diagnosis of HCL. The aim of this study was to investigate the specificity and the sensitivity of anti-TRAP antibody immunoreactivity in bone marrow trephine biopsy materials in the diagnosis of HCL.

Material and methods We divided the cases into two groups; a study and control group. The study group included the cases diagnosed as HCL by clinical, haematological and histopathological investigations. The cases in the control group were the cases characterised by non-HCL neoplastic or reactive lymphoid infiltrations, which raised the suspicion of HCL in the differentia l diagnosis. Immunohistochemical staining techniques were applied to paraffin block sections of biopsy materials with anti-TRAP antibody.

Results and conclusion We found that there is a statistically highly significant difference between TRAP immunoreactivities of the study and the control groups, and HCL can be diagnosed by TRAP immunoreactivity in the bone marrow trephine biopsy materials with the specificity of 98,27 % and with the sensitivity of 100 %.

P-391

Cathepsin L participation in collagen degradation during the recovery from experimental hepatic fibrosis

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Introduction Cathepsin L is one of the most important lysosomale cysteine proteinase, indispensable for proteolitic processes. The enzyme is capable to degrade the main components of the intercellular matrix. Aims: 1. To determine the participation of cathepsin L in collagen degradation in liver and to reveal the intracellular and/or extracellular localization of the enzyme activity. 2. To determine which cells of fibrotic liver are sources of cathepsin L.

Methods Biochemically cathepsin L activity and hidroxyproline level was determined in normal, CCl4-induced fibrotic rat liver and at 7, 14, 21, 30, 60 days after discontinuation of treatment. The activity of cathepsin L in fibrotic rat liver at the 7th and 21st days was investigated electron-histochemically. CBZ-Phe-Arg-4MBNA (Bachem) served as the substratum.

Results Biochemically in fibrotic liver an increase of cathepsin L activity as compared with normal liver was revealed. During fibrosis regression the maximums of enzymatic activity at the 7th day (170%) and at the 21st day (155%) after cessation of CCl4-treatment were fixed. At the 14th day of recovery some diminution of cathepsin L activity was noted. At the 60th day after toxin abolition enzyme activity was close to control. A close correlation between cathepsin L activity and liver hidroxyproline level was found. Electron-histochemically reaction product was revealed in the Kupffer cells and fibroblast phagolysosomes containing fragments of collagen fibrils. We also found extracellular localization of cathepsin L in both terms of investigation. The reaction product was noted on the hepatocyte, Kupffer cell and fibroblast plasmalemma and on adjacent collagen fibrils.

Conclusions Cathepsin L is directly implicated in collagen resorbtion during liver fibrosis regression. Detected extracellular activity of cathepsin L suggests that in addition to the intracellular proteolysis, cathepsin L is secreted by hepatocytes, macrophages and fibroblasts in intercellular space and can take part in extracellular collagen degradation. Ryvnyak Victor Laboratory of Morphology "Nicolae Testemitanu" State University of Medicine and Pharmacy Bd. Stefan cel Mare, 165 Kishinev, MD-2004 Moldova victorrivneac@yahoo.com

P-392

Solitary necrotic nodule of the liver. A report of three cases

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Introduction The solitary necrotic nodule (NNS) of the liver is an uncommon lesion with a round appearance near the surface of the liver, with a dense collar of hyalinised collagen surrounding a necrotic core.

Case reports We present three cases of NNS. Two patients underwent surgery to remove liver metastasis. We suggest a relationship with the previous hepatic surgery and with the microwave tissue coagulator. The other case have an history of abdominal traumatism by a seizure. The radiological image of NNS was misinterpreted as a tumor and the diagnosis by FNAC or frozen section could not exclude a metastasis. The potential risk of a malignant secondary tumour existed, and that was why limited liver resection was recommended. Several pathogenetic mechanisms have been avocated (infection, parasitic, traumatic, ischaemic, post chirurgical, iatrogenic, etc.), all of them having an identical morfological appearance.

Conclusion NNS is a rare non-tumorous lesion that might be misinterpreted as a tumor. The correct diagnosis can only be histological. It is important to differentiate the NNS from metastases due to the great influence on the treatment.

P-393

The significance of morphometrical analysis in staging of fibrosis in liver biopsies from patients with chronic hepatitis C

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Introduction Prognosis of the course of chronic hepatitis C (CHC) depends on fibrosis stage and its progression temps in the liver. Semi quantitative methods are currently regarded as a routine diagnostic procedure. But it is obvious that these methods are subjective. Morphometrical analysis with calculation of fibrosis index (FI) is an alternative method of evaluation. The aim of the study was to compare data obtained after morphometrical analysis of collagen formation to semi quantitative methods results and find out accordance with fibrosis stage.

Materials and methods Liver biopsies from 42 CHC patients were used. We applied V.J.Desmet (1994) semi quantitative method and software SigmaScan Pro 5.0 for morphometrical analysis of sections. All sections were stained by Masson Trichrome method for the detection of collagen. Fibrosis index (FI) was calculated as collagen area divided all section area.

Results 54,5% of patients had clinically significant level of liver fibrosis (Desmet F2, F3, F4). F1 Desmet stage was corresponding FI 0,01-0,02, F2 Desmet (moderate fibrosis) - FI 0,03-0,06 and

high level of fibrosis (F2 and F3) - IF 0,07-0,3. 15/42 cases (35,7%) showed differences between semi quantitative and morphometrical results. Four patients (9,5%) without fibrosis by semi quantitative methods showed IF corresponding moderate fibrosis (F2).
Conclusion Our results suggest that morphometrical analysis is beneficial in the evaluation of fibrosis stage and should be used for prognosis and monitoring the course of chronic viral hepatitis.

P-394

Phenotypic comparison of hepatic mucinous cystadenomas with mesenchymal stroma and ovarian mucinous cystadenomas

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Introduction Hepatic mucinous cystadenomas with mesenchymal stroma have been recognized as rare neoplasms that occur only in females and have recently been postulated as being hormonally dependent. Because of the similarity of these neoplasms to ovarian mucinous cystadenomas, we compared the immunohistochemical profiles of these neoplasms arising in two different organs. The aim of this study was to establish whether these two tumors were phenotypically similar.
Materials and methods We employed the following immunohistochemical stains, Estrogen Receptor, Progesterone Receptor, Inhibin, CK7, CK20 and Alcian Blue/ Periodic Acid-Schiff, to 6 cases of ovarian mucinous cystadenomas and to 5 cases of hepatic mucinous cystadenomas with mesenchymal stroma.
Results Both the ovarian and hepatic neoplasms showed similar results with all of these stains.
Conclusions Phenotypically, we have been unable to establish any difference between these two neoplasms. There appear to be two possible explanations for the origins of these tumors: One is that of a displaced ovarian anlage in the developing liver and the other would be that some of the primitive hepatic stem cells are capable of acquiring ovarian characteristics

P-395

Assessment of periodontum condition in hepatitis C infected patients with rapid and slow dynamics of advanced fibrosis

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Aim Analysis of the relationship between the stage of periodontium disease and the extent and dynamics of fibrosis in patients infected with chronic hepatitis C.
Material and methods The study included 28 patients whose blood serum revealed the presence of HCV-RNA via RT-PCR, while biopsies showed chronic inflammation and either septal fibrosis, or liver cirrhosis. Fibrosis was evaluated according to Scheuer's 4 point scale, with Azan- staining method and the authors' own modifications. Fibrosis dynamics (FU/Y) was evaluated as a quotient of the number of points assessing fibrosis and the number of years of the disease. Periodontitis was determined by the following criteria: inflammation limbus, gum swelling, gingival pockets depth, presence of supragingival and subgingival calculus and gomphiasis. Each criterion was assigned either 0 or 1 point within the cumulative scale of 0-4 points.
Results All patients with advanced liver fibrosis were found to show signs of periodontosis. The patients whose periodontium condition scored 4 points with the mean value of FU/Y amounting to 0,49, while those who scored 3 points showed mean values of FYU/Y equalling 0,79. The group of 19 patients with high values of periodontium disease showed significantly higher values of periodontum disease stage (most frequently assessed at 4 points) compared with the group of nine patients with low FU/Y values (mean value = $p < 0,05$). Periodontium score of the patients with cirrhosis and bridge fibrosis showed similar values.
Conclusion The degree of periodontal disease was found to be dependent on the pace of fibrosis dynamics.

P-396

Molecular evidence for a toxic memory effect in chronic liver disease

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Introduction Alcoholic liver disease shows a toxic memory effect: patients with a history of alcoholic hepatitis show even after prolonged withdrawal from alcohol rapid reappearance of alcoholic hepatitis upon reexposure to alcohol. This type of liver injury can be mimicked in a mouse model by feeding 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC). After 2 months of DDC intoxication mouse livers revealed ballooned hepatocytes containing numerous Mallory bodies. Within four weeks of recovery from intoxication most Mallory bodies disappear but rapidly reappear within three days of reintoxication indicating the existence of a toxic memory effect. The aim of this study was to investigate the molecular basis of the toxic memory effect.
Materials and methods cDNA-libraries were generated from normal and DDC-intoxicated mouse livers by subtractive suppressive hybridization. These gene libraries were used to produce cDNA-arrays for expression profiling. RNA was isolated from mouse livers at different stages of intoxication, recovery and reintoxication and hybridized to the cDNA-arrays. Expression profiles were analysed by Genesight- and Imagene software.
Results and conclusion Results obtained demonstrated several groups of genes differently expressed in short-term intoxicated naive and reintoxicated mice. These genes comprised Cyp2A5 as well as genes involved in the cellular response to oxidative stress and methionine metabolism. The sustained elevated level of Cyp2A5 during recovery and the enhanced induction upon reintoxication could explain why certain drugs lead to enhanced liver toxicity upon repeated administration even after long drug-free intervals.

P-397

The role of keratin in toxic liver injury

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Introduction Alcoholic (ASH) and non-alcoholic (NASH) steatohepatitis as well as certain chronic cholestatic liver diseases and copper toxicosis are associated with severe alterations of the hepatocytic intermediate filament cytoskeleton. Diseased livers reveal ballooned hepatocytes with a loss of cytoplasmic keratin filaments and the formation of keratin-containing cytoplasmic inclusions named Mallory bodies. These alterations can be reproduced in mice by chronic intoxication with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC). The aim of this study was to investigate the role of keratin alterations in toxic liver injury we compared the effect of DDC-intoxication in keratin 8 null mice (obtained from H. Baribault, La Jolla) as well as keratin 18 null mice (obtained from T. Magin, Bonn).
Materials and methods Both knock-out mice were crossed into the same genetic background and their responses to toxic injury monitored by gene expression profiling using cDNA-arrays containing liver-specific gene libraries.
Results and conclusion A much more pronounced toxicity of DDC was found in keratin 8 null mice whereas keratin 18 null mice behaved like wild-type mice. Keratin 8 null mice revealed increased expression of several genes particularly related to the oxidative stress response. The different toxicities in keratin 8 and keratin 18 null mice indicate that keratins fulfill in addition to their structural role non-skeletal functions and that keratin 8 acts as a modulator of toxic injury in hepatocytes.

P-398

Evaluation of FAS, FASL and TGF-beta1 molecules in hepatocellular carcinoma

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Introduction TGF-beta, FAS, FASL molecules take part in the regulation of apoptosis. FAS is a cell surface receptor present in most of the cells. FASL can be secreted from the surface of the cells and when it binds to FAS, they activate the caspases to trigger the apoptosis. Although the mechanism of TGF-beta is not clear yet, it plays a role in tumorigenesis. TGF-alpha may indicate inhibitory or stimulatory functions in conjunct with the stage of the tumors and cooperation with oncogenes and growth factors. We aimed to evaluate the correlation among the expressions of FAS, FASL, TGF-beta1 molecules in HCC.
Material and method In this study fifty cases diagnosed as hepatocellular carcinoma (HCC) between the 1992-2002 years were included. TGF-beta1, FAS, FASL expressions were evaluated immunohistochemically.
Results FAS, FASL expressions were observed together in 84% of tissue samples with HCC and 100% of the nontumoral liver tissue samples. It was noticed that FAS and FASL staining were more densely in the lymphocytes of the tumorous areas and neighboring normal tissues than the control group. There was no statistically significant correlation between FAS, FASL, TGF-B1 staining except the positive correlation between the TGF-beta1 and FAS. The poor differentiated cases weakly expressed FAS, FASL and TGF-beta1. Where as the majority of the cases stained cytoplasmic positive, some cases stained either cytoplasmic or membraneous positive.
Conclusion In HCC, tumor cells use variable pathways for apoptosis. The lymphocytes near the tumorous tissue have an important role in this process.

P-399

Dynamics of liver histology in patients with chronic hepatitis C after interferon-alpha and ribavirin therapy

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Introduction Interferon-alpha therapy for chronic hepatitis C improves hepatic inflammation and may change the evolution of disease to cirrhosis and hepatocellular carcinoma. Accurate assessment of liver fibrosis is necessary for morphologic diagnostics. The aim of this study was to evaluate the effect of Interferon-alpha and Ribavirin on liver histology; to compare the semi-quantitative (according to Ishak) and quantitative methods for evaluation of fibrosis.
Materials and methods Twenty-two patients (13 males, 9 females at mean age 40 years) with chronic hepatitis C were studied. Within 18 to 60 months from the pre-therapy biopsy and at least 6 months after the end of treatment patients were followed with second liver biopsy. Histological activity in paired liver specimens was assessed by Knodell modified score. Fibrosis was evaluated according to Ishak staging system. In addition, fibrosis was quantified in Masson trichrome stained sections using morphometric image analysis. Interferon-alpha (mean dose 495 ME) and Ribavirin were administered for 6 to 18 months. Patients were classified as complete sustained responders (n=8), relapsed after therapy cessation (n=3) and “non-responders” (n=11).
Results After treatment reduction of the histological activity score (at least one grade) was observed in 15 patients (68%). Lobular and periportal necroinflammatory lesions were decreased in both responders and non-responders ($p<0.01$). After therapy, dynamics the fibrosis degree and calculated fibrosis ratio were not significant. Fibrosis stage was improved in only 4 sustained responders (18%); remained unchanged in 7 patients, and progressed in 10 patients (45%).
Conclusions Improvement of liver histology after Interferon-alpha and Ribavirin treatment was constricted to the patients who achieved hepatitis C virus eradication. Morphometry was the informative method for evaluation of fibrosis dynamics in addition to fibrosis assessment with semi-quantitative classification systems.

P-400

Neonatal hepatitis and intrahepatic bile duct paucity: the value of quantitative analysis of histopathological parameters

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Introduction Liver biopsy is one of the diagnostic procedures for Neonatal Hepatitis (NH)and Intrahepatic Bile Duct Paucity (IHBP). In this study we aimed to evaluate the prognostic benefits of quantitative analysis of histopathological changes in NH and IHBP.
Material and methods We studied 28 liver biopsies in which 18 were NH and 10 were IHBP. The relationships between histopathological and prognostic parameters were analyzed. Hepatic histopathological changes such as portal and periportal fibrosis, portal and lobular inflammation, bridging necrosis with fibrosis, pericellular fibrosis, giant cell transformation and extramedullary hematopoesis were evaluated and according to their absence (0) or presence (1) a histopathological score was assessed. The sum of these data was accepted as total pathological injury score. Clinical prognostic parameters consisted of the patients height, presence or absence of hepatosplenomegaly and ascites, serum levels of ALT, albumin, bilirubin, and the value of protrombine time. The sum of these data was accepted as the clinical score. The patiens were also divided into two groups whose clinical scores were <4 as good prognosis and ≥4 as bad prognosis. For statistical analysis Pearson's chi-square, Mann-Whitney-U and ROC curve tests were used.
Results We found a statistically significant negative relation between prognosis and total pathological injury score ($p=0.042$), so as pericellular fibrosis ($p=0.016$).
Conclusion During the interpretation of liver biopsies of NH and IHBP, scoring of histopathological changes should be done in order to assess the clinical prognostic outcome. Moreover, the presence of pericellular fibrosis should be evaluated and reported.

P-401

Immunohistochemical analysis of hepatocellular carcinoma and intrahepatic cholangiocellularcarcinoma using markers supposed to indicate respective levels of diffentiation of the hepatic cell lineage

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It is widely recognized that there are diagnostically problematic cases among primary hepatic tumors. They cause difficulties in distinguishing between hepatocellular carcinoma (HCC) and intrahepatic cholangiocellularcarcinoma (CC). Meanwhile, recent progression of study on hepatocyte-diffrentiation has revealed that both mature hepatocyte and biliary epithelial cell should originate from the common cell lineage called as hepatic-progenitor-cell (HPC), bipolar-cell (BC) and/or intermediate-hepatocyte-like-cells. Thus, we performed immunohistochemical analysis for HCC and CC using markers supposed to indicate respective levels of differentiation of the hepatic cell lineage, in order to identify the origin of the tumors particular in the diagnostically problematic cases.
Materials and method Surgical specimens of 21 cases of HCC and 7 cases of CC were used. Ordinary immunohistochemical staining was performed with following antibodies; hepatocyte monoclonal antigen (Hep), c-kit, CK7, CK19 and CK20. The results were compared from the view of the histopathological findings and immunohistochemical reactions.
Results Rate of positive (>50%) and focal positive (>5%) were Hep; HCC 76%, CC 29%, c-kit; HCC 17%, CC 44% and CK19; HCC 23%, CC; 100%. CK7 and CK20 were similar as previously reported. In addition noteworthily, double positive for Hep and c-kit was observed on two cases of CC having apparent glandular formation.
Conclusions 1) HCC had a lower positive rate for Hep compared with previous reports. 2) CC with positivity for Hep might exist. 3) According to the c-kit staining pattern, we considered that there might be a peculiar variant of tumor cells in CC differentiating both hepatocyte and biliary epithelial cell.

P-402

Morphological consequences of starvation upon the liver

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Introduction Proteus anguinus, the sole species of the European Cave salamander and the remarkable cave dweller of underground waters in the Karst of Slovenia is well adapted to poor and discontinuous food supplies. Remarkable accumulation of lipid droplets and glycogen deposits in the liver beside slow metabolism allow Proteus to survive for long periods without food. The aim of this study was to establish morphological changes in the liver during starvation with particular emphasis on mobilization of the liver energy reserves.
Material and methods The liver was examined after food deprivation periods of one, four and eighteen months, respectively using light and transmission electron microscopy. Glycogen and proteins concentrations were determined spectrofotometrically. Total fat in the liver was estimated by Folch method.
Results Intense depletion of glycogen was present during first month of starvation. The glycogen wasn't formed in clumps typical for normal liver tissue. After eighteen months of fasting glycogen was re-synthesized, dense glycogen deposits appeared. Lipid droplets were less condensed. Numerous peroxisomes formed clusters with small and round mitochondria. Particular mitochondria increased in size and lost cristae. Lysosomes and autophagic vacuoles were numerous, too. Dilated intercellular space included electron dense granular material.
Conclusions Carbohydrate and lipid reserves in the liver of Proteus serve for metabolic needs during food deprivation. While in many other vertebrates fasting causes depletion of liver carbohydrates, these reserves are maintained in large amounts in Proteus. Results of our study confirm that a detailed morphological analysis of the liver could be a relevant informative method to prove metabolic adaptability of this organ.

P-403

Evaluation of T lymphocyte subpopulations in chronic hepatitis- relationship with histological lesions

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Background Intrahepatic T lymphocytes are believed to be involved in immunopathogenesis of chronic viral hepatitis. The aim of this study was to assess the correlation between morphological lesions and T lymphocyte subpopulations in liver and peripheral blood (PB) of patients with chronic hepatitis.
Methods and results 51 liver biopsy specimens from patients serologically confirmed (15 HBV and 36 HCV) were studied by histopathological and immunohistochemical methods. T cell subtypes (CD4, CD8, CD57) were tested by using monoclonal antibodies on tissue samples and by flow cytometry in PB.
Results In PB, 80% of patients have T lymphocyte CD8+ decreased and 65% of them have augmented values of T lymphocytes CD4+. In liver biopsy the prevalent subpopulation were CD4+ cell, being approximately twice higher than CD8+ cells. The observed histological lesions were quantified by Ishak score as moderate\severe chronic hepatitis.
Conclusions Decreased subpopulation of CD8+ T lymphocyte might contribute to the severity of liver disease. Relatively more frequent cell type was CD4 positive T cell, having a significant association with a higher inflammatory activity in both types, B and C virus hepatitis.

P-404

The role of survivin in regulation of proliferative and apoptotic activity of hepatocellular carcinoma

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Introduction Imbalance between proliferation and apoptosis is an important factor in progression of hepatocellular carcinoma (HCC). Aims: The percentage of proliferative (PI) and apoptotic cells (AI) in HCC compared to non-tumor liver was investigated. The role of unprocessed as well as active form of caspase-3, which is an important apoptotic protein was of great interest for this study. Expression of survivin, an inhibitor of apoptosis was investigated, too.
Material and methods Twenty patients with HCC were included in the study. Two samples were obtained from each patient, one of HCC and one of the surrounding non-tumor liver. MIB-1 antibody was used to determine the proliferative activity. Immunohistochemical staining for the detection of active form of caspase-3 was used to determine the percentage of apoptotic cells and for detection of procaspase-3 and survivin expression, as well.
Results AI was higher in HCC (1,532 ± 1,05) compared to non-tumor liver (0,426 ± 0,62), but the proliferative activity in HCC was much higher than apoptotic activity. 75% of HCC showed positive staining for pro-caspase 3. Positive correlation between expression of pro-caspase 3 and active form of caspase 3 proved to be statistically significant. 70% of HCCs were survivin positive. Stronger expression of survivin was detected in HCC compared to non-tumor liver. Positive correlation between survivin expression and the grade of tumor diferentiatiation as well as a significant association between survivin expression and both AI and PI was also detected.
Conclusion Our results indicate that survivin promoting cell proliferation in HCC has an important role in HCC progression.

P-405

The histopathological effects of offloading on liver of rat embryo

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Introduction Ofloxacin is an antibiotic from fluroqinolne with a wide range and widely used in various infection diseases. Since there is little information about ofloxacin side effect on liver of rat embryo. This preliminary study was planed to see what kind of changes occurs in development of liver in Rat embryo. The aim of present study was to determine the histopathological effects of offloading on liver of rat embryo.
Material and methods Fourty nine Wistar rats were selected and randomly divided into two groups; control (n=21) and test (n=28). The test group has been received 14mg/kg (PO) Ofloxacin daily during pregnancy. However, the control group just received plate. After delivery, liver tissue of neonatal in both groups were taken and prepared for light microscopy. Staining method was H&E.
Results Microscopic study of liver tissue specimens showed that in the test group hepatocytes were vesiculated. Degeneration and vacuolated pycnotic nuclei were seen when compared to control group. Weight of neonatal in test group was reduced when it was compared with the control group ($P<0.05$).
Conclusion Since Ofloxacin had side effects on liver of rat embryo, it is not suggested to be used during pregnancy in human.

P-406

Gene expression profiling of human hepatocellular carcinoma

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Introduction While the underlying etiology of HCC has been well characterized the molecular mechanisms of hepatocarcinogenesis are poorly understood. The extremely poor prognosis of HCC and the limited value of the current standard clinical markers underscore the need for improvent of diagnosis and treatment of this deadly disease. Aims: We performed gene expression profiling using cDNA microarrays in order to identify novel molecular markers and therapeutic targets for the treatment of HCC.
Material and methods We used the method of subtractive suppressive hybridization (SSH) to generate cDNA libraries that are highly enriched with clones representing genes both up- and downregulated in the diseased liver tissue. The expression pattern of 4600 clones representing ~ 4000 genes was analyzed in 26 surgically resected hepatocellular carcinomas, 4 focal nodular hyperplasias, one adenoma, and 6 cirrhotic livers by competetive

hybridization on custom made cDNA microarrays using a pool of non-neoplastic human liver as reference. The expression data were verified independently by quantitative RT-PCR (TaqMan).
Results We found 500 genes which were consistently deregulated in more than one third of the HCCs under investigation. Of these, 250 genes had been previously implicated in HCC, whereas 210 known genes as well as 40 novel genes (with unknown function) had not yet been linked with liver cancer.
Conclusions The present study revealed a number of genes specifically deregulated in hepatocelluar carcinoma. Future work will aim to evaluate the medical relevance of the encoded proteins which will ultimately help to identify potential new markers for better diagnosis/ prognosis of HCC and new targets for therapeutic intervention.

P-407

Actinomycosis: a report of 10 cases - an experience of the Anatomy and Pathology Laboratory of Casablanca Hospital

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Introduction Actinomycosis is a suppurative and granulomatous chronic infectious disease caused by Actinomyces sp. and most commonly affecting the cervicofacial area. Aim: To study the clinical characteristics of patients with actinomycosis, with regard to clinical history, presentation, method of diagnosis and comparing to the literature.
Patients and methods From January 2000 to July 2002, a retrospective review was performed on all cases of histologically proven actinomycosis.
Results Ten patients were studied, 5 men and 5 women, 5-65 years old. Two patients had a history of surgical procedures and/or dental manipulations. Two patients had a long history of diabetes mellitus. Locations were the liver (patient n 1), the feet articulation (patient n 2), the lung (patient n 3), the jugular lymph node (patient n 4), the skin (patients n 5 and 6), mandibular (patient n 7), the psoas muscle (patient n 8), the appendix (patient n 9) and the large bowel (patient n 10). Three patients showed a tumoral presentation (patients n 3, 8 and 10) and two patients had a tuberculosis disease presentation (patients n 2 and n 4). The patient n 9 had a clinical presentation of an inflammatory large bowel disease. One patient was diagnosed by cultures positive to A. israelii on microbiologic study and the others patients by surgical biopsy and detection of an actinomycosic granuloma. Special stains were used in all cases. Three patients received the classic initial regimen of iv.
Conclusion Actinomycosis is an uncommon disease in our country. In these cases gastrointestinal locations were predominant. Two uncommon locations were present. There was no IUD associated infection. Tuberculosis endemic in our country can mislead the diagnosis.

P-408

Further autopsy hazards: Mycobacterium tuberculosis in the autopsy room

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Introduction Tuberculosis has been the major occupationally acquired disease in the autopsy room. The risk of encountering tuberculosis at autopsy has fallen with the decreased incidence of the disease and greater emphasis has been placed recently on the hazards of viral diseases. However, tuberculosis may still be found at autopsy. The aim of this study was to assess the magnitude of exposure to Mycobacterium tuberculosis at autopsy in a large general hospital setting.
Methods Retrospective search of the autopsy records from 1991-2000. The patient record and histological slides were reviewed. The quantity of mycobacteria was assessed from the Ziehl-Neelson stains as scanty, moderate or abundant.
Results 13 cases of active Mycobacterium tuberculosis were identified in the 10-year period in which 3740 autopsies were performed (1 per 374 autopsies) of which 10 cases were unsuspected (77%). These included 7 cases of miliary tuberculosis and 2 with pulmonary infection. 3 cases contained abundant organisms. Patients tended to be middle aged and male with complex clinical histories. 3 patients were dead on arrival at hospital.
Conclusion The risk of unexpectedly encountering tuberculosis at autopsy continues and has implications for the health of autopsy room staff, autopsy room ventilation and design, protective equipment and for the involvement of the public health service.

P-409

Testing of the histological parameters for predicting the liver fibrosis in hepatitis C virus infection in drug abusers

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Introduction The course of chronic hepatitis C virus infection is difficult to predict. Although the disease is frequently asymptomatic, the development of fibrosis and cirrhosis are the major complications. Among patients suffering from chronic hepatitis C (CHC), special group are young drug abusers. Immunological toxicity of narcotics modulates the total immunologic response to the viral infection with characteristic morphologic expression of this disease. The aim of this study was to asses which pathological features are associated with liver fibrogenesis and are predictive to fibrosis in CHC drug abusers.
Methods Eighty liver biopsies with CHC from drug abusers (43 actively consumers; 26 free from drugs 3-6 months before liver biopsy and 11 free for more than one year before performing liver biopsy) stained by HE, Trichrom Masson, Perls and reticulin were analyzed semi quantitatively using standard questioner for determination of patohistological features for histological activity index. Results of fibrosis were correlated to all of them.
Results The fibrosis was 0, 1 and 2 in this group of young patients (mean age 22, 3 years). There were no correlation between any of the patohistological parameters and fibrosis, even for lobular necrosis.
Conclusion Histomorphological changes and the extent of fibrosis are in correlation to the duration of drug consumption. Morphological expression of immune toxicity of narcotic drugs in CHC patients make this disease special entity to which it is not possible to apply standard histopatologic parameters, nor the parameters predicting liver fibrosis.

P-410

Clinical, parasitological and morphological features of amebiasis in Vilnius

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Introduction Among the acute bowel infections there is tendency of increasing cases of unknown diarrhoea. The occurrence of this disease in Lithuania is presented by only a few confirmed cases per year, mostly imported by travellers from abroad. The most pathognomic clinical features of the disease are hemocolitis without severe tenesmus. Fresh feces microscopy has improved the diagnosis. Parasitological morphological data were very useful**.** The aim of this study was to define prevalence of amebiasis among acute infectious diarrhoeal patients. To provide commonest clinical symptoms and syndromes of amebiasis. To improve routine morphological diagnostics in suspicion of amebiasis in Vilnius.
Material and methods Diarrhoeal patients with confirmed amebiasis were analysed during the four-year period in VU Hospital for Infectious Diseases and statistical analysis of epidemiological and clinical data was performed. Morphological data of biopsies were analysed too.
Results Common manifestations of amebic enterocolitis diarrhoea such as blood in stool, abdominal pain or cramps, tenezmus, fever, and gastrointestinal symptoms were present in all patients. Asymptomatic patients were as cysts carriers. Systematic complications were observed in one patient with hepatic abscesses. One patient had sigmoid ameboma. All patients were males.
Conclusion Amebiasis is a rare parasitic infection and makes up to one percent of all bowel infections. Amebiasis can be suspected in cases with abdominal cramps and bloody diarrhoeal episodes without severe tenezmus and with travel history. Fresh faeces microscopy was the main diagnostic method. It is very important to recognize the E histolytica trophozoites from histiocytes in the biopsies.

P-411

Conjunctival epithelial neoplasia and HIV infection in Africa

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Introduction Epithelial tumors of the conjunctiva are rare diseases in Europe. In sub-Saharan countries, an increasing incidence was observed in connection with the HIV epidemic. The definite causes of these tumors are not known. Aims: Based on tumor material from Africa, we search for possible cancerogenic agents and its pathogenetical interaction in conjunctival carcinoma.
Materials and methods Conjunctival specimens of 68 patients from three African centers with the following histological lesions were examined: papilloma (1 case), pinguecula (5), conjunctival intraepithelial neoplasia (8 CIN I, 12 CIN II, 18 CIN III), squamous cell carcinoma (SCC, 24 cases). Immunohistological reactions for p53 and p16 were done. 23 of 38 tested patients were HIV positive.
Results and conclusion 39 of the 44 benign and precancerous lesions were associated with histological signs of a solar damage. 62 of the 68 cases showed immunohistologically an overexpression of p53, among them all SCC. This finding might indicate a p53 mutation as mostly seen in solar induced skin cancer too. 50% of the cases with CIN III and SCC had an overexpression of p16. Corresponding findings are known from transformed or neoplastic cervical epithelium in strong correlation with an active HPV (type 16,18) oncogene expression. No significant differences occurred between HIV positive and HIV negative cases in comparison to the frequency of different histological lesions as well as the p53 and p16 expression. The average manifestation age for CIN III and SCC in HIV positive cases (30 resp. 38 years) was 20 years lower than that of HIV negative cases (51 resp. 58 years). The results suggest a multifactorial tumorgenesis (UV radiation, oncogenic viruses, HIV infection) for the conjunctival carcinoma in Africa.

P-412

Craniofacial Botryomycosis: an unusual South African case

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Introduction Botryomycosis is an enigmatic pyogranulomatous infection, which may be caused by a variety of non-filamentous bacteria including Staphylococcus aureus and Pseudomonas aeruginosa. While the exact pathogenesis remains elusive, it is acknowledged that the interrelationship of host and agent factors is fundamental. We describe the clinicopathological features of a rare and particularly mutilating case of delayed onset post traumatic craniofacial botryomycosis.
Methods An immunocompetent 57-year-old Black male presented with multiple sinuses draining granules involving the frontal, parietal, orbital and maxillary areas. Nodulo-fibrous thickening was also present. This had evolved over a three-year period. Apart from the history of a head injury requiring cranioplasty 27 years earlier, no other predisposing factors were found.
Results The diagnostic features of clumped bacteria (cloxacillin sensitive Staphylococcus aureus), Splendore-Hoeppli phenomenon, granulomata and intense fibrosis were confirmed histologically. There was no response to intravenous cloxacillin and the patient died before surgical debridement could be done.
Conclusion This case report serves to highlight several points. Firstly, it illustrates that when considering the diagnosis of post traumatic botryomycosis, the time interval between the traumatic event and the onset of clinical features may span decades. We submit that this is due either to the small size of the bacterial load or their low virulence. Secondly, it is our contention that the poor response to antibiotics is due to the severe, impenetrable fibrosis. Finally, the destructive nature of the disease underscores the urgency in diagnosis and treatment.

P-413

Tuberculous meningitis in adulthood

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Introduction Tuberculous meningitis is a very frequent clinical manifestation of postprimary tuberculosis in childhood as the result of hematogenous dissemination of a pulmonary lesion; in adult patients tuberculous meningitis occurs less frequently, mainly associated with extrapulmonary disease, consequent to the rupture of a subependymal tubercle.
Case reports We report 4 cases of tuberculous meningitis in adulthood diagnosed by autopsy examination – median age 38,25 yrs – in an autopsy series of 20 tuberculous patients in 10 years. All but one of the patients had disseminated disease (lung, lymph nodes, liver, spleen and kidneys). None of the patients presented subependymal tubercles. One patient had dual mycobacterial infection - miliary tuberculosis due to Mycobacterium tuberculosis in all the extracranial affected organs and tuberculous meningitis with Mycobacterium avium-intracellulare (MAC). Cerebrospinal fluid examination revealed high cellularity and high albumin concentration in all cases; glucose levels were low, except in one case with normal glucose content – the MAC infected patient. One patient had associated systemic lupus erythematosus (SLE) and low CD4+ cells count. The histopathologic appearance consisted of a granulomatous meningitis with giant cells and epithelioids in three cases and polymorphous inflammatory infiltrate including numerous positive bacilli in Ziehl-Neelsen stain in MAC infected patient.
Conclusion The overall appearance suggested a hematogenous spread of the infection, the immune status correlating with the occurrence of the meningeal disease; in our series we had an immunodepressed patient (SLE) and another patient with miliary tuberculosis and superimposed MAC infection. Also, MAC meningitis had a different clinical and histopathologic appearance.

P-414

Surgical extrapulmonary tuberculosis - 12 years of experience

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Aim The authors reviewed 390 cases of surgical extrapulmonary lesions diagnosed as tuberculosis in No.1 County Emergency Hospital Department of Pathology from Craiova, Romania and assessed the diagnosis by means of new diagnostic tools.
Material and methods The samples were fixed in formalin, embedded in paraffin wax and stained with hematoxilin-eosin, van Gieson and Ziehl-Neelsen stains. Immunohistochemical staining with antibodies for CD3, CD20, CD68, LCA and S100 were used. PCR technique was used in 27 cases with controversial diagnosis.
Results The diagnosis was confirmed in 363 cases. The most frequent affected sites were the lymph nodes, followed by digestive system, urinary system and male genital system. Immunohistochemical stains cleared up the diagnosis in 38 cases. PCR was used in 27 cases with atypical granulomas, establishing the diagnosis of tuberculosis in 10 of them.
Conclusions Tuberculosis in extrapulmonary sites discovered in surgery departments is more and more frequent. In cases with controversial diagnosis of granulomatous lesions, immunohistochemistry and PCR techniques are required for clearing up the diagnosis.

P-415

Molecular analysis of caseous granulomas

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Introduction Clinical presentation and lesion morphology are often suggestive, but not conclusive for Mycobacterial (MB) infections. Rapid and accurate specific diagnosis is mandatory since appropriate therapy can be chosen only on the basis of the precise identification of the causative agent. One of the most promising tools for rapid and sensitive identification of MB species is represented by amplification of specific sequences of the 16S rRNA MB specific gene on the fragmented DNA (max 200 bp) obtained from fixed tissues.
Methods DNA was extracted from 15 cases selected from the archives of the Department of Pathology, University of Padova, with histopathologic features of granulomas, caseous necrosis, and Langhans-type giant cells. Ziehl-Neelsen staining for acid-fast bacilli and PCR/direct sequencing of a 155 bp variable region of the MB 16S rRNA gene were performed. H37Rv genome was employed as positive control.
Results MB specific sequences have been identified in 12 of 15 samples analyzed (80%). In two DNA samples (13%) an inserted DNA product was detected and it is currently under sequencing, while in one sample (7%) no evidence of MB infection has been gained. Agreement with Ziehl-Neelsen was present in 8 cases (53%).
Conclusion Our study suggests that PCR is a rapid, sensitive method for the diagnosis of MB in routinely processed formalin-fixed, paraffin-embedded histological specimens and is highly recommendable in cases of chronic inflammation without definite evidence of granulomatous inflammation.

P-416

Cerebral abscesses caused by Cladosporium bantianum - A report of six cases

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Introduction Cladosporium bantianum is a pigmented highly neurotropic fungus which can be identified easily in tissue sections due to their golden brown colour. Cerebral lesions caused by this fungus are rare.
Case reports We present six cases diagnosed at our institute over a period of 36 yrs. (1966-2002). There were 5 Males and 1 Female. All patients belonged to the second to fourth decade and had presented with symptoms of space occupying lesion. Radiological investigations revealed well circumscribed lesions with ring enhancement. The smallest lesion measured 0.1x0.1cm and largest measured 3x1.5x1 cm. Only two of the six cases had detectable predisposing debilitating illness. One 48 yr. old male had past history of Liver Tuberculosis and had taken anti-Tuberculosis treatment and a 26 yr. old female had systemic lupus erythematosus and was on immunosuppressants. We received four surgically excised specimens and two autopsy specimens of the entire brain. Histopathology revealed necrotic areas, micro-abscesses and mainly foreign body type of giant cells. Lying within the giant cells as well as

freely within the tissue were numerous golden brown to deep brown spherical forms of Cladosporium bantianum measuring 8-10 microns in diameter and their hyphal forms measuring 4-5 microns in diameter, 10-12 microns in length with septae and occasional branching. These were confirmed by Gomori's methenamine silver stain for fungus and species identification was carried out by culture studies. All the four patients with surgical excision showed recurrence within 3-4 months. Three succumbed to the disease and one is still alive.

Conclusion Cladosporium bantianum can affect both healthy as well as immunocompromised individuals. It has a fatal outcome. Cases are being presented because of the relative rarity.

P-417

Necrobiotic changes of skeletal muscle simulating myxoid sarcoma. Report of a case with immunohistochemical and electron microscopic studies

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Background Skeletal muscles are known to undergo bizarre reactive changes in response to traumatic injuries and may produce pseudosarcomatous lesions which may give diagnostic challenges to pathologists. Such lesions usually produce proliferative myositis in which atypical spindle cells of fibroblastic and myofibroblastic nature predominate and may simulate the appearance of fibrosarcomas and myofibrosarcomas. However with proper setting of previous trauma history, their true nature can be easily appreciated. We are reporting an unusual case of myxoid cellular lesion found in the abdominal rectus muscle involved in a post hysterectomy abdominal hernia which simulated the appearance of an extraskeletal myxoid chondrosarcoma (EMC). Its true reactive nature was recognized by electron microscopic (EM) study.

Design Light brownish gelatinous material aggregating 2.5x 2.5x1.0CM was found around the sheath of rectus abdominis muscle of a 48 year old female during a repair surgery of posthyterectomy ventral hernia. The tissue was fixed in buffered formalin and a representative portion of the tissue was fixed in phosphate buffered 2.5% glutaraldehyde and post fixed in osmium with en bloc staining with uranyl acetate. Epon embedded thin sections were stained with uranyl acetate and lead citrate. For immunochemistry, paraffin sections were stained for S100 protein, myosin, and actin by routine ABC-peroxidase techniques.

Results On routine examination, the lesion was characterized by loosely arranged spindly to epithelioid cells in bundles with abundant myxoid stroma. The lesional cells varied in size with frequent bizarre hyperchromatic nuclei. Mitosis was rare. The cells showed weakly positive diffuse cytoplasmic stain for all of above antibodies. With the initial impression of EMC, an expert opinion confirmed the diagnosis of grade II EMC. However, EM revealed the lesional cells to be consistent with skeletal muscle cells having of varying stages of degeneration and regeneration. No ultrastructural characteristics seen in the tumor cells in EMC were present. The attending physician was advised of careful follow-up and close observation without any additional treatment. The patient remains with no evidence of disease by 7 year follow-up in spite of incomplete excision of the lesion.

Discussion and conclusion The myxoid gelatinous material found in the abdominal rectus muscle presented herein showed histopathologic characteristics of EMC, however, electron microscopic studies revealed the lesion to be necrobiotic process of the rectus muscle. Evidence of no disease in 7 years of follow-up with incomplete excision supports the conclusion the lesion was not malignant as EM study indicated. This case exemplifies another variety of pseudosarcomatous reaction of skeletal muscles important in the differential diagnosis in the pathologic evaluation of cellular myxoid lesions.

P-418

Peritoneal psammocarcinoma. A case report with ultrastructural study

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Background Psammocarcinoma (PC) is a very rare variant of low grade serous tumors that appears to arise from the peritoneum and ovaries. This tumor has been defined as a serous neoplasm displaying 1) destructive invasion, 2) no more than moderate nuclear atypia, 3) no areas of solid epithelial proliferation except for occasional nests no more than 15 cells in diameter, and 4) at least 75 % of papillae or nests are associated with or completely obscured by psammoma bodies. PC shares some clinicopathologic features with serous tumor of low grade malignant potential (LMP), but may have been under-recognized at surgery and gross examination. The present case was incidentally found during a routine hysterectomy procedure for leiomyomas. The PC involved the omentum, peritoneum and diaphragm. We report, to our best knowledge, the first electon microscopic (EM) findings of PC.

Design Tumor tissue was fixed in buffered formalin for routine examination. In addition, a representative tissue was fixed in phosphate buffered 2.5% glutaraldehyde and post fixed in osmium with en bloc staining with uranyl acetate for EM study.

Results This tumor met the diagnostic criteria of psammocarcinoma. There were numerous psammoma bodies with or without nests of epithelial proliferation with minimal nuclear atypia. More than 75% of papillae were either associated with or completely replaced by psammoma bodies. Multifocal involvement was noted on the surfaces of both ovaries and fallopian tubes. Ultrastructurally, the tumor cells showed markedly villous surfaces with abundant long slender villi. Their length/diameter ratio (LDR) was 4 with some variation of their diameter. The cytoplasm contained abundant cytoplasmic organelles and intermediate filaments. Intracytoplasmic lumens were absent. Decalcified psammoma bodies resembled cut surfaces of geode.

Discussion and conclusion Our case met the proposed criteria for this tumor that arose de novo in peritoneum. Ultrastructurally, this tumor shared some findings with serous tumors (junctional complexes, irregular microvilli), but differed by the lack of prominent desmosomes and frequent intercellular lumens. PC differed from mesothelioma in that significantly greater microvillus LDR, prominent desmosomes, tomofilaments, intracytoplasmic lumens, external lamina and glycogen were rountine findings in the latter. Our findings justify classification of PC in the group of serous epithelial tumors arising from the coelomic mesothelium.

P-419

Application of electron- and immunoelectron microscopy in the differential diagnosis of pituitary adenomas

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Aim Evaluation of practical implementation of electron microscopic techniques in diagnosis of pituitary adenomas. **Material and methods** Material consists of 203 consecutive cases of surgically resected pituitary tumors. In 184 pituitary adenomas diagnosed clinically, there were 68 patients with symptoms of acromegaly, 32 patients with hyperprolactinemia, 33 cases of Cushing's syndrome and 1 case of TSH hypersecretion. There were 50 clinically nonfunctioning tumors. All cases were diagnosed by histological examination and immunohistochemical identification of known pituitary hormones (growth hormone, prolactin, ACTH, TSH, FSH, LH and alpha-subunit). The cases evaluated at light microscopy level as pituitary adenomas were examined with electron microscopy and immunoelectron microscopy using a postembedding immunogold technique.
Results Immunohistochemical investigation showed that about 40,1% of specimens of pituitary adenomas contained more than one pituitary hormone. Additionally, immunopositivity was discovered in 29% of tumors from patients with clinical and biological signs of silent pituitary adenoma. Ultrastructural analysis provided proper diagnosis of selected cases, e.g. silent corticotroph adenoma or distinction between densely- and sparsely granulated growth hormone secreting adenomas. Double labeling immunoelectron microscopy using gold particles of two different sizes greatly facilitated the distinction among mixed growth hormone/ prolactin, mammosomatotroph and acidophil stem cell adenomas.
Conclusions Electron microscopy analysis was valuable to provide a useful informations regarding the diagnosis. Our results confirmed practical value of WHO recommendation for five-tier evaluation of pituitary adenomas (clinical, neuroimaging, histological, immunohistochemical and ultrastructural). On the basis of our results and the published data, diagnostic postoperative algorithm for proper pathologic management has been proposed.

P-420

Apoptosis, sarcomere disruption and myofibrilar degeneration in skeletal muscles of stable COPD patients: an ultrastructural study

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Introduction In patients with chronic obstructive pulmonary disease (COPD), the diaphragm muscle shows signs of injury, even during clinical stability. In addition, these patients have a markedly increased susceptibility to additional diaphragm injury following inspiratory loading. We have hypothesized that, if sytemic factors play a role in diaphragm injury, limb muscles may also be affected. The purpose of this study is to investigate whether apoptosis occurs in the diaphragm and quadriceps of COPD patients, and to evaluate fiber injury in non-respiratory muscles.
Methods Samples from both diaphragm and quadriceps muscles were obtained in 22 patients undergoing thoracotomy for a low-stage lung neoplasm (LN) and stratified into two cohorts according to pulmonary function: LN+COPD (n=11) and non-COPD patients (LN+non-COPD, n=11). In addition, samples form quadriceps muscle in 7 healthy age-matched volunteers were also included (control group). All samples were processed for ultrastructural analysis. The proportion (%) of normal nuclei (NN) and those showing early (EAN) and advanced (AAN) signs of apoptosis were determined in all samples. Digital images of longitudinal fiber sections (1950x) were used to determine the area fraction (%) of abnormal myofibrilar structures (AAb).
Results The level of apoptotic signs in the diaphragm of both COPD and non-COPD groups was minimal and very similar to that of control limb muscle. In contrast, quadriceps muscle of both COPD and non-COPD groups showed a two- and three-fold increase respectively in the apoptotic nuclei when compared to controls, mainly due to EAN. Apoptotic bodies were not found. In control quadriceps AAb was 1,7±0,3%, mostly due to sarcomere disruption, while quadriceps in COPD patients was associated with an increased AAb (21±7%, $p<0,005$) including myofibrilar disorganization and degeneration.
Conclusions In patients with COPD, the diaphragm is not altered by apoptotic phenomena. Muscle remodeling through apoptotic mechanisms as well as through structural changes, such as sarcomere disruption and myofibrilar degeneration, seem to be enhanced in the quadriceps muscle of these patients, regardless of the severity of pulmonary function impairment, suggesting that additional factors such as the degree of physical inactivity either related to the disease or to the preoperative status could also be involved. (Supported in part by Grants BIOMED, FIS, FUCAP and ARMAR)

P-421

Differential CD68 expression in chromophylic (papillary) renal cell carcinoma and clear cell renal cell carcinoma: an immunohistochemical and ultrastructural study

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Background Renal chromophylic (papillary) carcinoma (CPRC) has a propensity for storing hemosiderin in tumor cells. By transmission electron microscopy (TEM), some tumors show a prominent lysosomal component, while in other cases, cells contain mostly isometric lipid vacuoles. It has been proposed that the latter would merely represent a papillary variety of clear cell renal cell carcinoma (CCRC). The aim of this study has been to analyze the lysosomal content in chromophylic (papillary) renal cell carcinoma

by both immunohistochemical study of CD68 expression and TEM, and to compare these results with those of conventional CCRC.
Design Cases of CPRC (n=21) and CCRC (n=12) are the subject of the study. The presence and relative amount of lysosomes and isometric fat vacuoles was investigated by TEM (n=11). Immunostaining with CD68 antibody (PG-M1, Dako, Denmark) was performed in representative sections. The presence, distribution and percentage of CD68 staining in tumor cells was compared with TEM data.
Results Expression of CD68 was observed in all cases of CPRC. The mean percentage of positive cells was 63% (1-95%). Distribution of positive cells was diffuse in 67% of cases. Of the 11 TEM cases, 9 contained abundant fat vacuoles, but only 4 had extensive isometric vacuolation by light microscopy. Electron-dense lysosomes were less abundant than fat in 7 cases, 4 of which had massive CD68 expression. Detailed examination of fat vacuoles in these cases suggested they were membrane-bound and of probable lysosomal origin. CD68 expression was also observed in 11 cases of CCRC, all of them with focal distribution, involving a mean of 14% tumor cells (1-50%). These differences were statistically significant ($p<0.001$).
Conclusions Diffuse CD68 expression is typical of CPRC and may be a useful auxiliary diagnostic criterion. The isometric fat vacuoles in CPRC are often membrane-bound and of probable lysosomal origin. These findings support the notion that the lipid-containing, vacuolated variety of CPRC is different from CCRC.

P-422

Nucleolar features and prognosis of breast cancer: an ultrastructural morphometric study

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Introduction Several cytological features are taken into account in infiltrating duct carcinoma of the breast. The aim of this study has been to assess the prognostic value of the nucleolar area by means of ultrastructural morphometry.
Materials and methods Forty-six cases of ductal carcinoma of the breast were included in the current study. All biopsies were processed for electron microscopy and electron micrographs were taken at x3,600 magnification. Nucleolar area measurements were done using SigmaScanPro software and statistical analyses were performed. Nucleolar area measures were categorized in terciles. A Kaplan-Meier estimation was performed to assess those measures related with disease-free interval. Measures that reached statistical significance were dichotomized at the cut point with maximum likehood in a Cox Regression model and then were included in a multivariate analysis.
Results Statistically significant differences were found in terciles per area ($p=0.019$) and per variation coefficient ($p=0.012$). In terms of nucleolar area measurements, any given nucleolar area with a standard deviation 3 1.5, showed a relative risk for recurrence of 4.05 (1.23-13.36) ($p=0.02$).
Conclusions Ultrastructural morphometry shows that nucleolar features, particularly those related to size heterogeneity, have a significant impact on prognosis.

P-423

Determination of ploidy in different lesions of prostate

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Introduction Any lesion in prostate is followed by different proliferative activity of cells. One of the markers, which relate to proliferative activity, is ploidy. It can be determined by cytometry, morphometry or by other methods. Aims: Study of DNA ploidy status in different lesions of prostate by morphometry.
Materials and methods Samples from 80 patients with atrophy, benign prostatic hyperplasia (BPH), squamous cell metaplasia (SCM), atypical adenomatous hyperplasia (AAH), basal cell hyperplasia (BCH), prostatic intraepithelial neoplasia (PIN) and prostatic carcinoma were processed in formalin, dehydratated in graded alcohols, embedded in paraffin, stained by Feulgen-stain. Integral optical density was assessed by morphometry on image analysis system. Integral optical density of lymphocyte was counted as 2c ploidy. Integral optical density at different prostatic lesions was divided on 1c.
Results Average ploidy at atrophy was 2.0, BPH - 2.2, SCM – 2.4, BCH – 2.6, AAH - 2.8, low grade PIN was 4.2. Average ploidy at high grade PIN was 5.1, high grade carcinoma – 5.2, moderate grade carcinoma – 5.9, low grade carcinoma – 7.9.
Conclusion Increase of ploidy status from atrophy to BPH, SCM, BCH, AAH, PIN and to carcinoma was observed. Regarding to ploidy lesions may be divided in benign and neoplastic processes, with cutoff point between them as 4.5.

P-424

Pseudohyperplastic prostatic adenocarcinoma in transurethral resection of the prostate

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Background the pseudohyperplastic prostatic adenocarcinoma (PHPA) is a recently described variant of adenocarcinoma, studied in needle biopsies and prostatectomy specimens. PHPA is characterized by malignant glands that simulate benign hyperplastic glands of variable size with complex architecture, papillary enfolding, luminal undulations branching or cystic dilations, columnar cells with macronuclei and nucleoli enlargement. The aim of this study was to define the frequency and microscopic aspect of PHPA in specimens originated in transuretral prostatectomy (TUP).
Material and methods 250 specimens of transurethral prostatectomy were analized from the Surgical Pathology Department at INCMyNSZ. 150 cases were initially diagnosed as benign glandular hyperplasia (BGH) and 100 as conventional adenocarcinoma (CC)
Results 2 cases (1.3%) with PHPA were identified in specimens diagnosed as BHG. These specimens demonstrated PHPA in two

microscopic areas of 3 mm. Both patients are well and free of disease 2 and 4 years after diagnosis. The review of cases diagnosed as CC, three specimens had 2 microscopic segments of PHPA. In the outcome the patients developed early bone metastasis. The histopathology study revealed papillary projections, nuclear enlargement, crawled glands, macronucleoli (5/5); cystic glandular dilatation, straight luminal borders, pink amorphous secretions (4/5); nuclear hypercromasia and transition to small cell carcinoma (3/5). Other changes observed were corpora amylacea, double nucleoli and mitoses.
Conclusions the PHPA is a rare neoplasm observed in TUP specimens and is observed in only few areas. The false negative frequency in BGH is 1.3%, and 3% in usual adenocarcinoma. The outcome for these cases is related to the main conventional adenocarcinoma component.

P-425

Vascular Endothelial Growth Factor: An important angiogenic mediator in chronic urinary bladder lesions

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Vascular endothelial growth factor (VEGF) is an overriding growth factor mediating tumor anigogenesis. This study was performed to assess VEGF levels of expression in both serum and tissue in benign, premalignant and malignant urinary bladder lesions with or without schistosomal infestation in a trail to study the angiogenic phenotype of these lesions and to address any possible role of VEGF as a prognostic biologic marker of bladder cancer. Also the impact of schistosomal infestation on the VEGF level of expression was assessed.The study included 62 patients: 27 chronic cystitis cases (17 schistosomal and 10 non-schistosomal), 5 cases with schistosomal premalignant lesions (metaplasia, dysplasia and leukoplakia) and 30 cases with bladder carcinoma (5 schistosomal SqCC, 14 schistosomal TCC and 11 non-schistosomal TCC). Eight cases with normal urothelium served as controls. VEGF serum concentration was measured by the enzyme immunoassay (ELISA) while, its tissue expression was detected by immunohistochemical staining.. Significantly higher levels of VEGF where detected in the premalignant and malignant cases in tissue ($p< 0.01$ & $p< 0.001$ respectively) and in serum ($p< 0.01$) compared to controls. Also an aberrant increase in VEGF serum and tissue levels was recorded in malignant cases compared to those with chronic cystitis ($p< 0.001$ & 0.01 respectively). Schistosomal infestation was found to have a positive impact on VEGF tissue and serum levels. Tissue VEGF positive rates was 100% in all the premalignant and malignant schistosomal associated cases. On stratifying malignant cases according to their histopathological grade and stage, an evident stepladder increase in VEGF serum and tissue levels was recorded. VEGF serum levels were well correlated with their corresponding tissue level which may indicate that VEGF serum levels are reflecting intratumoral angiogenic status. Although both VEGF serum and tissue levels were positively correlated with tumor histopathological grade and stage, yet this correlation was more evident with the tissue expression ($r= 0.7$, $r= 0.6$, $p< 0.001$ in tissue, while $r= 0.5$, $r= 0.4$, $p<0.01$, $p=0.05$ in serum with grade and stage respectively). In conclusion, our data indicate that VEGF expression level in both serum and tissue may be used as a valuable angiogenic marker for prediction of prognosis. This may help in selecting patients for more intensive surgical or chemotherapeutic approaches. Serum VEGF concentration may be used as a non-invasive prognostic parameter especially in high risk patients who need continuous follow up.

P-426

Microvessel density in urothelial tumors of the upper urinary tract

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Background Angiogenesis in tumors of the urinary bladder is relatively well studied, in spite of some conflicting results and usually correlates with bad prognosis. Few data are available about microvessel density (MVD) in urothelial tumors of the upper urinary tract (UUT). Our purpose was to investigate the MVD in patients with urothelial tumors of the UUT and to check for its relationships with stage, grade, and survival.
Patients, material, and methods There were investigated 32 patients with urothelial carcinoma of the UUT. Routine-stained slides were reviewed by two independent pathologists for the final diagnosis, T stage, and grade. Only cases with invasive and mixed papillary-invasive tumors were included. Two cases had lymph node metastasis. Additional slides were stained for FVIII, CD31, and CD34. MVD was estimated using the hot-spot method and mean value, calculated on 10 consecutive fields from the tumor area and 10 from the deeper connective tissue (x200). All cases had a follow-up of minimum 5 years.
Results Best results were obtained with CD34 and CD31. In the tumor area, mean values of MVD were 9.7 for T1, 23.78 for T2, and 37 for T3. The hot-spot method consists of choosing the field with maximum density of blood vessels, and results showed 16 for T1, 34.16 for T2, and 49.66 for T3. The comparison between MVD and G showed a significant increase from G1 to G3 in the mean values and maximal density as well: 6.1/10 for G1, 23.76/36.5 for G2, and 33.07/41.75 for G3. No relationship was found between MVD and N positive cases. Nine patients with high MVD died within 5 years. The extra-tumor MVD had no prognostic significance.
Conclusions Our results show a strong correlation between tumor areas MVD, T, G, and survival. MVD had no value as a predictor of lymph node metastasis.

P-427

Is there any prognostic significance for HER2 protein in urothelial tumors of the upper urinary tract?

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Introduction HER2 protein is overexpressed in about 20-30% of breast tumours and in 28% of bladder tumours. Until now, only two articles were published about the expression of HER2 in urothelial tumors of the upper urinary tract, and its signifiance is still uncertain. Our purpose was to investigate the incidence of the overexpression

of HER2 protein in urothelial tumours of the upper urinary tract, and its relationships with the stage and grade. The final purpose was to conclude on the prognostic value of HER2 protein in patients with tumours of upper urinary tract. Patients,

Material and methods There were investigated 26 cases with tumours of the upper urinary tract. Routine HE stained slides were reviewed for the pathologic diagnosis and grade. Additional slides were stained with Her-2/neu (Dako HercepTest). Interpretation of results was based on the score system (from 0 to +3) already used in breast cancer. Results obtained with Her-2/neu were compared with stage and grade.

Results Eight from 26 cases were positive for HER2 protein (30.7%). No relationship was found between HER2 overexpression and 'G'. All but one case with G3 had a +3 score for HercepTest.

Conclusions The overexpression of HER2 protein does not correlate with tumour stage, and has no predictive value for lymph nodes metastasis. It strongly correlates with 'G'.

P-428

Immunohistochemical correlation between pattern of postatrophic hyperplasia and carcinoma of the prostate

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Introduction Some authors consider postatrophic hyperplasia (PAH), a glandular hyperplasia that mimics some patterns of prostate carcinoma (PCa), without any correlation with neoplastic and preneoplastic condition of the prostate. The association of PAH with PCa recently suggested (Mucci et al., 2001; Tsujimoto et al., 2002) induced us to analyze the role of PAH as precursor of PCa.

Material and methods Formalin fixed and paraffin embedded archival sections (60 selected patients undergoing radical prostatectomy - mean age = 66.8 yr) were studied. Twenty cases (10 with PAH and 10 without PAH) were selected for each group: A) BPH (Benign Prostatic Hyperplasia), B) PCa-GHS<7 (Gleason Histological Score) and C) PCa-GHS=>7. Slides were immuno-cytochemically treated with PSA, 34ßE12, CD99, vimentin, CD34, AR, NSE, chromogranin A, PGP9.5, p53, bcl2, Ki67/MiB1, incubated with streptavidin-biotin complex (SBC) and revealed by diaminobenzidine (DAB).

Results Immunohistochemical investigations revealed the presence of basal cells (34ßE12) only in PAH. The nuclear cell cycle proliferation (MIB1/Ki67) and p53 mutation were quite similar in number of positive nuclei between PAH and PCa-GHS<7. The Chromogranin A were positive in PAH, randomly in PCa-GHS<7, and in nests in PCa-GHS=>7. Vimentin stained nests in PAH, whereas were negative in PCa. The bcl2 were positive in PAH and negative in PCa-GHS<7.

Conclusion These findings suggest a strong overlapping of cell cycle nuclear proliferation and mutation markers, supporting a possible role of PAH as a precursor of PCa.

P-429

Eosinophilic cystitis: clinico-morphologic aspects

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Collaborative study called for review of archived urinary bladder biopsy specimens with inflammatory lesions for the period 1995-2002. Features consistent with the diagnosis of eosinophilic cystitis were identified in 15 cases, thus positioning it at 6th place with an incidence of 5,63% from all inflammatory lesions. The distribution of clinical signs manifested in these patients often deceptive of interstitial cystitis or tumor processes is as follows: polakisuria, hematuria, suprapubical pains are evident in 11/73,3%/ patients; 3/20,0%/ presented with acute obstructive pyelonephritis as a result of bladder wall remodeling and 1 case /6,66%/ revealed combined features of gastroeneteritis and cystitis. Inflammed bladder wall and reduced bladder volume is estimated in 13/86,6%/ cases, ulcerations in 2/13,3%/ papillomatous and solid pseudotumoral lesions are 5/33,3%/ and 4/26,6%/ respectively. As all clinical signs are nonspecific, the only way to set a proper diagnosis is through bladder biopsy. Morphologic confirmation is based on the standard protocols. Histochemical (positive Ziehl-Neelsen stain) and ultrastructural identification of Charcot-Leyden's crystals are evident in some cases.

P-430

Adenocarcinoma of the male urethra in association with nephrogenic metaplasia

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We describe a case of adenocarcinoma of the urethra associated with so-called nephrogenic metaplasia in a 75 year-old male patient that showed a polypoid tumor of the proximal penile urethra. Histopathology examination of the biopsy specimen revealed two distinct patterns. The first showed a tumor composed of papillary fronds and glands lined by columnar epithelial cells with eosinophilic and clear cytoplasm, and contained atypical nuclei with frequent mitotic figures. This tumor was interpreted as adenocarcinoma. The second pattern was different, it consisted of small tubules some with mild cystic changes lying in an edematous stroma. The tubules were lined by a single layer of flattened cells with faintly eosinophilic cytoplasm and bland nuclei, mitotic figures were not identified. This area was interpreted as nephrogenic metaplasia and showed a direct transition into the adenocarcinoma. The immunohistochemical study revealed the presence of low-molecular-weight and some high-molecular-weight keratins in both types of lesions. The adenocarcinoma cells also showed nuclear staining for p53, in contrast, the cells of nephrogenic metaplasia were negative. The nephrogenic metaplasia as well as the adenocarcinoma cells were not stained with PSA and PAP. The histogenesis of adenocarcinoma of the lower urinary tract remains unclear. The association and direct transition of the nephrogenic metaplasia into the adenocarcinoma in this case suggest the proposed histogenetic pathway that the urethral adenocarcinoma may arise by malignant transformation of nephrogenic metaplasia.

P-431

Correlations between usual molecular markers and apoptotic index in bladder cancer

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Introduction Transitional cell carcinoma (TCC) is the second most common malignancy in the genito-urinary tract. The recurrence rate is particulary high after first presentation. Aims. Before treatment we assess to determine the p53protein (p53), bcl-2 oncoprotein (bcl-2) expression and apoptotic index (AI) in papillary non-invasive (Ta), papillary invasive (T1) and in situ carcinoma (TIS) in TCC.
Materials and methods We analyzed a total of 30 patients who underwent transurethral resection for primary TCC. The tumor stage (T1/Ta/TIS) and grade (G1/G2/G3) was determined after haematoxilin-eosin stained. Immunostaining was performed using the indirect tristadial Streptavidin-Biotin method for p53 and bcl-2.Apoptosis was detected by using APO-DIRECTTM TUNEL Assay kit.
Results Positive immunostaining for p53 was detected in Ta (48%), and TIS (67%); bcl-2 was present in Ta (4%) vs TIS (30%). According the grade the percentage of p53 positive cases increased from 52% (G1) to 80% (G2);in the same time the positivity of bcl-2 was lower in G1 (9%) than G3 (20%). An increased AI was found in G3 tumors.
Conclusions The majority of tumors was included in T1 (60%) and G1 (56%). The intensity and positivity of p53 and bcl-2 increased with grade of TCC. There is no significant correlation between frecquency of apoptosis and pathological stage of TCC, but our study reveals an obvious relation between AI, p53, bcl-2 expression vs grade of TCC.

P-432

Growth factor expression in cultured, human prostatic stromal cells with upregulated oestrogen receptors

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Introduction Benign prostatic hyperplasia (BPH) is associated with a shift in sex hormone balance towards an increase in the oestrogen:androgen ratio. This is accompanied by elevated levels of oestrogen receptor alpha (ER), predominantly in the stroma. Recently, we described a simple, in vitro model to create moderate upregulation of ER in human, prostatic stromal cells. The aim of this study was to investigate the hypothesis that in BPH upregulated ER and the action of androgens differentially regulate expression of several stromal growth factors.
Materials and methods Eight human, prostatic stromal cell strains were subjected to a procedure to upregulate ER alpha by exposing them to 1 micro mol beta-estradiol for 10 days, followed by passage and growth in the absence of steroids. Four cell strains received instead 100 n mol of dihydrotestosterone for 48 hr. Immunoexpressions of ER alpha, AR, and six growth factors (FGF-2, FGF-7, IGF-1, TGF-beta1, NGF and eNOS) were quantified by flow cytometry.
Results Expression of ER was significantly increased above control (6 out of 8 cell strains). Expressions of all six growth factors were elevated but not significantly so. However, there was a significant positive correlation between the change in ER (expressed as percentage of control) and the change in FGF-2 and FGF-7. Exposure of four cell strains to dihydrotestosterone reduced the expression of ER and all six growth factors in comparison with oestrogen-treated cells. This reduction was significant for ER and FGF-7.
Conclusion Upregulated ER in prostatic stroma have a greater modulating effect on synthesis of certain growth factors than the direct action of androgens and hence may play a major role in the development of BPH.

P-433

Periacinar retraction clefting in the prostatic needle core biopsies

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Introduction The diagnosis of prostatic adenocarcinoma in needle core biopsy is based on major and supportive criteria. One of the supportive criteria is the presence of retraction clefting around neoplastic glands. The aim of our study was to analyze needle core prostatic biopsies in order to determine the frequency of periacinar clefting spaces in tumors and non-tumorous prostatic tissue.
Materials and methods We analyzed a consecutive series of 137 prostatic cancer cases diagnosed by needle core biopsy to determine the frequency and extent of periacinar clefting. All tumors consisted of at least 30 neoplastic glands. Specimens were fixed in 10% buffered formaldehyde, embedded in paraffin, cut at 5µm thickness and routinely stained with hematoxylin and eosin. Clefting was analyzed on 10 neoplastic and 10 normal glands in three different high power fields.
Results One third or more of 30 analyzed glands with clefts affecting more than 50% of circumference were significantly more common in tumors (51.8%) than in benign glands (8%). More strict criterion that designated as positive cases with at least 50% of neoplastic glands (fifteen out of thirty) with clefts that affected more than 50% of circumference revealed clefts in the malignant cases only (15.3%) but not in benign cases (0%). Fifteen or more glands with clefts regardless of their extension were also more prominent in malignant cases (86.9%) than in benign cases (20.4%).
Conclusion We conclude that retraction clefting represents a reliable criterion for diagnosis of prostatic adenocarcinoma.

P-434

Unusual causes of ureteral obstruction

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Objective To report a series of cases of ureteral obstruction due to unusual pathological lesions, most of them being clinically and/or radiographically suspicious for malignant tumors.
Materials and methods A retrospective study was done to

analyze the causes of ureteral obstruction in a series of patients managed at the Department of Urology, Timisoara County Hospital between 1996 and 2002. There were selected only those particular cases of ureteral obstruction, others than cases with lithiasis, stenosis of the ureteropelvic junction and transitional cell carcinoma. An additional case was send for evaluation from another institution was also included in the study. For three of the selected cases we performed additional histochemical (Alcian-Blue-PAS) and immunohistochemical study (anti-CK, anti-PSA, anti-vimentin, anti-smooth muscle actin, ABC and LSAB system, visualization with DAB).

Results We identified 8 cases of ureteral obstruction due to unusual pathological lesions. They occurred in 5 men and 3 women. The mean age of the study group was 50 years (range 34 and 58 years). There were 3 malignant and 5 benign lesions that produced ureteral obstruction. In 5 cases, the compression was extrinsic and only in 3 cases the obstruction was intrinsec. The site of ureteral obstruction was in the lower ureter in 2 cases, in the lower and the middle ureter in 3 patients and in the upper ureter in 3 cases. The pathological lesions were: ureteritis cystica - 2 cases, nephrogenic adenoma of the ureter - 1 case, retroperitoneal fibrosis - 1 case, reproperitoneal sarcoma - 1 case, pelvic endometriosis -1 case, prostate cancer metastasis - 1 case, signet ring cell carcinoma of the colon invading the ureter - 1 case.

Conclusions After discarding the more common causes of extrinsic or intrinsic ureteral obstruction, the proliferative-metaplastic lesions of the urothelium, the retroperitoneal fibrosis and sarcoma, the endometriosis and metastatic/invasive carcinoma should be considered in the diagnosis.

P-435

Immunohistochemical study of androgen receptor and TGF alpha in human benign and malignant prostate

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Aims That androgen receptor(AR)and TGF alpha might be prognostic of hormonal response and biological behaviour in prostate carcinoma.

Methods AR and TGF alpha were studied in paraffin sections from 50 men (35 treated for prostate cancer and 15 for BPH by open, transurethral prostatectomy or prostate biopsy) No patient had undergone previous treatment for the prostate disease. The sections were stained with HE to establish morphological characteristics (Gleason Score) and then were studied with indirect immunoperoxidase method applying monoclonal antibodies to AR(F 39.4.1 Biogenex) and antiTGF alpha (clone 88, Biogenex). Results were semiquantified using Histoscore.Nuclei were graded for intensity of AR staining (0-absent,1-weak,2-moderate or3-strong) and the percentage of these intensity levels and a total intensity score were determined. We compared the AR and TGF alpha characteristics of prostate cancer (PCa) with BPH.

Results Increasing Gleason grade was correlated with decreasing intensity score and greater heterogeneity of AR staining and increased (+++) TGF alpha immunoreactivity.

Conclusions Our data indicate that AR and TGF alpha content could be useful predictors of endocrine response and biologocal behaviour of prostate cancer.

P-436

Penile leiomyosarcoma-morphological and immunohistochemical study

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Introduction Our case is about a 48 years old male patient who was admitted with the diagnosis of a penile tumor, reason why a total penectomy was performed.

Material and methods Fragments of the tumor and penile tissue were fixed in formalin 10%, embedded in paraffin and sections were stained with HE and VG. Immunohistochemical tests have been done: actin, vimentin, S100, AE1-AE3, HMB45, PCNA.

Results On macroscopic examination, the tumor was bulky, ulcerative nodular, occupying the glans and the distal part of the shaft; it was gray white coloured, with hemoragic areas. Microscopic examination revealed a tumorous proliferation of elongated cells with moderate nuclear pleomorphism and mitosis (typical and atypical), organised in a fascicular pattern, well vascularised; the tumor was superficially ulcerated and invaded the corpora cavernosa. The tumor was diffusely positive for actin and vimentin, focally positive for S100, negative for AE1-AE3 and HMB45, and PCNA was 70%.

Conclusions The morphological appearance and immunhistochemical results led us to the diagnosis of penile leiomyosarcoma, moderately differentiated.

P-437

Do human papillomaviruses infect proximal parts of the male genital tract?

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Introduction Human papillomaviruses (HPV) play an essential role in the pathogenesis of squamous cell carcinoma of the uterine cervix. The male urogenital tract is considered to be a reservoir of HPV infection. Our previous finding of HPV in the squamous metaplastic epithelium with dysplasia in the epididymis prompted us to start a study focused on the presence of HPV in the male genital tract. Aims: Verifying the presence of HPV in the epididymis, ductus deferens and seminal vesicles.

Materials and methods Samples from three groups (A, B, C) of patients were analysed by means of nested PCR for the presence of HPV DNA extracted from a paraffin-embedded tissue sections. Types of HPV were determined by DNA nucleotide sequencing of the PCR products. Group A consisted of 22 patients (mean age 61, age range 39-87, except for one patients 19 years old), group B consisted of 22 patients (mean age 73, age range 43-87, except for two patients 23 and 24 years old) and group C consisted of 44 patients (mean age 65, age range 50-82). Patients in group A were surgically treated for non-tuberculous epididymitis (41 epididymal

and 8 ductus deferens samples), patients in group B were orchiectomised for the diagnosis of prostate cancer, teratocarcinoma of the testis and/or epididymal cyst (normal 22 epididymal and 7 ductus deferens samples) and subjects in group C were treated by radical prostatectomy or cystectomy for prostate or urinary bladder cancer (44 samples of seminal vesicles without inflammatory changes).
Results HPV DNA was detected in 7(31%) of patients in group A(HPV types 6,16, 33, 35, 55, 73), in 1 (0.5%) patient in group B(at present unclassified type) and in 5 (11.4%) patients in group C(HPV types 16, 18 and 52).Neither koilocytes nor dysplastic changes were observed.
Conclusion Our data confirmed the presence of HPV, both low- and high-risk types, in the male genital tract. They support the idea of a male reservoir of HPV infection.

P-438

Spermatogenetic function of the testis in patients of different varicocele grades

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Introduction Varicocele is one of the main reasons of male infertility. Surgical repair may improve sperm quality. The aim of this study is to investigate a grading system of varicocele and its correlation with the histological appearance using a multiple biopsy method.
Materials and methods Bilateral double testicular biopsies of 53 young infertile grade 2-4 varicocele patients were examined histologically. Spermatozoa numbers counted in ten round shaped tubules, severity of atrophy and presence of maturation arrest was recorded. Distribution pattern of observed spermatozoa numbers was evaluated.
Results Grade 2 and 3 cases showed normal distribution. In grade 4 cases five peaks were found indicating group inhomogenity. Lower grade varicoceles presented with milder histological changes (mild atrophy or normal spermatogenesis) while in higher grade varicoceles more marked alterations (moderate-marked atrophy and/or maturation arrest) were seen.
Conclusion Grade 4 varicocele cases represent an inhomogeneous group, which should be divided into more categories. Modification of the grading system is required. Spermatogenetic activity of varicocelic patients cannot be predicted from clinico-radiological examinations so before ART a diagnostic testicular biopsy is recommended.

P-439

Synchronous double urogenital cancer – A case report

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We present a case of synchronous double urogenital cancer, comprised of invasive transitional cell carcinoma of the urinary bladder and adenocarcinoma of the prostate. Cystoprostatectomy was performed in a 68-year-old male patient with previous cytological diagnosis of transitional cell carcinoma of the urinary bladder and no evidence of prostatic malignancy on preoperative clinical investigations. Gross analysis of the urinary bladder revealed a cauliflower tumor mass filling the entire lumen and infiltrating the wall of the bladder. The size of the prostate was slightly increased. Histological analysis of the urinary bladder specimens showed poorly differentiated transitional cell carcinoma, with only a few foci of preserved papillary architecture, infiltrating deeply into the muscle layer of the bladder wall. The specimens taken from the prostate gland for the purpose of staging of the neoplasm excluded the existence of infiltration of the prostatic urethra with the previously described neoplasm, but foci of moderately differentiated adenocarcinoma (Gleason score 6) of the prostate were discovered in its vicinity. The subsequent extensive sampling of the prostate showed organ confined adenocarcinoma. The review of the literature showed that the reported prevalence of synchronous existence of double primary malignant neoplasms of the urinary tract (transitional cell carcinoma of the bladder and prostatic adenocarcinoma) varies between 27% and 33%. The prevalence is noted to rise with increasing age. This leads to a conclusion that during standard cystoprostatectomy analysis, extensive sampling of the prostate is needed, even in the absence of macroscopic changes and preoperative clinical data suggesting prostatic neoplasm.

P-440

Multiple synchronous renal cell tumors

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Introduction The use of the nephron-sparing surgery demands detection of multiple renal cell tumors.
Materials We performed a retrospective analysis of nephrectomies and autopsies performed in our clinic.
Results Of 120 renal cell tumors 26 (21, 7%) were multifocal. Most of the patients were men. Twelve cases were present where the histological subtype of the largest tumor was clear cell. Satellite tumors were clear cell carcinoma (7), papillary adenoma (5), angiomyolipoma (3), renomedullary fibroma (2), Bellini duct carcinoma (1). Six multiple renal cell tumors were found in T1, two in T2, four in T3A stage of the primary tumors. Four patients had bilateral neoplasms. In thirteen cases only multiple papillary adenomas and carcinomas were found. Usually tumors were no more than 3 cm in diameter. In six cases multiple tumors originated on a background of nephrosclerosis of various genesis (chronic pyelonephritis, gouty nephropathy, amyloidosis).
Conclusion When conservative surgery of kidney cancer is performed, it should be remembered that 20% of patients with renal cell tumor have multiple neoplasms, which may cause local recurrence. The origin of multiple renal cell tumors may be bound up with hereditary predisposition or nephrosclerosis creating multifocal tumor field.

P-441

Loss of Parkin expression in prostate carcinoma

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Introduction Prostate cancer is a leading cause of cancer-related deaths in men and is the most common malignancy in American males. The heterogeneity of prostate cancer defines the current clinical challenges. One challenge is to identify in early, still potentially curable, stages of prostate cancer, molecular markers that predict aggressive or metastatic behavior. Prostate cancer is characterized, like many other cancers, by molecular and genetics aberrations involving chromosome 6. Parkin, mapped at the long arm of chromosome 6, is a 465 amino acid protein with a molecular mass of 52 kDa, an ubiquitin-like domain at its amino terminus and two RING finger motifs flanking a cysteine-rich region. Our aim was to investigated whether Parkin alterations play a role in development and progression of prostate carcinoma
Materials and methods We examined Parkin expression, by Western blot analysis in eight prostatic carcinomas derived cell lines as well as in normal prostate, and in three prostatic intraepithelial neoplasias (PIN) and in twenty primary prostatic carcinomas by immunohistochemistry.
Results Parkin protein was undetectable in six out of eight prostate cancer cell lines tested and barely detectable in the remaining two whereas normal prostate showed a substantial expression. Preliminary results using immunohistochemistry showed that Parkin expression was absent in PIN lesions, and in the prostatic carcinomas analyzed.
Conclusion Our findings suggest that Parkin might be a useful tool to detect early alteration in prostate cancer.

P-442

The KDR/Flk-1 receptor of vascular endothelial growth factor in urothelial bladder cancer: relation with clinicopathologic parameters and patients' survival

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Introduction The impact of different angiogenic inducers and inhibitors in the switch of angiogenesis in urothelial bladder cancer, is not clarified. KDR/flk-1 is a receptor-tyrosine kinase that binds specifically vascular endothelial growth factor (VEGF) on vascular endothelium, mediating its full biologic spectrum. Although KDR/flk-1 expression was at first reported to be restricted to the classic endothelium there is increasing evidence that KDR/flk-1 is also expressed by normal and tumor cells other than endothelial. Aims: As data about KDR/flk-1 protein in urothelial bladder cancer is very limited, we investigated its expression in a series of 114 urothelial carcinomas in relation with clinicopathologic parameters and patients' survival. Moreover, prompted by studies suggesting that KDR/flk-1 may have broader functions apart from angiogenesis we evaluated KDR/flk-1 expression in relation with markers of cellular proliferation and apoptosis (Ki-67, p53, bcl-2).
Materials and methods Immunohistochemistry (ABC-HRP) was performed for the detection of KDR/flk-1 (mouse/A-3, SC-6251, Santa Cruz), Ki-67, p53 and bcl-2 using monoclonal and polyclonal antibodies. Statistical analysis was univariate (Pearson's x2, Mann-Whitney test, Log-Rank test) and multivariate (Cox's model).
Results KDR/flk-1 expression was observed in the cytoplasm of cancerous cells in 78/114 cases. No significant associations were observed between KDR/flk-1 expression and clinicopathologic parameters, Ki-67, p53 and bcl-2 expressions. On the other hand, widespread KDR/flk-1 expression in more than 50% of cancerous cells, was associated with patients' increased survival, in univariate and multivariate analysis ($p=0.0119$, $p=0.042$, respectively).
Conclusion Although non endothelial KDR/flk-1 expression has not yet been elucidated, its association with better patients' survival may be related with the failure of non-endothelial KDR/flk-1 to mediate angiogenic and mitogenic effects, that has been reported in some previous in vitro studies.

P-443

TIMP-2 protein expression in relation with cellular proliferation, apoptosis and survival of urothelial bladder cancer patients

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Introduction The role of tissue inhibitors of matrix metalloproteinase (TIMPs) in cancer progression remains a controversial matter since apart from the well established inhibitory action of TIMPs on MMPs, several new functions are now attributing to TIMPs such as growth promoting activity, involvement in apoptosis control and in angiogenesis. The aim of this study was to elucidate the role of TIMP-2 in urothelial bladder cancer, we investigated its expression in a series of 106 urothelial carcinomas in relation to clinicopathological parameters, patients' survival and the expression of the Ki-67 proliferation index.
Materials and methods Immunohistochemistry (ABC-HRP) was performed for the detection of TIMP-2 and Ki-67 proteins, using monoclonal and polyclonal antibodies. Statistical analysis was univariate and multivariate.
Results TIMP-2 stromal cell expression was observed in 28/106 cases and associated significantly with urothelial carcinomas of high histologic grade ($p<0.0001$) and advanced stage ($p=0.001$) as well as with poor patients' survival ($p=0.0002$). TIMP-2 expression in cancerous cells was found in 58/106 cases. TIMP-2 either stromal or cancerous cell expression correlated significantly with Ki-67 expression ($p=0.02$, $p=0.044$ respectively).
Conclusion The association of TIMP-2 with Ki-67 supports the growth promoting activity of TIMP-2. Moreover, TIMP-2 expression in stromal cells may be a valuable marker of adverse prognosis in urothelial cancer patients.

P-444

Investigation of telomerase protein subunits (hTEP-1 and hTERT) and RNA component (hTR) in relation with clinicopathological parameters and patients' survival in urothelial cell bladder carcinomas

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Introduction Telomerase is a large ribonucleoprotein complex that stabilizes and extends telomeres of eukaryotic chromosomes regulating cell replicative potential and lifespan. Human telomerase is composed of an RNA subunit (hTR) and several protein components including the catalytic telomerase reverse transcriptase (hTERT) and telomerase-associated-protein-1 (hTEP-1). Aims: To clarify the role of telomerase in bladder cancer we investigated for the first time the expression of hTERT, hTEP-1 and hTR in 132 urothelial carcinomas, in relation with clinicopathological parameters, patients' survival and markers related with proliferation and apoptosis (Ki-67, bcl-2, caspase-3).
Materials and methods Immunohistochemistry (ABC-HRP) using monoclonal and polyclonal antibodies and RNA in situ hybridization were performed for the detection of hTEP-1, hTERT, Ki-67, bcl-2, caspase-3 proteins and hTR respectively. Statistical analysis was univariate and multivariate.
Results hTEP-1 positive cytoplasmic expression associated significantly with the lower grade of carcinomas (p=0.027). Cytoplasmic hTEP-1, nuclear hTERT, cytoplasmic hTERT and hTR correlated inversely with stage of the disease (p=0.003, p=0.018, p=0.0001 and p=0.06 respectively). Cytoplasmic TEP-1 and hTERT demonstrated inverse associations with p53 expression (p=0.001 and p=0.047 respectively). Cytoplasmic hTERT associated with caspase-3 expression (p=0.004). Cytoplasmic hTEP-1 and nuclear hTERT correlated significantly with increased patients' survival in univariate (p=0.04) and multivariate (p=0.007) analysis respectively.
Conclusions 1) Telomerase expression associates with early stage urothelial carcinomas. 2) The correlations between cytoplasmic TEP-1 or cytoplasmic hTERT and p53 are supported by in vitro studies implicating p53 in telomerase regulation. 3) Nuclear hTERT seems to carry a favorable impact on patients' survival. This may be due to the induction of genomic instability, telomere-telomere fusion, aneuploidy and prevalence of more aggressive clones, known to be associated with telomerase inhibition.

P-445

Gleason score and nodal metastases of prostate cancer

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Introduction Gleason score is important predictor for differentiation and biological potential of prostate cancer. Node – negative (p N0) prostate cancer is the best result after radical prostatectomy which is indicated in patients with localised prostate cancer. The aim of the study was to analyze Gleason score in patients with nodal metastases of prostate cancer (PC).
Material and methods We analyzed 49 cases of radical prostatectomies due to localised prostate cancer in the period 1996-2000 in Clinic of Urology in Clinical Centre of Serbia, Belgrade. Nodal metastases (stage of disease), average age of the patients, Grade and Gleason score of tumors and premalignant lesions were analysed. Immunohistochemical staining was performed and pathological analyzed. We used statistical analysis ANOVA and H^2 test.
Results The average age of the patients was 65.6 years (range 44-76, pick 61-70). Most cases 25 (51%, $p<0.001$) were in pT2a N0M0, in pT2b N1M0 9 (18.36%), in pT3bN0M0 10 (20.4%), in pT3bN1M0 3 (6.12%), in pT4aN0M0 2 (4.08%). Positive nodal status was present in 12 cases: 9 (18%) in pT2bN1M0 - iliac 3 (right 2, left 1), obturatory 6 (right l, left 5) and 3 cases in pT3bN1M0-iliac left 1 and obturatory 2 (1 right and 1 left). We found Gleason score 8 in 9 cases (18.36%) in pT2bN1M0 versus 7 cases (14.5%) without nodal metastases. Gleason score 9 was found in 3 cases (6.1%) in pT3bN1M0 versus one case without nodal metastases ($p>0.05$). Gleason score 3 was present in 6.1%, 4 in 12.2%, 5 in 8.1%, 6 in 16.3%, 7 in 24.5%. Grade 1 tumors were found in 9 cases (18%), grade 2 in 11 (22%), and grade 3 in 29 (60%). HG PIN was present in 18 cases (36.7%), LG PIN in 10 (20.4%).
Conclusion Gleason score differ, but not significantly in prostate cancer with nodal metastases. Pelvic lymphadenectomy is necessary for staging purposes in prostate cancer. Radical prostatectomy is the most adequate method in surgical treatment of localised prostate cancer.

P-446

Immunohistochemical and molecular evaluation of c-erbB2 gene amplification and c-erbB2 protein overexpression in prostate cancer

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Introduction The amplification of oncogene c-erbB2 and overexpression of C-erbB2 protein found in some human cancers is associated with more aggressive development of tumor. The previous data suggested an independent role of c-erbB2 protein as a prognostic factor in human breast cancer. However, the role of c-erbB2 gene and its protein product in prostate cancer cells still remain unclear. The aim of our study is to show what is the role of c-erbB2 protein in prostate cancer and to examine association between c-erbB2 gene amplification and its protein product expression in the histologicaly graded prostate tumor.
Materials and methods We have analyzed c-erbB2 gene amplification by RT PCR and c-erbB2 protein expression by immunohistochemistry in 39 cases of paraffin embedded slides. The tumors were divided according to WHO by Mostofi classification: 13 cases of G1 - well differentiated cancer; 13 cases of G2 - moderately differentiated cancer; and 13 cases of G3 - poorly differentiate cancer. These groups are equivalent to 1-4, 5-7 and 8-10 Gleason classification respectively.
Results The correlation between c-erbB2 protein expression and

well, moderately and poorly differentiated cancer respectively existed ($p<0.05$). There was no significant correlation between c-erbB2 gene amplification and c-erbB2 protein expression in all examined groups.
Conclusion Our results suggest that the c-erbB2 protein expression is an independent factor on the c-erbB2 gene amplification in prostate cancer cells. It is possible that there are other ways, except gene amplification, for overexpression of c-erbB2 protein in prostate cancer.

P-447

Comparison of needle biopsy and surgical specimen in prostatic carcinoma

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In Finland carcinoma of the prostate is the most common form of cancer in men. The number of radical prostatectomies is increasing in most countries. The radical prostatectomy is normally preceeded by a needle biopsy. At the university Central Hospital of Helsinki 599 radical prostatectomies were performed between 1988 and 2001. in 474 patients, the preoperative needle biopsy was available. In many cases the preoperative PSA value was also available. The mean PSA value dropped from 20 to less than 10 ng/ml. The percentage of seminal vesicle invasion dropped from 35 to 10. Except for the earlier years, the men Gleason score was about 1 point higher in the radical prostatectomy specimen than in the biopsy. The mean Gleason score has increased, while the stage and PSA values have decreased indicating that the criteria for the Gleason grading has changed ovewr the years. This makes the comparison of treatment results very difficult.

P-448

Immunohistochemical profiles as an aid in borderline prostatic lesions

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Introduction Distinguishing atypical adenomatous hyperplasia (AAH) from small foci of prostatic adenocarcinoma in needle biopsies of the prostate often presents as a challenge for pathologists. The aim of the study was to establish characteristic immunohistochemical patterns in benign and malignant prostatic lesions so they can be used to classify foci of AAH as benign or malignant.
Materials and methods Using immunohistochemistry, prostatic needle biopsies from forty-five patients were evaluated for cytokeratin 903, p63 and alpha-methylacyl-CoA Racemase (P504S). Twenty-five of these biopsies were benign and twenty contained prostatic adenocarcinoma.
Results 96% of the benign prostatic biopsies were positive for cytokeratin 903 and p63 and 96% were negative for P504S. Only one case of benign prostatic tissue was focally positive for P504S. When evaluating cases with adenocarcinoma 95% were positive for P504S and 100% were negative for cytokeratin 903 and p63. P504S immunostain showed consistently positive staining in areas of low and intermediate grade adenocarcinoma and areas with high grade PIN. High-grade prostatic adenocarcinoma was frequently negative for P504S. Sensitivity and specificity for cytokeratin 903/p63 were 96% and 100% while sensitivity and specificity for P504S were 95% and 96%.
Conclusion Cytokeratin 903 was as sensitive and as specific as p63 and can be used interchangeably in cases of AAH. The combination of the two antibodies did not improve sensitivity or specificity. A combination of negative cytokeratin 903 or p63 with positive P504S may be helpful in positively identifying prostatic carcinoma in cases of AAH.

P-449

Myofibroblastic stromal reaction in prostatic adenocarcinoma

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Introduction Reactive stromal changes that occur in different human cancers probably are involved in local tumor spreading and progression. It seems that myofibroblast plays very important role in stromal reaction to certain types of human cancer.
Material and methods We have analyzed 47 prostatic carcinomas in radical prostatectomy specimens to determine the proportion of myofibroblasts around normal and neoplastic acini. Myofibroblast showed positive staining for alpha-smooth muscle actin (SMA) and vimentin. Positive cells were counted in the hot spots areas under high power magnification (HPF). Results were expressed as negative (-) for no staining, (+) for positive staining in up to 10% of stromal cells, (++) in 10-25% of cells and (+++) for more than 25% of positive cells. Stromal blood vessels were counted in ten HPF. The degree of desmoplasia was also analyzed by Mallory staining and expressed semiquantitatively as weak, moderate and strong.
Results and conclusions Highly increased number of myofibroblast was observed around neoplastic acini in 16 (34.1%) cancer cases, moderate in 10 (21.2%) and sparse in 21 (44.7%) cases while there were occasional myofibroblastic cells adjacent to normal acini. There was a positive correlation between the degree of stromal desmoplasia and the proportion of SMA and vimentin positive cells in adjacent stroma. The number of vessels in the adjacent stroma was also significantly increased in comparison with normal glands ($p<0.05$). It seems that myofibroblasts are present in the stroma and may play a significant role in the desmoplastic stromal reaction in prostatic adenocarcinoma.

P-450

C-kit expression in sarcomatoid renal cell carcinoma. Potential treatment with imatinib (STI-571) for this ominous tumor

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Background Sarcomatoid renal cell carcinoma (SRCC) is an uncommon subtype of Renal Cell Carcinoma (RCC) with a poor prognosis. Currently, there is no specific useful treatment for it.

Few immunohistochemical studies on SRCC have been published, and c-Kit expression has not been reported yet.
Material and methods We reviewed 215 cases of RCC diagnosed at our Department from 1995 to 2002. Among them, 20 cases (9.3%) were SRCC. Formalin-fixed, paraffin-embedded material was available in 19 cases and we performed immunohistochemical staining against c-Kit (rabbit polyclonal antihuman antibody CD117, dil. 1:100, Dako) on them.
Results Among the 20 SRCC, 2 (10%) showed no epithelial differentiation, and in the other cases the epithelial component was conventional RCC in 10 cases (50%), papillary CCR in 5 (25%), and chromophobe RCC in 3 (15%). There was no sex predominance and the average patient age at the time of diagnose was 61 years. Sixteen cases (80%) presented an advanced stage at the time of diagnose, and 13 (65%) died of disease. The immunohistochemical study showed that 17 cases were positive for c-Kit (89.5%), 7 diffusely (>50% of the sarcomatoid cells positive), 6 moderately (10-50% of the cells), and 4 focally (<10% of the cells).
Conclusion The high expression of c-Kit in SRCC in our series and the existence of target therapy against cells expressing this marker (Imatinib (STI-571, Gleevec, Novartis, Basel, Switzerland) suggests its potential use in the clinical management of these patients in order to improve their prognosis, especially in disseminated cases.

P-451

Does HPV play a role in the development of bladder TCC? A comparison of PCR and immunohistochemical detection methods

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The role of human papilloma virus (HPV) in the development of bladder carcinomas remains controversial. Varying techniques have been used to identify this virus in transitional cell carcinoma (TCC). Here we used two different methods to detect the presence of mucosal HPV in 78 TCC samples. The first, immunohistochemistry, involved using a polyclonal antibody that reacts with the L1 capsid protein of most known papilloma viruses. The second method is used on a general primer-mediated polymerase chain reaction (PCR) assay. This involves using type-specific oligonucleotide probes to identify 22 different mucosal HPV genotypes from a 150bp PCR product from the L1 region of the HPV genome. The two methods produced contrasting results, 78% of the samples were positive for HPV antigen and 2% were positive for HPV DNA. The reason for such conflicting results is that polyclonal antibodies are not specific and are likely to cross react with other antigens. PCR is specific, more sensitive, and it is widely used to detect HPV in a variety of clinical samples. The results obtained in our study implies that HPV does not play a role in the development of bladder carcinoma, and PCR remains to be the best method to detect HPV in TCC.

P-452

Does TGF-α1 prevent carcinoma spreading?

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Introduction Most human carcinomas including prostate cancer are associated with stromal reaction typified by changes in growth factor expression and over-accumulation of extracellular matrix. Our study deals with the differential TGF-α1 production in neoplastic focuses and in adjacent paraneoplastic stroma in prostate.
Materials and methods Tissue samples from radical prostatectomies were examined. To verify malignant material or benign the immunohistochemical staining against high molecular weight cytokeratin was performed on sections and in heterogeneous primary cultures of the same specimens. The immunohistochemical identification of TGF-α1 was also performed. The ELISA technique for quantitative analysis of TGF-α1 soluble form in culture supernatants was applied.
Results Majority of selected cancer cases were histologically estimated as small-sized glands with large desmoplastic fibro-muscular stroma. The immunohistochemical staining for TGF-α1 in malignant focuses was much more significant than in neigh-bouring benign areas on sections in contrast to, the cultures obtained from neoplastic or paraneoplastic portions of prostate tissue which stained positively for TGF-α1 without any difference. Considering that immunohistochemistry shows only latent TGF-α1 we evaluated its bioactive form in culture supernatans. We found dramatically high supernatant levels of active TGF-α1 in benign cultures compared to the cultures from the malignant areas.
Conclusion Our data demonstrate that desmoplastic alterations in benign areas adjacent to malignant glands was associated with elevated expression of TGF-α1 active form. So significant alteration in factor production and accompanied tissue remodeling can be considered as a protective reaction of surrounding stroma against tumor invasion.

P-453

Primitive neuroectodermal tumor of the kidney: A case report

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Introduction Primitive neuroectodermal tumors (PNETs) represent a family of neoplasms, of presumed neuroectodermal origin, that mostly presenting as bone or soft tissue masses in the trunk or axial skeleton in adolescents and young adults. The aim of this study was to report a case of PNET arising from kidney which is a rare localization.
Methods A 32 year-old-man presented with abdominal pain and abdominal mass. The imaging methods revealed a mass nearly replacing the right kidney and he underwent a right nephrectomy.

Results On gross examination the tumor measured 16 cmx14 cm with cystic areas and necrosis. Microscopically, it was a highly cellular neoplasm of consisting sheets of small-round-to-oval cells with irregular nuclei. Immunohistochemical stains were positive for vimentin and CD99. Microscopic and immunohistochemical findings were compatible with PNET.
Conclusion Although rare, PNET must be included in the differential diagnosis of renal tumors especially in children and young adults. We present a case of renal PNET as an unusual case and discuss the differential diagnosis

P-454

Pathologic stage of urinary bladder cancer at presentation in a non-selected patient population in a high-incidence region of the United States

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Introduction Most studies of pathologic stage in bladder cancer (Ca) are retrospective, using selected, usually hospital-based, patient populations. The aim of this study was to determine the pathologic stage of bladder Ca at initial presentation in a non-selected patient population in a high-incidence region of the northeastern United States.
Materials and methods All patients newly presenting with bladder Ca in the state of New Hampshire (U.S.A.) from July 1998 to December 2001 were identified through the State Tumor Registry. Slides were classified according to WHO and ISUP criteria.
Results Of 265 cases, 15 were diagnosed as non-malignant or technically inevaluable, leaving 250 Ca cases. 14 (5.6%) were carcinoma-in-situ (stage Tis). 205 (82.0%) were papillary, of which 150 (73.2%) were low-grade and 55 (26.8%) high-grade. Of the low-grade cases, only 7 (4.7%) were invasive, all limited to lamina propria (stage T1). Of the high-grade cases, 19 (34.5%) were non-invasive (stage Ta), 31 (56.4%) invaded lamina propria (stage T1), and 5 (9.1%) invaded muscle (stage 2). 26 cases (10.4%) had no papillary component and all were invasive; 14 (53.8%) were stage T1 and 12 (46.2%) were stage T2. 5 cases (2.0%) were non-urothelial, including 2 squamous cell Ca, 2 small cell Ca, and 1 adenoCa; 2 of these presented as stage T1 and 3 as stage T2. Overall, 74 cases (31.4%) were invasive (at least T1) at the time of presentation.
Conclusions This population-based series establishes the distribution of bladder tumor stage at initial presentation and documents a smaller percentage of invasive cancers than most hospital-based series

P-455

The relationship between mitotic index and nucleolar organizer region with histologic grade in transitional cell carcinoma of urinary bladder

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Background and object Transitional cell carcinoma (TCC) of urinary bladder is the second most common cancer of the genitourinary tract, that the prognosis and treatment is related to several parameters such as proliferative cell markers. In this study relationship between two proliferative cell markers:1-Mitotic Index(MI)& 2-AgNOR and tumor grade were studied.
Methods and materials In a cross sectional descriptive study 75 paraffin blocks of patients with TCC of the urinary bladder in the period from 2000 to 2002 from pathology department of shahid Mostafa Khomeini hospital, were obtained and from each one 3 micron sections were provided. Tumor grade on basis of WHO/ISUP grading system, was determined and number of mitosis on 100 tumor cells were counted. In the other section, after one-step silver nitrate staining, the number of NOR also on 100 tumoral cells were counted and the mean of it in each cell were determined.
Results The high grade tumors possessed more AgNOR per nucleus than did the low grade ones. Significant relationship between tumor grade and MI (P=0.000, rs=0.801) and AgNOR (P=0.000, rs=0.731), and also between MI and AgNOR (P=0.000, rs=0.638) were found. The regression analysis revealed relationship between MI & AgNOR. (AgNOR=1.58+0.45 MI)
Conclusion The MI and AgNOR have direct relationship with bladder TCC's grade. As statistical results didn't show difference between MI and AgNOR to predict the grade, we prefer to use MI for this purpose.

P-456

HER2 in invasive urothelial carcinoma of the bladder

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Introduction The human epidermal growth factor receptor 2 (HER2) is overexpressed not only in breast, but in a range of other tumor types including, ovarian, salivary gland, endometrial, pancreatic, non-small cell lung (NSCLC) and bladder cancer. However, non-standardisation in HER2 status evaluation or population selection has generated conflicting results among the incidences of HER2 expression in individual tumor types and bladder cancer. The aim of the present study was to evaluate HER2 overexpression and gene amplification with standardized routinely performed assays (DAKO HercepTest, FISH-Vysis, CISH) on a selected population of poorly differentiated urothelial carcinomas of the bladder.
Materials and methods We analyzed 55 formalin-fixed, paraffin embedded tissue samples of urothelial carcinomas of the bladder at G3/pT3-4 as well as 22 cases at G1/2 pTis (2)and pTa-1 (20) using HercepTest to verify the HER2-status. Staining and scoring was performed according to the manufacturers instructions. According to diagnosis of HER2, all HercepTest positive (2+) tissue samples were used for further evaluation with fluorescence in situ hybridisation (FISH; Vysis) and chromogenic in situ hybridisation (CISH) to approve the expected gene amplification. This procedure is in agreement with the standardized guidelines for breast cancer.
Results 30 (60%) cases were considered as HER2 positive (3+ and 2+) in the primary tumor and therefrom 16 cases (32%) 2+ positive and 14 cases (28%) 3 + positive. Out of the 16 cases with 2+ positivity, only two cases showed a FISH/CISH positivity. No polysomy for chromosome 17 was detected.
Conclusion Our results indicate that HER2 may not only be a useful prognostic parameter in poorly differentiated invasive

bladder cancer, but accurate determination of overexpression and verification of gene amplification may also have therapeutic importance.

P-457

The prognostic importance of tumor volume (TV), non-tumor volume (NTV), TV/NTV, fibronectin distribution, β-hCG and PSA expression in localized prostatic adenocarcinoma

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Aim To assess the importance of fibronectin (FN) distribution, β-hCG and PSA expression in localized prostatic adenocarcinomas with regard to tumor volume (TV), non-tumoral volume (NTV), TV/NTV and with other clinicopathological prognostic parameters.
Material and method Fifty-one radical prostatectomies were whole mounted, serially sectioned and examined for TV, NTV, TV/NTV, capsular invasion, vascular invasion, seminal vesicle invasion and Gleason score. The FN distribution, β-hCG and PSA expression were examined immunohistochemically. A FN-positive staining was defined as a constant diffuse, or pericellular demarcation of FN-positive fibers surrounding tumor cells at the invasive border. In lack of such staining pattern, FN-negative staining was recorded.
Results Both TV and TV/NTV showed very strong positive correlation with grade, capsular invasion, vascular invasion, seminal vesicle invasion, β-hCG and PSA expression ($p<0.01$). β-hCG was detected only in 16 cases (31.4%). A significant relationship was found between β-hCG expression and grade, capsular invasion, vascular invasion, seminal vesicle invasion, PSA expression ($p<0.01$). The FN-positive staining reaction was significantly associated with lower incidence of capsular, and seminal vesicle invasion ($p<0.05$). TV and grade was found higher in tumors with negative FN staining ($p<0.05$). PSA expression showed significant relationship with grade, vascular and capsular invasion ($p<0.01$). NTV statistically showed no relationship with all parameters.
Conclusion These data suggest that, TV, TV/NTV, FN distribution, β-hCG and PSA expression in prostatic adenocarcinoma have strong prognostic importance irrespective of Gleason score. The FN distribution could be important for the invasion of the tumor cells during cancer progression. These may allow stratification of patients to type of treatment and may allow selection of expectant management for men with localized prostatic adenocarcinoma.

P-458

Neuroendocrine differentiation in prostate cancer: correlation with biochemical failure after radical prostatectomy

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Introduction Focal neuroendocrine differenatiation (NED) is a commom feature of prostate cancer (PC), occuring in 50%-100% tumors studied. Published results of the evaluation of NED as a prognostic factor in PC after radical prostatectomy (RP) have been contradictory. The aim of this study was to correlate NED in PC in relation to biochemical failure after RP.
Materials and methods Radical prostatectomy specimens from 36 PC patients without preoperative hormonal or radiation therapy were followed for 13 to 73 months. Neuroendocrine (NE) cells were identified using a panel of immunohistochemical NE markers: chromogranin A, serotonin, and neuron-specific enolase. NED was scored as NE-negative (0 to 1+) or NE-positive (2 to 3+) per 10HPF. Biochemical progression (BP) was defined as a prostate-specific antigen (PSA) level greater than 0.2 ng/ml in two consecutive measurements. Kaplan-Meier method was used to estimate BP-free survival time for each risk group. The Cox proportional hazards regression analysis was the model for BP time using pathological and clinical variables.
Results The total of 23(64%) patients developed BP after RP. The average time for BP was 16 months. The preoperative PSA serum level range <10 ng/ml in 52% of the patients. Postoperative staging confirmed that 23 (64%) of patients had pT2 stage and 13 (36%) of patients had pT3 stage. NED demonstrated in the 14(61%) PC patient with BP. The average time for BP was 10 months. Tumor volume ($p<0.05$), pathological stage ($p <0.05$), tertiary Gleason grade ($p= 0.01$) and NEDF ($p<0.01$) significantly affect the level of postoperative PSA progression.
Conclusion The expression of NED in PC correlates with tumor volume and tertiary Gleason grade and represents an independent prognostic factor of BP after RP.

P-459

Expression of Vascular Endothelial Growth Factor (VEGF) and correlation with microvessel density in benign hyperplasia and prostate carcinoma

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Aim The present study is scheduled to evaluate the levels of VEGF expression in benign prostatic hyperplasia (BPH) and prostate carcinoma and to correlate them with angiogenesis.
Material and method Formalin-fixed, paraffin-embedded tissue sections from patients who underwent radical prostatectomy or transurethral resection of the prostate were studied. Our material consisted of 32 cases of BPH and 30 cases of prostate carcinoma. Microvessel density (MVD), as determined by CD31, and expression of VEGF were investigated by immunohistochemistry using the monoclonal antibodies CD31 (DAKO) and VEGF (Neomarkers), respectively.
Results Expression of VEGF was detected in 26/32 (81,25%) cases of BPH and in all (100%) invasive prostate carcinomas. Cytoplasmic immunoreactivity for VEGF was detected in epithelial and stromal cells. VEGF expression was stronger in carcinomas, when compared to BPH ($p<0.05$), while it was minimal to absent in normal adjacent prostate tissue. A positive correlation between VEGF expression and Gleason score was found ($p<0.05$). Microvessel density (MVD) was higher in BPH and prostate carcinomas, when compared to normal adjacent prostate tissue. A statistically significant association was noted between VEGF and MVD expression ($p<0.001$).

Conclusions Our results indicate that generation of new capillary blood vessels begins in BPH and keeps progressing in invasive prostate carcinoma. VEGF seems to be a key functional regulator of angiogenesis in the prostate gland.

P-460

Paratesticular solitary fibrous tumor

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Aim To describe an additional case of paratesticular solitary fibrous tumor.
Methods and results A 46-year- old man presented with a paratesticular mass. Histological examination showed a well-circumscribed lesion composed of spindle cells without atypia, arranged in a fascicular pattern, intimately intertwining with thick collagen fibers. Tumor cells were strongly positive for vimentin and CD-34. Diagnostic criteria, clinical features and treatment of this condition are discussed.
Conclusion Solitary fibrous tumors are spindle cell neoplasms originally described in the pleura, but may occur in many different sites. Intrascrotal solitary fibrous tumors are uncommon and few cases have been reported.

P-461

The relationship between histologic grade and ABO blood groups in transitional cell carcinoma of the urinary bladder

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Introduction According to the statistics of 1996, the urinary bladder cancer is the fifth common cancer in Iran. The majority of urinary bladder tumors are transitional cell carcinomas. Different factors are effective in the prognosis of these patients. One of these factors is the histologic grade. Another factor is to determine the ABO blood groups and the relationship of it with the histologic grade. Thus, this study is conducted to shed some lights on this issue.
Materials and methods A cross sectional study was conducted in 198 patients with urinary bladder transitional cell carcinoma during 1996 - 2000.
Results Of 198 patients, 43 were female (21.7%) and 155 were male(78.3%). The mean age of patients was 63.7+/-12. There was no significant statistical relationship between of histologic grade and ABO blood groups. There was no significant differences between the ABO blood groups frequency in patients with urinary bladder transitional cell carcinoma and general population.
Conclusion Although the existing differences between Asian, European and American studies might be due to racial effects, it is better to carry out more studies in this respect in order to comment in this area with more confidence and certainty.

P-462

Prostatic mucinous carcinoma with signet ring cells treated by radical prostatectomy

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Introduction A variable degree of mucin secretion may be present in prostatic carcinoma (PC), but true mucinous tumors are rare. Prostatic mucinous carcinoma (PMC) is a variant of high-grade adenocarcinoma, with a 77.8% rate of prostate-specific antigen (PSA) elevation and a similar rate of response to endocrine therapy. The aim of this study was to report on a case of PMC with signet ring cells treated with radical prostatectomy (RP).
Materials and methods A 70-year-old man presented with acute urinary retention and long history of difficult voiding. The serum PSA was 8.9 ng/ml. The transperineal biopsy of the prostate gland was done and the histology showed a poorly differentiated PC with large lakes of extracellular mucins. Isotope bone scan was normal and a CT-scan did not show evidence of extracapsular spread of the tumour. Radical prostatectomy was performed. Patient died seven month after RP.
Results Histopathologically, over 70% of the prostate samples were infiltrated by a poorly differentiated PC with mucinous and signet ring patterns. Mucin secretion is evident as large pools of mucin and periodic-acid-Schiff-positive diastase-resistant intraluminal and citoplasmic globules. Immunohistochemical staining for PSA and prostate-specific-acid phosphatase was positive, and negative for carcinoembryonic antigen and cytokeratin 20.
Conclusion The diagnosis of PMC with signet ring cells requires exclusion of an extra-prostatic origin, mainly within digestive tract. Since only small number of MPC cases treated with RP have been reported, treatment and prognosis of this tumour remain unclear.

P-463

Expression of CD10 antigen in Testicular Germ Cell Tumors (TGCTs)

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Introduction CD10 is a well-known marker for hematolymphoid neoplasms and is expressed as well in various nonhematopoietic tissues and solid tumors. A systematic study of CD10 expression in TGCTs, however, is lacking. The aim of this study was to investigate the immunoreactivity for CD10 in the different patterns of TGCTs.
Material and methods Immunostaining with Mab against CD10 (clone 56C6) in 59 unselected cases of primary (46 cases: 18 classical seminoma (CS); 9 pure embryonal carcinoma (EC); 18 mixed-non-seminomas; 1 spermatocytic seminoma (SS) and metastatic (13 cases) TGCTs. In mixed tumors, the different histological patterns were evaluated separately.
Results CD10 was expressed in 18 (78%) out of 23 cases in the CS component, in 15 (100%) of 15 teratomas, in 13 (93%) of 14

endodermal sinus tumors (EST), in 11 (100%) of 11 cases of choriocarcinoma (ChC) or syncytiotrophoblastic giant cells (STGC) and in 1 (4%) of 23 EC. CD10 was focally positive in 28 (80%) of 35 cases of intratubular germ cell neoplasia (ITGCN) and was negative in one SS.
Conclusions Except for EC and SS, CD10 is expressed in the majority of histological types of primary and metastatic TGCTs. CD10 is useful to highlight minute foci of EST, ChC or STGC in EC. A TGCT is to be taken into account in the differential diagnosis of a CD10 positive metastatic tumor of unknown origin. Further studies are needed to evaluate the eventual significance of CD10 as a prognostic factor or as a potential therapeutic target in TGCTs.

P-464

Morphological changes of stromal components of the aging testis from patients with prostatic carcinoma

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Introduction The authors assesed the correlations between age and morphologic aspects of stromal components in testes from 28 cases with orchiectomy for prostate adenocarcinoma.
Material and methods The samples were fixed in Bouin's fluid, embedded in paraffin wax and stained with hematoxilin-eosin, Goldner and Gömöri stains. Immunohistochemical staining with antibodies for smooth muscle Actin, Vimentin, Collagen IV and CD34 Classs I were used.
Results The seminiferous tubules basement mebrane (BM) narrowed and became discontinuous with age and with the severity of tubular sclerosis. Lamina propria (LP) generally became more thick with a decrease of myoid cells number in the inner layers and an increase of the collagen fibers right under the tubular BM and of the fybroblasts number in the outmost layers. The amount of peritubular capillaries remained relatively constant but the number of intralaminar capillaries decreased with the severity of LP sclerosis. The amount of Leydig cells increased with age especially in areas with tubular sclerosis.
Conclusions The stromal components presented morphological changes related with age with a 'mosaic', focal distribution. The evolution toward sclerosis in the LP seems to be influenced rather by Leydig cells hyperplasia than by vascular changes.

P-465

The expression of CD44 molecule in renal cell carcinoma

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Introduction It is generally accepted that the prognosis of renal cell carcinoma (RCC) differs according to the histological type, and, whatever the histological type, the tumor stage and nuclear grade are considered the main prognostic factors. However, in many cases of conventional renal cell carcinomas (CRCC), these parameters are insufficient to predict the clinical behaviour of these tumors. Therefore, several studies have focused on the evaluation of new markers. The CD44 proteins form a broadly distributed family of cell surface adhesion molecules involved in cell-cell and cell-matrix interactions. De novo expression of CD44s and its variant isoforms has been associated with aggressive behaviour in various tumours. The aim of this study was to analyse the prognostic value of CD44s and CD44v6 in renal cell carcinoma.
Materials and methods The expression of CD44s and CD44v6 was immunohistochemically evaluated on 75 RCC: 62 were CRCC, 9 papillary, 3 cromophobe and 1 collecting duct carcinoma. The expression was compared to usual clinicopathological parameters.
Results In normal renal tissue no staining was observed. The CD44s molecule was upregulated in 18 CRCC (29%), 4 papillary, one cromophobe and collecting duct carcinoma. Only 5 tumors were focally CD44v6 positive. The statistical analysis showed the correlation between CD44s expression and Fuhrman nuclear grade ($p<0,0025$) as well as Ki67 proliferative index ($p<0,0003$).
Conclusion The expression of CD44s molecule appears to mediate a more agressive phenotype to RCC.

P-466

Amplification of CerbB2 expression in bone metastasis of prostatic cancer: A pilot immunohistochemical and cytogenetic study

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Introduction Her-2-neu expression is reported to increase in androgen-independent prostatic cancer. Consequently, it has been suggested that Trastuzumab, an antibody targeting the extracellular domain of Her-2, could be used in this setting. The possibility of assessing Her-2 expression by immunohistochemistry could be used as a rationale for the treatment of advanced cases with this drug. The aim of this study was to assess whether Her-2 expression is increased in bone metastases of prostatic cancer compared to the respective primitive tumors, and to determine whether this overexpression could be related to gene amplification.
Materials and methods In this pilot study, primary tumor needle biopsies and bone metastases from 7 patients diagnosed with prostatic cancer were immunostained wit C-erb-B2 antibody (Dako, Glostrup, Denmark), and the results scored using a three-tiered system. In addition, in a bone marrow sample from one of the cases, HER2 gene amplification was investigated using FISH.
Results There was a significant increase in Her-2 expression in four cases, with the same level of expression in primary and metastatic tumors in the remaining three patients. In addition, FISH showed no amplification of HER2 gene.
Conclusion HER-2 overexpression is not a constant event in prostate cancer metastatic to bone. Preliminary evidence suggests that in cases with overexpression, it is not related to gene amplification. A larger study will be required, however, to definitively determine the real therapeutic meaning of these findings.

P-467

Sarcoid-like granulomas (SGs) in renal cell carcinoma (RCC)

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Introduction SGs are known to occur in association with a variety of hematologic and solid neoplasms. Concerning RCC-associated SGs, only isolated case reports have been published. The aim of this study was to disclose the prevalence and clinical significance of SGs in a series of RCCs from a single institution.
Material and methods Clinicopathologic review of 210 consecutive cases of RCC surgically treated at the HUB between January-1999 and March-2003. Immunohistochemical staining with CD68, panB and panT antibodies.
Results SGs were seen in 7 cases, both within the tumor and/or in the nontumor tissue nearby. Patients were 4 females and 3 males aged 55 to 76(median 68) years. Tumors were 5 conventional clear cell RCCs and 2 chromophobe RCCs. There were 5 pT1, one pT2 and one pT3b. Two tumors were Fuhrman grade 3 and 5 were grade 2. In each case, the lymphocytes in the SGs were predominantly T cells. One patient was already known to have a CREST syndrome. In another one sarcoidosis was subsequently diagnosed. In the remaining 5 no systemic disease has been found to date.
Conclusions SGs are found in about 3.5 % of RCCs. Morphology and immunohistochemistry allow no distinction between local sarcoid-like reactions and sarcoidosis. The clinical profile of the patients and the pathologic features of the tumors are not different from those of RCCs unassociated with SGs. The finding of tumor-associated SGs must be reported to the clinicians in order to rule out a systemic granulomatous disease, thus avoiding misinterpretation of clinical symptoms or overstaging of tumors.

P-468

Metanephric adenoma in the kidney coexistent with paraganglioma in the bladder: A case report

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We report a case of metanephric adenoma in the kidney and paraganglioma in the bladder in a 32 year-old woman. The patient presented with hematuria. Abdominal USG and BT revealed a mass in the lower pole of the kidney and in the posterior wall of the bladder. Left nephrectomy and transurethral resection of the mass in the bladder were performed. The tumor in the kidney which measured 4x3x2 cm. had a yellow to white cut-surface and well-defined margin. Histological examination demonstrated a monomorphous population of small cells with bland cytology arranged generally in tubular and occasionally in papillary structures. No atypia and mitotic activity were present. Immuno-histochemically tumor was diffusely positive for EMA, focally positive for pancytokeratin and negative for vimentin. The tumor in the bladder was characterized by well-defined nests of cuboidal cells with granular cytoplasm which were seperated by highly vascularized fibrous septa. Sustentacular cells were demonstrated with anti-S100 protein immunohistochemically. We didn't encounter the coexistence of these two tumors in the literature. We think that it is a coinsidence.

P-469

Co-occurrence of extra-adrenal myelolipoma, renal cell carcinoma and thymoma – a case report

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A case of never reported in literature of co-occurrence of a very rare tumour – extra-adrenal myelolipoma (EAML) with two other neoplasms, was presented in the report. 72-year man after a short hospital stay due to heart ischaemic disease died with the symptoms of acute circulatory insufficiency. An autopsy, which confirmed clinical diagnosis, was performed. A tumour of the size 15x11x8 cm, which compressed the heart and aorta, was found during the mediastinum autopsy. The tumour was covered with a sack, lobular, gray and beige at the cross-section with blood extravasation, necrosis foci and calcifications (histopathologic result – medullary thymoma). The compressed heart was moved to the middle, right side of the chest. In the adipose capsule of the left kidney, near the lower pole, an encapsulated solid fatty tumour of the size 7x5x5 cm was found. The tumour was connected to the kidney. Histopathologic result – myelolipoma. The tumour was composed of fat cells with normal marrow hematopoietic elements including megakaryocytes. The third tumour, 2cm of diameter, was found on the front surface of the right kidney (histopathologic result – multilocular cystic clear cell, renal cell carcinoma). None of the tumours was diagnosed during the patient's life.

P-470

Signet ring cell carcinoma of the prostate: pathologic and immunohistochemical analysis of two cases

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Introduction Signet ring cell carcinoma of the prostate is extremely rare and was first described by Giltman in 1981. To date, there have been 39 cases reported in the literature.
Materials and methods We evaluated two cases formaline fixed paraffine embedded tissue by histochemical (mucicarmine, alcian blue) and immunohistochemical methods (PSA, PSAP) to confirm our histological diagnosis.
Results Both our cases were high grade adenocarcinomas Gleason score 9 (4+5) and were positive for PSA and PSAP. One case contain a small amount of acid mucin, but both were negative for neutral mucin. The combined results from our and from previously reported cases: PSA positive in 88.8% (32/36), mucicarmine stain positive in 41.9% (13/31) and alcian blue stain positive in 50.0% (18/36) of cases.
Conclusion Signet ring cell carcinoma is variant of high grade adenocarcinoma of the prostate but results of histochemical and immunohistochemical staining are variable.

P-471

Value of ISUP (1998) and WHO (1999) criteria in the prediction of prognosis in urothelial tumors

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Introduction The 1998 ISUP classification of urothelial tumors was followed by a modified WHO classification in 1999. The purpose of this study has been to assess the value of both systems in the prediction of progression, in a large series of cases with detailed follow-up.
Material and methods 500 "superficial" (pTa + pT1) urinary bladder tumors are the subject of this work. All tumors were reassessed strictly adhering to published criteria. Progression (increased grade or stage) was used as the end-point.
Results With a relative risk (RR) of 1 for G1, progression RR for G2 was 2.98 (1.05-8.46) and for G3 10.09 (3.82-26.65) ($p<0.001$). With a risk of 1 for pTa, RR for pT1 was 5.64 (3.01-10.56) and for pT2 4.80 (1.13-20.38) ($p<0.001$). When combining grade and stage, RR for pTa (G2+3) was 2.60 (1.06-6.39) and for pT1 (G2+3) was 10.03 (4.16-24.20) ($p<0.001$).
Conclusions Strictly adhering to the 1999 WHO grading and staging criteria, both systems result in a very efficient prediction of progression of urothelial carcinoma. Grouping G2 and G3 into a single high grade category does not improve these results. "Superficial" infiltration (pT1) introduces a relevant risk of progression when restrictively identified, thus suggesting that pT1 should not be lumped with pTa for therapeutic purposes.

P-472

Neuroendocrine expression in prostate cancer

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Introduction Presence of neuroendocrine (NE) cells that are suggested to be important regulators of cell growth and differentiation represents uncommon finding in benign and neoplastic prostate epithelium. In order to correlate cellular expression with histological differentiation in benign epithelium and primary prostate cancer, expression of chromogranin A (CgA) had been determined.
Materials and methods HE and immunohistochemical analysis with antibody against CgA was performed in order to determine the coexistence of NE differentiation in tissues of twelve patients with prostatic adenocarcinoma (PC).
Results NE differentiation was found in 8 (66,6 %) tumors. Among them, 5 (41,66%) had CgA positive cells scattered or focally distributed, 3 (25%) intermediated and intensive. There was a significant correlation between the extent of NE features and Gleason score ($p<0,05$).
Conclusion These data support the concept that NE differentiation in human prostate cancer has a positive correlation with histological differentiation, i.e. patients with Gleason score > 7 had higher cellular expression of CgA.

P-473

The p130 retinoblatoma gene protein and p53 expression in transitional cell tumors

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Background The retinoblastoma family of proteins (p105, p107 and p130) are implicated in cell cycle regulation, differentiation and apoptosis. p105 (retinoblastoma supressor gene) alterations seem to be correlated with worse prognosis in urothelial neoplasms, and the status of the other two proteins has been less studied. p53 mutations are usually associated with intranuclear accumulation of p53 protein, and have also been correlated with worse prognosis in urothelial neoplasms.
Design Formalin-fixed, paraffin-embedded archival tissue from 18 low-grade and 17 high-grade transitional tumors was immunostained with polyclonal and monoclonal antibodies against the Retinoblastoma Family of Proteins, and with monoclonal antibody against p53 protein. The avidin-biotin-peroxidase (ABC) method was utilized with every antibody (Dako, clone Rb1 for p105; Santa Cruz, clone sc-318 for p107; Santa Cruz, clone sc-317 for p130 and Dako, clone DO-7 for p53). Immunostaining was scored quantitatively by 3 pathologists, following previously described criteria, to study p105, p107 and p53. Nuclear and/or cytoplasmic location was determined by 3 pathologists to study p130.
Results p130 expression: among 18 low-grade tumors, 12 (66.67%) showed nuclear positivity and 4 (22.22%) showed a granular cytoplasmic pattern of staining. Among 17 high-grade tumors, 1 (5.88%) showed nuclear expression and 13 (76.47%) showed a granular pattern of staining. p53 expression: among 18 low-grade tumors, 1 (5.55%) showed positivity and among 17 high-grade tumors, 14 (82.35%) stained positively. We found no stadistically significative differences regarding p107 and p105 immunohistochemical stainings.
Conclusion Due to granular cytoplasmic pattern of staining for p130 is more frequent among high-grade transitional neoplasms, we propose that p130 granular cytoplasmic immunohistochemical pattern of staining may be considered a marker of tumor progression in transitional cell tumors. The prognostic value of p130 cytoplasmic positivity among low grade cases, as well as the prognosis of tumors with p53 overexpression and p130 granular cytoplasmic pattern will be discussed.

P-474

Are there any relationship between Bellini duct carcinoma and urothelial cell carcinoma?

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Introduction Two collecting duct carcinomas (CDC) and 1 renal papilla urothelial carcinoma (UC) with similar outcome. Aims. To study the common features of CDC and UC.

Methods CDC's underwent nephroureterectomy under suspicion of urinary tract neoplasm. UC had radical nephrectomy for uncontrolled acute pyelonephritis.
Results All were male. Mean age was 67 years.

CASES	SIZE&SITE	PT	CK903	Ulex Eur(UE)	AE1/AE 3	CK2 0	Vim
CDC-1	All Kidney	4	-	+++	+++	-	-
CDC-2	5 cm Medullary	3	+++	+++	+++	-	-
UC-3	1cm renal papilae	3	-	+/-	+	-	-

All had atypical cells in nearby collecting ducts. UC had carcinoma in situ in adjacent urothelium. Median survival was 5.6 months.
Discussion Aggressiveness of our 3 lesions and ambiguous immunohistochemistry results on case 3, made us consider that CDC and UC of the renal papilla may share biological features. Normal collecting duct and the urinary tract have the same mesonephric origin. LOH 1q characteristic of all distal nephron tumors, have been described in UC. Immunophenotype of CDC includes positivity to CK903 which is also positive in UC. This overlappings shows us that these tumors are difficult to categorize and we may be in front of a spectrum of lesions. This may explain that some CDC may behave like UC and share some aspects, incuding their response to therapy.
Conclusions CDC are aggressive tumors, and general pathologists should be able to recognize them. Immunohistochemistry is needed for diagnosis. Possible relationship between UC and CDC may have therapeutical implications.

P-475

Serous papillary cystic tumor of borderline malignancy arising in testis - Case report

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Primary adenocarcinoma is one of the rarest types of malignant testicular tumors. There is a small number of reported patients of testicular tumors of the serous or mucinous ovarian type. A 63 years old white man presented with painless scrotal swelling. He was given antibiotic therapy, no regression appeared. Left high inguinal orchiectomy was performed. On histopathological examination the diagnosis was serous papillary cystic tumor of borderline malignancy arising in testis. The prognosis is poor. In these patients who are not diagnosed and treated early. The histopathological features, treatment and prognosis of this unusual malignancy are reviewed.

P-476

Morphological specialties of prostate at tuberculosis of lungs

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The aim of researching is appear to examine of ofteness of come across of specific prostatitis and it's morphological form in autopsy material at patients with tuberculosis of lungs. Morphological researches of prostate including layer cut macro and micro preparations section was made and was paint by hemotoxilin-eosin and by Van-Hison. In Since Research Institute of Pulmanology and Phthisiology in Tashkent city was died 25 man at the age 20-60 age from 2001 till 2002y. The average age was 36 years. From that patients on the autopsi material at 14 (56%) was discovered tuberculosis of prostate, at 11(44%) nonspecific defeat of prostate. At 14 died patients with tuberculosis of prostate was found different types of tuberculosis inflammation: (1) aexudative - at 7, productive - at 2, and mixed - at 5., from them (2) infiltrative - at 8 and destructive - at 6 patients, aexudative type of inflammation was also diagnosed at 11 patients with nonspecific defeat of prostata. Patomorfological data's not always can help in diagnostic of tuberculosis of prostate it ties with patomorfose of tuberculosis under influence of treatment.

P-477

Cancerogenesis in prostate: ploidometric differential diagnostics

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Background The problem of unification in diagnostics of various prostate tumours and tumour-like lesions has gained a special significance in the modern oncology. Aim: Use the ploidometry research for studying the epithelial cell clones in the prostate, forming the different stages of cancerogenesis.
Methods Computer ploidometry test was carried out for nuclei of epithelial cells in biopsy specimen of the prostate, taken from 80 patients in different stages of cancerogenesis. Histological preparations of 8 micron thickness, stained by Feulgen's technique, were subject of the research. Nuclei's ploidy was defined by means of the computer image analyzer "Imager-CH" ("Avtan-San" software version). The integral brightness of nuclei of 396 small lymphocytes, found in histological sections, was tested to define the "tissue ploidy standard" (2c), which, in its turn, was used for obtaining the data on ploidy of 1332 epithelial cell nuclei of the prostate gland. Inter-phase cell nuclei of clones with normal and hyperplastic prostate gland, areas of prostate intraepithelial neoplasia (PIN-I– low grade – and PIN-II– high grade) and adenocarcinoma in different stages of dedifferentiation (well, moderate, poorly) were the subjects of investigation. The research determined that while the cancerogenesis progressed, the proliferative activity (indirect type information) of prostate gland cells and the ploidy of nuclei naturally increased.
Results In comparison with the average ploidy value of the normal epithelial cells nuclei (2.8 c), the average ploidy index increases 1.07 times in hyperplasia, 1.32 times in PIN-I, 1.53 times in PIN-II, 1.92, 1.96 and 2.4 times correspondingly of well, – moderate, – poorly adenocarcinoma. Taking into account the requirements of the oncological practice, it is advisable to single out only the four main stages of cancerogenesis. These stages differ substantially in the characteristics of the average ploidy of nuclei and the type of their cell clones histograms: namely normal prostate tissue and its hyperplasia; benign stages – slight intra-epithelial neoplasia, low grade (PIN –I); marginal stage – non-invasive intra–epithelial neoplasia, high grade (PIN–2); malignant stages – infiltrating adenocarcinoma (with and without metastasis).
Conclusions Computer ploidometry, as the new line of patohistological investigations may be used in the differential diagnostics

of prostate tumor and tumor-like lesions. Use of ploidometry data, obtained by means of the computer analyzer opens up the new opportunities in specifying the differential morphological diagnostics of tumors with various localization (basis of quantipathology).

P-478

Glucagonoma in Mexico. Clinical study, histopathology, immunohistochemistry and electron microscopy of six cases

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Introduction Glucagonoma is an alpha-cell pancreatic neoplasm with incidence of one in 20 million; the malignancy rate is unknown. The clinical, pathologic, immunohistochemistry and electron microscopy characteristics are described in a Mexican cohort of patients.
Material and methods We reviewed the pathology archives of INCMyNSZ and HOCMNSXXI for pancreatic tumors with neuroendrocrine morphology and expression of immunohistochemistry markers for neuroendocrine and glucagon-related peptides. Complete clinical history and follow-up were achieved.
Results Six cases of glucagonoma were obtained. Electron microscopy was available in two cases. Three man and three women with median age of 61.16 years, were admitted with glucagonoma syndrome complaints. The tumor localization was: head of pancreas (3/6) and tail (2/6), with average size of 6.4 cm. Two cases showed hepatic metastasis. The histopathologic study was constant with contrasting architectural, cytologic and mitotic count in all the cases. Expression of neuroendocrine markers was observed in all 6 cases, with positive glucagon stain, and negative for secretion markers (insulin, PP, serotonin, calcitonin). In electron microscopy, dense core granules were identified in two cases.
Discussion There are 200 cases of glucagonoma described in English literature; no one is originated from Latin American population. In this series, the neoplasms have equal prevalence for female and male patients, all from elderly group. The morphologic feature extremely varies in each tumor, the malignancy criteria were correlated in two cases with vascular invasion and this coincides with metastasis. A peculiar finding are the only expression of glucagon in tumoral cells, and two malignant cases with liver metastasis.

P-479

Primary neurilemoma of the thyroid gland: A case report

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Introduction Primary nonepithelial tumors of the thyroid gland are rare.
Case report We present the case of a neurilemoma of the right lobe of the thyroid gland in a 16-year-old female patient. The tumor was asymptomatic and measured 6.5x2.5 cm in size. Histologic examination was consistent with an Antoni A-type neurilemoma. The tumor cells were strongly positive for S-100 protein. A loboisthmectomy was done.
Conclusion Only 12 other cases of neurilemomas of the thyroid gland have been reported in the literature. The tumor can be sporadic or associated with type 1 neurofibromatosis. The diagnosis can be made cytologically and a large exision can be avoided. The diagnosis is based on pathology that rules out malignancy.

P-480

Withdrawn

P-481

The synergetic role of granulosa cells in Ham's F10 and follicular fluid in primary stages of human embryos growth and development

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In early stage of embryonic growth and development the exchange of metabolite in uterus is carried via granulosa and endometrial cells. Numerous investigations have been carried to prepare a normal medium for cultivation of embryonic cells. The cultured embryos with supportive and protective cells, such as granulosa cells are usually called co-culture, show many beneficial effects such as better cleavage and nutritional status. These techniques have obviated the existing developmental barrier in this respect. Some authors have suggested that the use of natural media which contain follicular fluid have shows a better and faster clavage of zygot and nutrition status. Therefore to obtain a better culture media we have investigated 5 media preparation: 1) follicular fluid, 2) Follicular fluid and granulosa cells, 3) Ham's F10 with 10% human albumine, 4) Ham's F10 with 10% human albumine and granulosa cells, and 5) synthetic serum substitute (3S). The secondary metaphasic oocytes of 27 patient were evaluated and fertilized by intracytoplasmic sperm injection technique (ICSI) (n= 117). The injected oocytes have been transfered to one of the above media. The oocytes developmental process were evaluated approximately 21 and 43 hours after ICSI steps for two pronuclei formation and cell cleavage episodes respectively. The results showed that harvested fertilized oocytes were more abundant in 3S and follicular fluid media than in the other. After 43 hours of ICSI 18% of embryos were in 2-cell stages (n=21), 41.9% were in 4-cell stages (n=49), 11.1% were in 8 – cell stages (n=13) and 29.1% of them (n= 34) did not undergo cell devision at all. The maximum and minimum of uncleaved oocytes have been observed in Ham's F10 and 3S media respectively. The Co-culture media revealed the highest 8 – Cell stage embryos. The number of embryos have been significantly higher in 3S media than in Ham's F10 (P=0.0088). The best quality of embryos was only observed in Hsm's F10 alone and together with granulosa cells co-cultured media. The above results suggest that co-culture of embryos with granulosa cells made higher cleavage of embryo with the best grade of embryo quality and growth in Ham's F10 media decreased cleavage meanwhile increased cell fragmentation of embryos.

P-482

Effect of calcitriol and of its analogues on expression of calcitonin gene in cultured cells of medullary thyroid carcinoma

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Introduction Calcitriol and its analogues affect function of several normal and neoplastic cells, including cells of medullary thyroid carcinoma originating from parafollicular cells. In the cells expression of calcitonin gene takes place, yielding due to alternative splicing calcitonin (CT) and calcitonin gene related peptide (CGRP). The aim of this study was examination of effects of calcitriol and of its two analogues, PRI-1906 and PRI-2191 on proliferation of two cell lines in vitro and on expression of calcitonin gene.
Material and methods The studies were performed on MTC 6-23 (rat cell line) and TT (human cell line) cells originating from medullary thyroid carcinomas. The cells were cultured with calcitriol or its analogues at 10(-9) to 10(-6)M for 5 days. The estimated variables included: cell number, proliferation-associated antigens (Ki-67, PCNA), CT and CGRP levels in medium (RIA), amounts of the two hormones and of their mRNAs in the cells (immunocytochemistry or hydridocytochemistry accompanied by image analysis).
Results Calcitriol and its analogues exerted low antiproliferative effect, more evident in MTC than in TT cells. PRI-2191 affected production and secretion of the two hormones. Evident correlation was noted in TT cells, positive for CT and negative one for CGRP. In MTC cells, the effect was slightly less intense. PRI-1906 exerted a similar effect in the two cell lines but only at higher concentrations of the analogue. On the other hand, calcitriol manifested its activity only in MTC cells. Effects of studied substances were also examined on CGRP/CT ratio of secreted hormones, which reflected corresponding ratio of alternative splicing of CT gene transcript. In TT cells the ratio decreased in presence of either analogue and in MTC cells the effect was observed following culture with calcitriol and the two analogues.
Conclusions Calcitriol and its analogues exert a weak antiproliferative effect and an effect on alternative splicing of calcitonin gene.

P-483

Allelotyping analysis of GEP endocrine tumors

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Background The molecular pathogenesis of GEP endocrine tumors is still largely unknown and no specific prognostic molecular markers have been yet identified. The purpose of this work was to identify specific chromosome regions or putative tumor suppressor loci that may be involved in the malignant evolution of these tumors.
Materials and methods 24 well differentiated endocrine tumors (WDETs) (11 pancreatic, 3 gastric, 10 intestinal) and 14 poorly differentiated endocrine carcinomas (PDECs) (1 pancreatic, 6 gastric, 7 colorectal) were allelotyped with an automated DNA sequencer (Applied Biosystems 310) using 38 polymorphic microsatellite markers covering chromosomes 1, 3, 5q, 6, 11, 17 and 18.
Results Regardless of the primary site, a significantly higher percentage of allelic imbalances (AI) was observed in PDECs than in WDETs ($p= 0.012$) with the only exception for 3 out of 8 nonfunctioning pancreatic tumors exhibiting multiple AI on chromosomes 11, 6q and 3p. A strong positive correlation between AI percentage and the Ki-67 proliferation index was detected considering both all tumors and WDETs alone ($p=0.002$ and $p=0.001$, respectively). Moreover, AI at 17p was the most frequent alteration observed in PDECs (92% of PDECs versus 4.8% of WDETs; $p<0.01$) together with 6q and 3p AI in gastric and 5q AI in colorectal PDECs, respectively.
Conclusion These findings suggest the existence of two different molecular profiles associated with PDECs and WDETs. Since no specific molecular markers of malignancy can be defined with certainty, the ploidy status and the evaluation of the global level of chromosomal instability of the tumors appear to be the most informative genetic factors with prognostic significance.

P-484

Mixed medullary-papillary thyroid carcinoma

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Mixed medullary-papillary carcinomas are rare neoplasms and must be distinguished from medullary carcinoma with entrapped follicles, papillary variant of medullary carcinoma and solid growth pattern of sclerosing variant of papillary carcinoma. These mixed tumors could arise from uncommited stem cells; it is also possible that these neoplasms could be collision tumors. The aim of our study was to show that these mixed tumors have some particular features. We studied 250 cases of thyroid carcinoma and for diagnosis we used immunohistochemical analyses for thyroglobulin, calcitonin, CEA, chromogranin and cytokeratin 19. We found 3 cases of mixed medullary-papillary carcinoma. These mixed tumors are not so frequent but the admixture of papillary and medullary components confer a different prognosis and a potential response to radioactive iodine treatment.

P-485

Genomic imbalances of sporadic and hereditary thyroid carcinomas

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Introduction Medullary thyroid carcinomas (MTC) are rare tumours, which can occur in a sporadic and a hereditary setting as well.
Material and methods Comparative genomic hybridization (CGH) was used to analyse 53 medullary thyroid carcinomas from 44 patients. 25 of the 53 MTC were characterized as hereditary.

Results Only four hereditary (4/25) tumours showed genomic imbalances. 28 MTC appeared as sporadic forms. In 21 of the sporadic tumours we detected DNA sequence copy number changes (mean: 7 per tumour, range: 1-15). The minimal common regions for the most frequent gains were narrowed down to 1q24-q42, 5p14, and 11q14 (48 percent), 1q43-q44, 5p15, and 11q21 (43 percent), 5p12-p13, 6p11.2-p22, and 11q22 (38 percent), and 6p23-pter, 6q12-q22, and 7p11.2-p15 (33 percent). Eight high-level amplifications were observed in four samples. 5p was affected twice. The minimal common regions for the most frequent losses were observed for 3p (48 percent), and 3q (38 percent).
Conclusion Only limited genomic alterations could be detected by CGH for hereditary MTC, which share a germline mutation of the RET proto-oncogene as initial step of cancerization. Additional genetic alterations, which are responsible for the progression of these tumours, are not identified yet. In contrast, sporadic MTC revealed a recurrent pattern of genomic imbalances. Our data are in accordance in part with the few cytogenetic data, which are reported in the literature and can help to rule out parts of the genome on which further studies should focus on.

P-486

Immunohistochemical analysis of tumor infiltrating immunocytes in thyroid tumors

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Introduction The presence of host inflammatory cells within or at the periphery of human tumors has long been recognized and is commonly viewed as representing a host tumor immune response. In the thyroid gland, the occurrence of chronic lymphocytic thyroiditis (CLT) in thyroid cancers has been reported and considered by some authors as a favorable prognostic sign.
Materials and method The inflammatory cell population in 46 consecutive thyroid tumors (22 papillary, 6 occult, 2 follicular, 1 myeloid carcinomas and 15 follicular adenomas), accompanied with chronic non-specific thyroiditis, were characterized imunohistochemically, using a panel of monoclonal antibodies against T-cells (anti-CD3, anti-CD4, anti-CD8), B-cells (anti-CD20), macrophages (anti-CD68) and dendritic cells (anti-S-100). The extent of lymphocytic infiltration was graded as: +(subtle), ++(moderate) and +++(intense and diffuse lymphoid aggregates).
Results Papillary carcinomas showed denser infiltrates than the follicular tumors or occult carcinomas: of the papillary cancers 63% (14/22) showed +, 27% (6/22) ++ and 10% (2/22) +++ peri- and intratumoral lymphocytic infiltration, whereas all follicular tumors and occult carcinomas exhibited + lymphoid aggregates, mostly in the peritumoral tissue. Tumor infiltrating lymphocytes (TILs) consisted of both B and T cells. B-cells tended to cluster focally in the stroma, whereas T-cells (mainly T8) contacted closely with tumor cells. TILs occurred in the setting of CLT (80% of the cases). Tumor infiltrating macrophages were found in 93% of the cases. Follicular tumors showed only small numbers of large, granule rich macrophages within the lumen of the follicles, whereas in papillary carcinomas they were found in increased numbers in the papillae or in intratumoral stroma. A significant proportion (75%) of papillary carcinomas showed an increased number of S-100(+) dentritic cells, in close contact with neoplastic cells, whereas 18%(3/17) of the follicular tumors exhibited only scant number of intratumoral dentritic cells.
Conclusions TILs are associated more often with papillary carcinomas. The observed low immune reaction in follicular tumors may be due to the capsular barrier.

P-487

CK-19, galectin-3, involucrin, p27 and Mib-1 expression in thyroid cells obtained by fine-needle aspiration citology and their histological counterparts: a preliminary report

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Introduction Thyroid cancer is the most frequent endocrine malignancy and its preoperative diagnosis is sometimes difficult. We investigate whether ck-19, galectin-3, involucrin, p27 and MIB-1 could be potential markers for diagnosis of conventional cytology.
Material and methods A preliminary immunohistochemical analysis was performed on 22 thyroid specimens (5 nodular hiperplasia-NH-, 5 follicular adenomas-FA-, 4 follicular carcinomas-FC- and 7 papillary carcinoma-PC-) from selected patients with thyroid nodules and their preoperative cytologic samples. We analysed ck-19(b170), galectin-3(9C4), involucrin (SY5), p27(1B4) and MIB-1 expression in both cell block and their histological counterparts, semiquantitatively.
Results This table corresponds to histological specimens.

Diagnosis	involucrin	galectin-3	CK-19	p27	Mib-1	Total
NH	-	-	2 (++)	5*	5•	5
FA	-	1 (+)	3 (+)	41	5•	5
FC	-	-	1 (++)	4,	4•	4
PC	6	7 (++)	7 (+++)	63	7•	7

*4 cases score 3+,1 case 2+/ 14 cases score 2+,1 case 1+/ ˛ 2 cases score 2+,2 cases1+/3 4 cases score 1+,2 cases 2+,1 case 0+/ • all cases score 1+
Results were similar in cell block, although involucrine was negative in three PC. Expression of Mib-1 and p27 is variably detected in benign and malignant proliferative lesions. Interestingly, galectin-3, ck-19 and involucrin are invariably detected in papillary carcinomas, although heterogeneous positive expression was observed into the same lesion.
Conclusions Our findings show that galectin-3($p<0.001$), ck-19($p<0.006$) and involucrin($p<0.001$) are useful to identify papillary carcinoma from other thyroid lesions; however, heterogeneous positive pattern expression is not useful for preoperative diagnostic of conventional cytology.

P-488

VEGF-C, VEGF-D, and VEGFR-3 in pheochromocytomas

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Introduction Pheochromocytomas are rare neuroendocrine tumors that are highly vascular. Their malignancy is extremely difficult to estimate on the basis of histopathological features. Vascular

endothelial growth factor receptor 3 (VEGFR-3) is a marker for lymphatic endothelium. Its ligands VEGF-C and VEGF-D are expressed in many neuroendocrine tissues. Our aim was to investigate the expression and the potential role of VEGF-C, VEGF-D, and VEGFR-3 in various pheochromocytomas.
Materials and methods Thirty-three pheochromocytomas, six of them metastasized, were studied using immunohistochemistry. Thirteen tumors exhibited histologically suspicious features, such as necrosis, mitosis count over 5/10 HPF, vascular or capsular invasion, but they not metastasized, and were thus called borderline tumors.
Results and conclusions VEGF-C immunohistochemical positivity was found in most pheochromocytomas. The expression of VEGF-C was heterogenous and varied also within different tumor groups (benign, borderline, and malignant tumors). However, it tended to be stronger in extraadrenally located tumors. VEGF-D positivity was also found in most tumors studied (10/18). Interestingly, all malignant tumors (n = 3) were totally negative for VEGF-D. VEGFR-3 expression was found in 15 out of 33 tumors, but the staining was mostly weak. The expression of VEGFR-3 did not seem to correlate with the expression of VEGF-C or VEGF-D.
In conclusion, VEGF-C, VEGF-D, and VEGFR-3 are expressed in various pheochromocytomas, however, the diagnostic value of these markers needs further investigations.

P-489

Mixed corticomedullary tumor of the adrenal gland. A case report

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Corticomedullary mixed tumors of the adrenal gland are quite rare, with only seven well-documented cases reported in the literature. We report the light microscope and immunohistochemical features of one case of this rare tumor. The patient is a 28-year-old woman who presented with hypertension, hirsutism, gain weight and ammenorhoea of 6-month duration. The surgically resected specimen consisted of a well-circumscribed, single adrenal mass, weighing 22,6gr, and 30 mm in diameter, surrounded by a rim of uninvolved adrenal cortical tissue. Paraffin-embedded cut sections were stained by both hematoxylin-eosin and PAS.The expression of inhibin, melan A, chromogranin A, synaptophysin, S-100 protein and ACTH was examined using the PAP method, with appropriate antibodies being applied. The tumor was composed of cords and nodules of adrenal cortical cells intimately admixed with pheochromocytes. Immunohistochemical investigations highlighted these two cellular components. The adrenal cortical cells revealed inhibin and melan A immunopositivity, whereas the pheochromocytes were strongly reactive with chromogranin A, synaptophysin, and ACTH, and the sustentacular cells with S-100 protein. Thus, we report one additional case of mixed corticomedullary tumor of the adrenal gland.

P-490

Bcl-2 and Fas antigen expression - immunohistochemical and molecular studies in chronic thyroiditis

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Introduction The study of apoptotic markers seems to be important in understanding the pathogenesis of chronic inflammatory processes of the thyroid gland. Aim: Intensity determinations of BCL-2 and FAS immunohistochemical reactions with the quantitative assessment of BCL-2 and FAS gene expressions in inflammatory affected thyroid glands.
Materials and methods The specimens were taken from 16 women aged 21 to 66. The type of inflammation and the intensity of lymphocytic infiltration were assessed according to the Mizukami and the Waterhouse and Doniach classifications, respectively. The expression of BCL-2 and FAS antigens was determined immunohistochemically. Moreover, BCL-2 and FAS genes were quantitatively analysed by RT-PCR assay using TaqMan technology.
Results Intensity of immunostaining for BCL-2 antigen in patients with chronic thyroiditis was stronger than in patients without lymphocytic infiltration. An increase in the intensity of lymphocytic infiltration did not affect BCL-2 mRNA levels. FAS antigen got stained in subjects with mixed inflammation; a presence of oxyphilic cells enhanced the stain reaction. In the case of oxyphilic thyroiditis, the changes in FAS antigen were accompanied by a concomitant increase of FAS mRNA levels. Small differences, between various samples of the same specimen, in BCL-2 and FAS mRNA and protein levels were found in patients with focal thyroiditis. These differences were related to the intensity of lymphocytic infiltration.
Conclusions The thyroid gland is characterised by a high degree of antiapoptotic activity. Each type of thyroiditis has its own characteristic pattern of BCL-2 and FAS expressed as mRNA and as protein.

P-491

The use of galectin-3 and cytokeratin 19 in differential diagnosis of thyroid tumors with follicular growth pattern

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Aim To study the use of galectin-3 (Gal) and cytokeratin 19 (CK19) in differential diagnostics of thyroid tumors with follicular growth pattern.

Material and methods A series of 31 thyroid lesions (10 follicular adenomas (FA), 9 follicular carcinomas (FC), 12 follicular variants of papillary carcinoma (FVPC)) was studied retrospectively by immunohistochemistry using monoclonal antibodies.
Results The staining intensity of either antibody was evaluated as positive or negative using different thresholds (0, 25 and 50% of positive cells). Generally, the intensity of staining of Gal was much higher than that of CK19. Using 0% threshold, 8/10 FA showed positivity of Gal and none was positive in CK19. In FC, 8/9 tumors were Gal positive, 4/9 were also CK19 positive. Among FVPC, all 12 cases were Gal positive and 11/12 were also CK19 positive. Using 50% threshold, only 1/10 FA was Gal positive, none was CK 19 positive. Among FC, however, all tumors were both Gal and CK19 negative. On the other hand, 9/12 of FVPC were Gal positive and 4/12 were CK19 positive. The sensitivity, specificity, negative and positive predictive value of Gal were 0.95; 0.20; 0.71; 0.67 for 0% threshold and 0.43; 0.90; 0.90; 0.43 for 50% threshold, respectively. For CK19 it was 0.71; 1.00; 1.00; 0.63 for 0% threshold and 0.19; 1.00; 1.00; 0.37 for 50% threshold, respectively.
Conclusions The use of Gal and CK19 may be helpful in differentiating FVPC from benign lesions. However, the use of these markers in differential diagnostics of FC from FA is of little value. Moreover, due to low intensity of staining, its use in preoperative diagnostics (e.g. in FNAC cytoblock specimens) seems to be questionable.

P-492

Adrenal cortical adenoma in association with Cushing's syndrome - case report

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Introduction Morphological feature of adrenal cortical adenoma by itself doesn't allow reliable distinction between tumors associated with any particular endocrine syndrome. Without being aware of the clinical setting and/or endocrinological data, it may be impossible to distinguish them from an incidental nonhyper-functional macronodule. The aim of this investigation was to represent the only case of adrenal cortical adenoma in association with Cushing's syndrome in our material.
Material and methods We have analyzed the only case of adrenal cortical adenoma with Cushing's syndrome according to its morphological changes, laboratory data, CT-scan as well as MRI of adrenal gland.
Results Gross examination showed a small ovoid adenoma. On section, it appeared yellow to golden yellow with dark brown foci, blood and degenerative changes. Microscopically, tumor contained cells with pale, lipid-rich cytoplasm, compact eosinophilic cytoplasm, arranged in trabeculae, short blunt cords, as well as in rounded nests. Nuclei were somewhat vesicular with a single small dot-like darkly stained nucleolus.
Discussion and conclusion Our data indicate that being aware of characteristic histologic feature in terms of architectural pattern and cell type for cortical adenoma associated with Cushing's syndrome, this could be the purpose of more comprehensive discussion.

P-493

Correlation of histopathology and sequential analysis of gene expression in follicular thyroid neoplasms

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Background The expression profile and diagnostic relevance of nuclear features, inflammation, and stromal changes have not been systematically evaluated in follicular thyroid carcinomas (FTC).
Design We selected 69 FT neoplasms (22 adenomas, 14 minimally invasive carcinomas, 22 widely invasive carcinomas, and 11 anaplastic carcinomas), classified using standard criteria. Histological variables including anisokaryosis, chromatin pattern, nucleolus, nuclear pleomorphism, nuclear/cytoplasmic ratio, necrosis, stromal changes, and tumor interstitial lymphocytes (TIL) were studied by histological diagnosis (significant if $P<0.05$). Oligo(dT)-primed double strand cDNA was produced from total RNA, and libraries were produced from 3 normal thyroids and 8 FTC. Sequential analysis of gene expression (SAGE) was performed (FTC/control expression factor$\geq$2, significance$\leq$0.01).
Results TIL were absent in 64/69 neoplasms. Minimally invasive carcinomas were distinguished from adenomas by conspicuous nucleolus, increased nuclear/cytoplasmic ratio, and coexistence of apoptosis and myxoid changes in adenomas. The most specific variables of high-grade carcinoma were coagulative necrosis, and nuclear hyperchromatism and pleomorphism. FTC SAGE showed ubiquitin C and sequestosome 1 down-regulation, and tumor protein translationally controlled 1 and malate dehydrogenase 1 up-regulation. This SAGE profile correlated negatively with proinflammatory molecules expression (Interleukin-1beta, chemokine XC-receptor 1, interferon-induced transmembrane protein 2, protein tyrosine phosphatase C), BRCA2, and protein phosphatase 4. The correlation was positive with proliferation markers (myb, mitogen-activated protein kinase 12, cyclin-dependent kinase 8, BRCA2/CDKN1A interacting protein, and RAS oncogene family).
Conclusions Proliferation up-regulation and apoptosis down-regulation results in prominent nucleolus and high nuclear/cytoplasmic ratio for low-grade carcinomas and hyperchromatism and nuclear pleomorphism for high-grade carcinomas. Tumor down-regulation of proinflammatory molecules explains TIL absence.

P-494

Papillary carcinoma of the thyroid with bone formation. A case report

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Introduction Papillary carcinoma is by far the most common type of thyroid malignancy. However, ossification in papillary carcinoma is very rare. A case of papillary carcinoma of the thyroid metastatic to cervical and supraclavicular lymph nodes with associated bone formation and extensive calcification is presented.
Case report A 67 year-old male with a history of gastric carcinoma was admitted to hospital presenting with lumbal pain. A computed tomography scan revealed a calcified nodule, measuring in 33 mm diameter in the thyroid. Total thyroidectomy and modified neck dissection were performed. The thyroidectomy

specimen showed a well circumscribed, calcified, rock-hard nodule measuring 4x3x2 cm. Histologic examination of the hard nodule after decalcification showed spicules of bone formation and papillary carcinoma. Foci of metastatic papillary carcinoma with bone formation were also seen in the cervical and supraclavicular lymph nodes. There was also bone marrow formation both in the thyroid nodule and in the metastatic lymph nodes. Immunohistochemically, the tumour cells were positive for thyroglobulin.
Conclusion Ossification in papillary carcinoma of the thyroid is an unusual finding. The mechanism of bone formation is not clear. The presence of ossification both in primary and metastatic tumoral masses suggests that ossification is not a dystrophic process in the preexisting benign thyroid tissue but somehow related to the papillary carcinoma in the present case.

P-495

Sex hormones receptors in human thymoma - clinicopathological analysis

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Estrogens regulate the development, maturation, and function of various organs, including the thymus. Therefore it is suggested that estrogens play important roles in the development of human thymoma. However there is no study about influences of sex steroids to human thymoma. In this study, we examined the immunohistochemical localization of sex steroid receptors for estrogen (ER)α and ERβ, progesterone (PR)-A and PR-B, and androgen (AR) in human thymoma (n=132), and correlated these findings with various clinicopathological parameters. We used reverse transcription polymerase chain reaction (RT-PCR) and real-time PCR to further study the expression of these receptors in 20 thymoma cases.
Results Immunoreactivity for all sex steroid receptors was detected in the nuclei of thymoma epithelial cells. The percentage of immuno-positive cases and H-score values for each receptor (mean±SD) were: ERα, 65.9% and 85.8±80.2; ERβ, 6.8% and 7.2±8.7; PR-A, 3.8% and 2.7±4.9; PR-B, 49.2% and 55.8±68.3, and AR, 15.2% and 14.1±11.7, respectively. Results of real-time PCR were consistent with those of immunohistochemistry especially results for ERα, PR-B, and AR. A significant positive correlation was detected between immunoreactivity for ERα and PR-B. ERα immunoreactivity was inversely correlated with tumor size, clinical stage, WHO classification and Ki-67 labeling index. In addition, the status of ERα immunoreactivity was significantly associated with a better clinical outcome in thymoma patients.
Conclusions Sex hormones receptors express in human thymoma, and especially ERα may be related to sex, clinical stage and pathological cell type.

P-496

Galectin-3 immunodetection using a biotin-free system in a series of 137 oxyphilic (Hurthle) cell tumors of the thyroid

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Introduction Galectin-3 was reported to distinguish follicular and papillary carcinomas of the thyroid from adenomas, but controversial results were obtained in oxyphilic cell tumors, possibly related to endogenous biotin reactivity. The aim of this study was to assess the role of galectin-3 immunodetection in a large series of benign and malignant oxyphilic tumors.
Materials and methods Galectin-3 expression was retrospectively analysed in 137 thyroid oxyphilic (Hurthle) cell tumors, including 42 adenomas [OA], 66 carcinomas [OC], 29 oxyphilic variant of papillary carcinoma [OPTC] and in 28 control cases of goiter with oxyphilic changes and Hashimoto's thyroiditis. A purified Galectin-3 monoclonal antibody and a biotin-free immunoperoxidase procedure were used in paraffin embedded specimens.
Results 60 of 66 OCs were focally (11 cases) or diffusely (49 cases) positive. 28/29 OPTC were focally (7 cases) or diffusely (21 cases) reactive. Overall, malignant oxyphilic tumors were positive in 93% (88/95). OA had a focal reactivity in 24% (10/42) of cases, with a preferential subcapsular immunostaining. Sensitivity and specificity were 93% and 76%, respectively. Control oxyphilic goiters were negative (0/13), as opposed to Hashimoto's thyroiditis (oxyphilic cells positive in 14/15 cases).
Conclusion The role of Galectin-3 immunoreactivity in the differential diagnosis of benign and malignant follicular lesions is confirmed also in oxyphilic cell tumors. The focal immunoreactivity observed in adenomas may be probably related to a true expression of Galectin-3, as confirmed by mRNA expression by means of in situ hybridisation, and could be related to early malignant changes in tumors still lacking the currently accepted morphological signs of malignancy.

P-497

c-Kit protein expression in Merkel cell carcinoma of the skin and other extrapulmonary poorly differentiated neuroendocrine carcinomas

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Introduction c-Kit gene encodes for a transmembrane tyrosine kinase receptor which is expressed in a variety of human normal tissues and malignancies, being small cell lung carcinoma one of the most frequent among the latter group. The search for c-kit expression in human tumors bears clinical implications since several tyrosine kinase inhibitors have been proposed in the treatment of c-kit positive tumors. The aim of our study was to investigate c-kit protein expression in a series of Merkel cell carcinomas (MCC) and of other extrapulmonary poorly differentiated neuroendocrine carcinomas (PDNECs).

Materials and methods A total of 39 samples, including 25 Merkel cell carcinomas (MCC) of the skin (from 24 patients, 17 of them with follow up information available), and 14 cases of extrapulmonary PDNECs from different sites (bladder - 5 cases, colon - 4 cases, gallbladder and pancreas - 2 cases, parotid - 1 case) were analyzed by means of immunohistochemistry for c-kit protein expression. Only a membranous staining was considered specific, and scored according to the percentage of positive cells.
Results c-Kit protein was demonstrated in 8/17 (47%) MCC, and in 11/14 (79%) of other PDNECs. In MCC, a correlation, slightly below statistical significance (p=0.08), was observed between the presence of c-kit expression and clinically aggressive behaviour.
Conclusion Our data indicate that c-kit expression may play a role in the growth regulation of extrapulmonary PDNECs. Therefore this group of tumors may be considered as a possible target for tyrosine kinase inhibitors therapy.

P-498

Carcinoid tumors in the thyroid gland. Primary or metastatic? Report of two cases

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Introduction The occurrence of well differentiated endocrine tumors (carcinoid) in the thyroid gland is unfrequent and may represent both a differential diagnostic problem with medullary carcinoma, and a potential pitfall in the searching for its primary or metastatic origin. Our aim was to determine the differential morphological and immunohistochemical profile of two cases of carcinoid tumors localized in the thyroid - one metastatic from the lung, and the other of unknown origin.
Materials and methods The first collected case had a clinical history of an atypical lung carcinoid diagnosed two years before, while the other lacked a clinical, instrumental and laboratory history suggestive of a possible primary carcinoid elsewhere. Paraffin embedded material was histologically reviewed and analyzed by immunohistochemistry for thyroglobulin, calcitonin, CEA, chromogranin A, S-100, TTF1, and Ki-67.
Results The first case (F/55) presented as multiple nodules in both lobes, composed of poligonal eosinophilic cells arranged in the typical organoid pattern. Foci of necrosis were present. The morphological features overlapped with those of the primary lung tumor. The second case (M/52) presented as a single nodule in the left lobe, morphologically resembling typical carcinoid without necrosis. Both cases were negative for thyroglobulin, calcitonin, S-100 and CEA, positive for chromogranin A and TTF1, and low proliferating (Ki-67 <10% in both).
Conclusion Our data suggest that, even in the absence of a clinically detected primary tumor, the occurrence of a carcinoid tumor in the thyroid gland should be firstly considered metastatic in origin, the lung being the major candidate for the primary localization (as determined by TTF1 immunostaining).

P-499

Telomerase expression and proliferation activity in gastroenteropancreatic tumours

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Intruduction Gastroenteropancreatic endocrine tumours have a variable prognosis and the prediction of biological behavior based on histopathology is not always possible. Telomerase is a reverse transcriptase that synthesis telomere DNA to stabilize telomere length.Telomerase activity is increased in different human cancers. The aim of this study was to determine whether the expression of telomerase (hTERT) correlates with the histological typing of endocrine tumours and Ki-67 proliferation index.
Material and methods Formalin-fixed, paraffin-embedded tissue sections from 40 gastroenteropancreatic endocrine tumours and 10 normal pancreas were stained immunohistochemically for human telomerase reverse transcriptase (hTERT) and Ki-67 antigen.
Results 11 cases were diagnosed as well-differentiated endocrine tumour, 26 were well-differentiated endocrine carcinoma and 3 were poorly-differentiated endocrine carcinoma according to WHO Classification of Endocrine Tumours. The percentage and intensity of positive neoplastic cells were assessed for each case. Expression of hTERT was observed in one well-differentiated endocrine tumour (9%), in 7 well-differentiated endocrine carcinomas (26.9%) and in 3 poorly-differentiated endocrine carcinomas (100%). There was a significant correlation between the expression of hTERT and histopathological diagnosis ($p<0.01$). Also the correlation of hTERT expression and Ki-67 index was statistically significant ($p<0.01$).
Conclusion Immunohistochemical detection of hTERT expression in gastroenteropancreatic endocrine tumours may be a helpful tool in addition to morphological findings and Ki-67 index in predicting the malignant potential of these tumours.

P-500

Proliferation, p53 expression and apoptosis in pituitary adenomas: relationship to tumor behaviour

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Introduction Pituitary adenomas are known to vary considerably from the stand point of their intrinsic aggresiveness that might imply a different therapeutical approach. The aim of this study was to estimate the relationship between proliferative activity (Ki67), p53 expression, apoptosis and pituitary tumor behaviour.
Materials and methods Immunohistochemical analysis of Ki67 and p53 expression were performed in 50 paraffin-embeded pituitary adenomas: 28 functioning; 22 non-functioning; 34 invasive adenomas. Apoptotic index (AI) was determined in 24 adenomas by the in situ end-labeling technique (TUNEL).
Results Ki67 expression in all adenomas was 3.68% (significantly higher in functioning comparatively with non-functioning pituitary adenomas - 4.45±0.5% versus 2.62±0.47%). We noticed a positive correlation between the proliferative activity and the tumoral invasiveness (Ki67 labeling index 4.82±0.4% in invasive and 1.25±0.3% in non-invasive adenoma). p53 nuclear positivity was identified in 50% of the invasive adenomas and only in 5% non-invasive adenomas, with a positive correlation between p53 and proliferative activity (Ki67: 5.58±0.4 in p53 positive versus 2.60±0.47% in p53 negative tumors). Apoptosis was found in 60% of tumors, higher in some of the invasive ones, with a positive correlation between the proliferation rates and apoptotic indices (AI: 4.54±1.22 in total versus 5.42±1.46 in invasive pituitary adenomas). Also, some differences were recorded in functioning

and non-functioning adenomas (AI: 5.14±1.62 versus 3.70±1.92).
Conclusions The data suggest that the assessment of proliferation, p53 expression and apoptosis might be used to evaluate tumor behaviour and susceptibility of adenomas to therapy.

P-501

Differentially expression of NESP55 in Langerhans islets of the human pancreas and overexpression in endocrine pancreatic tumors

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Introduction NESP55 is a novel member of the chromogranin family and is processed by endopeptides within the chromaffin granules in various rat and bovine endocrine and neuronal tissues, including adrenal glands, pituitary glands, some brain regions and intestine. Nothing is known about the distribution in human organs and tissues.
Materials and methods We investigated human pancreatic tissues as well as pancreas derived endocrine tumors. Therefore Langerhans islets of different pancreatic parts and 24 endocrine pancreatic tumors were immunohistochemically investigated. To compare the results with other endocrine tumors, eight intestinal carcinoid tumors were incubated too. Additionally RT-PCR was used to confirm our results.
Results In Langerhans islets, all different cell types presented with different amounts of NESP55, including insulin, glucagon, somatostatin and pancreatic polypeptide producing cells. All of the endocrine tumors derived from these cells showed an occurrence of NESP55. Interestingly, in no carcinoid tumor of the intestine reactivity with the NESP55 antibodies could be found.
Discussion and conclusion NESP55, as other chromogranins too, is an important marker for neuroendocrine tissues and tumors with a prognostically relevant impact by clearly differentiating endocrine from exocrine pancreatic neoplasms. Moreover, by use of NESP55, it seems to be possible to distinguish between pancreatic derived endocrine tumors and intestinal carcinoids, especially important for identifiing the primary tumor, when neuroendocrine liver metastases and clinically unspecific symptoms are found.

P-502

Clinical importance of vascular endothelial growth factor (VEGF) for papillary thyroid carcinomas

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Introduction Vascular endothelial growth factor (VEGF) is a major regulator of angiogenesis and may be produced by some cancer cells. Several recent reports have documented that increased expression of VEGF is associated with a risk of recurrence or decreased recurrence-free survival in papillary thyroid cancers (PTC). The aims of this study were to determine whether immunohistochemical expression of VEGF is related to local and distant recurrence of PTC and to evaluate the relationship between hypervascularization and VEGF expression in papillary thyroid carcinomas.
Material and methods VEGF expression was examined immunohistochemically in 48 papillary carcinomas. Ten normal thyroids were used as controls. Patients were followed for 61.7 months, (range, 24-143). Twelve of the patients had local and distant recurrences. VEGF immunostaining was evaluated semiquantitatively by two pathologists, blinded for clinicopathological data.
Results The difference between the recurrent (n=12) and nonrecurrent (n=36) carcinomas was statistically significant (p=0.001). VEGF expression was also stronger in papillary thyroid carcinomas than in normal thyroid tissues. The mean microvascular densities were significantly higher than those in normal thyroid tissues.
Conclusion These data indicate that VEGF staining is strongly associated with increased frequency of local and distant recurrence in PTC and that the immunohistochemical profile of the expression may be used as a marker for predicting which tumors may have metastatic potential. Key words: thyroid, VEGF, angiogenesis.

P-503

Bone mineral density and Pap smears

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Introduction Osteoporosis is an increasingly prevalent bone disease. The key to the treatment of osteoporosis lies in prevention and requires screening of non-symptomatic population, which may not be cost-effective. The present study attempted to find out possible correlation between morphologic characteristics in Pap smears, and bone mineral density (BMD) as measured by dual energy X-ray absorbtiometry (DEXA)
Materials and methods Four different lumbar spine and left hip regions were automatically scanned in 79 women whom Pap smears for routine cervical cancer screening were taken. The smears were grouped into atrophic and mature cell patterns, which can easily be recognised during routine screening. Using astereological analysis, the mean areas of squamous cells, their nuclei, cytoplasm and nuclear cytoplasmatic ratio were estimated.
Results The mean areas of cells and cytoplasm were significantly lower at lower T-scores ($p<0.01$), while the mean areas of nuclei were not ($p>0.5$). Nuclear cytoplasmatic ratio was higher at lower T-scores ($p<0.01$). T-scores of all hip and spine regions were significantly lower in atrophic cell pattern group. The study group indicated concurrently high sensitivity of 81% and specificity of 78%, with positive predictive value of 84%.
Conclusion Our results suggest that a significant number of women with low BMD could be identified parallel with the routine Pap test for cervical cancer screening without additional costs.

P-504

Value of repeat fine needle aspiration cytology in the management of thyroid nodules with an initial cytology result of benignity

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Introduction Management of benign thyroid nodules is controversial. Some studies recommend to repeat FNA in all benign thyroid

nodules, while others recommend to do it only if clinical changes are present. This study was done to determinate the usefulness of systematic repetition of FNA in patients with benign cytology result in the initial FNA.
Materials and methods Data were collected from 208 patients referred to Endocrinology Service for evaluation of thyroid nodular disease between 1998 and 2001. All patients underwent repeated FNA of thyroid nodule. Age of patients was 46.9+23.8 years, 91.8% were females, 74% had uninodular goiters, nodule size was 2.6+1.1cm. Time elapsed between both FNA was 14.3+9.7 months.
Results At second FNA, 175 aspirations had benign cytology, 21 were non diagnostic, 10 suggestive of follicular neoplasm and 2 had malignant cytology results. Excluding non-diagnostic aspirations, we get 6% of discordant results. Uninodular goiters as compared to multinodular have a higher rate of discordant results. We did not find other variables between groups of concordant and discordant results. Twenty-two patients with benign cytology underwent thyroid surgery, without any histological malignant result.
Conclusion 1- Uninodular goiters with an initial benign cytology have a significant rate of discordant results after repetition of FNA. However, in 30% of these cases the first diagnosis was based on limited material. 2-Our results show that 5 cases of thyroid carcinoma out of 208 patients with nodular goiter were misdiagnosed by initial FNA cytology. Therefore we recommend systematic repetition of FNA cytology in all patients with uninodular goiter.

P-505

Immunocytochemistry in FNA biopsy material

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Objective Assessing diagnostic value of immunocytochemical methods in destained cytology smear specimens prepared from fine needle aspiration (FNA) biopsy material.
Material and methods Investigated material comprised 135 samples from FNA biopsies that were diagnosed in 1999-2001. Alcohol-fixed cytology smears were typically stained with hematoxylin-eosin. For immunocytochemical studies, preparations were first destained using alcohol series. Following this, chosen antibodies were used either separately, or as a panel CKMNF, EMA, LCA, TG, CT, HMB-45, S-100, CHR, NSE, ER, PR, CD 68 (Dako LSAB 2 System, Peroxidase).
Results Immunocytochemical staining was helpful in 83% of cases. Initial diagnosis was confirmed in 45 cases. Immuno-cytochemical staining was important particularly in confirming diagnosis of poorly differentiated neoplasms. It was also helpful in identifying the primary site of metastatic carcinoma in 20 FNA cases. In 23(17%) cases diagnosis based on the investigated method failed. Precise diagnosis was not possible either due to negative staining with single marker/panel or when only single cells were stained positive.
Conclusion Immunocytochemical methods in destained smears allow basic as well as discriminating diagnosis even in cases of poor availability of cytologic material.

P-506

Fine needle aspiration of lesions in the thyroid bed.

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Introduction Clinical suspition of recurrent thyroid carcinoma based on ultrasound examination of thyroid bed with correlation to increased thyreoglobulin level or calcitonin level and/or radiomarker uptake on scintigraphy can be supported or dismissed by fine needle aspiration biopsy (FNAB). The aim of this study was assesment of specificity and possitive predictive value of ultrasound- guided FNAB of the lesions in the thyroid bed after total thyroidectomy for diagnosis of recurrent thyroid carcinoma.
Material and methods Ultrasound guided FNAB of thyroid bed lesions of diameter 0.4-3.2 cm performed by pathologist in 93 patiens after thyroidectomy. Immunohistochemistry stain for calcitonin was performed using DAKO antibody in 4 cases.
Results The recurrent papillary carcinoma was diagnosed in 18 (19.5%) patients, the recurrent follicular carcinoma in 7 (7.6%) patients, Hürtle cell carcinoma in 2 (2.3%) patients and medullary carcinoma in 5 (5.5%) patients (immunocytochemistry stain for calcitonin confirmed diagnosis in 3 patients). Pathologic correlation was able in 24 cases: 22 postoperative examinations confirmed recurrent carcinoma, and 2 cytologic diagnoses were false-negative. In the remaining 38 patients granulation, purulent inflammation and benign lesions were diagnosed. In 23 (24.8%) cases material was nondiagnostic. Specifity of the method was 96.8%, possitive predictive value 91.6%.
Conclusions Ultrasound- guided fine needle aspiration biopsy performed by pathologist is valuable and specific method in the diagnosis of recurrent thyroid carcinoma in thyroid bed.

P-507

The use of FNA in the management of thyroid goiter

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Introduction Fine-needle aspiration (FNA) is one of the most useful methods in the diagnosis of benign goiter. In consideration of the goiter; it is the most frequent lesion in the thyroid tissue, significance of FNA as a diagnostic method become greater.
Material and methods In 414 cases diagnosed as goiter we analyzed: 1) sex distribution, 2) age distribution, 3) correlation between clinical and cytological diagnoses and 4) goiter classification in accordance with cytological characteristics.
Results Goiter was five times more frequent in females than males (348 women, 66 men). The majority of the patients were between 41 and 50. The youngest was 13, and the oldest 78 years old. Almost 84% of cytologically diagnosed goiters had the same clinical diagnosis, in only 1.5% of all patients tumor was suspected clinically. We confirmed high correlation between clinical and cytological diagnosis. According to the cytological characteristics

all goiters were classified in 2 categories: goiters with and without degenerative changes. Hi-square test confirmed statistically significant difference in the presentation of such goiters (Hi-square test=10.4; p<0.01).
Conclusion FNA permits a substantial reduction or even elimination of other diagnostic procedures, such as imaging, with consequent saving in time and money. Furthermore, benign cystic lesion may be cured by FNA (a diagnostic and unexpected therapeutic value).

P-508

Diagnostic guidelines for Hashimoto's thyroiditis in fine-needle aspirates

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Introduction Fine-needle aspiration (FNA) is a very useful method for diagnosing various lesion in the thyroid gland, among them Hashimoto's thyroiditis (HT). Although many methods may be used, FNA is a necessary diagnostic procedure nowadays in order to confirm the diagnoisis of HT.
Material and methods Durind a period of 2 years citologically 600 aspirates were citologically analyzed, stained with HE and MGG. The main cytological guidelines in the diagnostic procedure were: 1. lymphocytic cellularity of smears, 2. oncocytic follicular cells, 3. a lot of cellular debris.
Results From 600 aspirates analyzed 35 (3,5%) were diagnosed as HT. There was a high statistical significance in sex and ages for the genesis of HT (χ^2=24,02; p=0,005). We analyzed correlation between clinical and cytological diagnosis: in 82 cases initial clinical diagnosis was "Thyroiditis", and among them 46 (56%) were cytologically confirmed. From 35 patients with an FNA diagnosis of HT the same diagnosis was susspected clinically in 23 cases (66%). Several authors noted a high incidence of thyroid carcinoma in patients with HT. We researched association of HT and thyroid tumors, and we did not confirm that HT is a premalignant lesion (χ^2=1,22, p<0,05).
Conclusion HT and its association with other lesions could be clearly diagnosed with FNA, and we recommend FNA as a first line investigation in HT.

P-509

Colposcopic and cytologic findings in HPV infection of the uterine cervix

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Introduction Uterine cervical carcinoma is a sexually transmitted disease. HPV infection, especialy types 16, and 18 oncogene is directly associated with occurrence of preinvasive and invasive changes in the uterine cervix ranging from 36 - 79%. Cyto-pathologic effect of this virus presence in a cell is called koilocytosis and occurs by the effect of fragment E4 on cytokeratin building protein. Koss observed these changes back in 1956, describing them as irregular cavities in cell protoplasm, but only later these cytologic findings were related to HPV infection. In our retrospective study we analyzed colposcopic and cytologic findings of 34 examinees in which the diagnose of HPV infection was established by biopsy.
Material and methods The mean age of examinees was 30,8, and in regards to parity and menarche there was no statistically significant difference between experimental and control group, since coefficient t was lower than 1,90 p<00,5. Therefore we concluded that the sample was representative.
Results Sensitivity of cytology in detection of HPV infection of the uterine cervix was 53,1% and the specificity was 64%. Positive predictive value was 85% and negative 65%. The most frequent colposcopic findings in the uterine cervix in case of HPV infection were aceto-white epithel in 34% and mosaic in 37%. Other colposcopic findings were significantly lower (punctate 19%, leucoplakia 7,3%). In examinees with colposcopically diagnosed HPV infection in external genitalia, the infection was also present in the uterine cervix in 82,6% which was statistically significant for X2 =29,7597>(X2 (1;0,16)=6,635).
Conclusion Based on the results presented above, we concluded that sensitivity of cytology in the detection of HPV infection of the uterine cervix was low and that it was necessary to combine that method with colposcopy and directed biopsy. In case of colposcopically diagnosed condylomas of the external genitalia and presence of pathologic colposcopic findings of the uterine cervix (aceto-white epithel, mosaic), the presence of HPV infection in the uterine cervix can be expected with high probability.

P-510

Fine needle aspirate of papillary thyroid carcinomas in correlation with histological diagnosis

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Material and methods 32 cases of thyroid aspirates were studied in correlation with histological findings. The patients were 9 – 89 years old (mean age 46,3). Cytological diagnosis was made according to morphological criteria such as arrangement of cells, nuclear atypia and special features (colloid, giant cells, psammoma bodies).
Results Cytological diagnosis was in agreement with the histological diagnosis in 29 cases (90,6%). 2 cases were follicular adenomas and one Hürthle cell tumor. In cases of disagreement between cytological and histological diagnosis the cytomorphological findings were analysed.
Conclusion Cytological evaluation of papillary thyroid carcinomas is of high diagnostic accuracy, although overlapping of cytological criteria between the various pathological entities may lead to false evaluation.

P-511

The value of universal short-term storage cell medium in processing FNAB samples for ancillary diagnostic methods

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Introduction The application of ancillary methods to FNAB samples is mainly impaired by a limited quantity of cells in the

samples, furthermore it is difficult to predict at patient's bedside which ancillary methods, if any, will be necessary. The aim of this study was to demonstrate the value of a universal short-term storage cell medium in processing FNAB samples for different ancillary methods.

Materials and methods After diagnostic smear preparation, the needle and syringe were washed out with the short-term storage cell medium in 6535 FNAB samples, obtained from 1998 to 2002. Microscopic smear analysis determined the choice of ancillary method used. Cell suspensions were then processed as required for each individual ancillary method.

Results We used 3200 (49%) FNAB samples stored in the medium for ancillary methods, while 3335 samples (51%) were discarded due to the clear morphology-based diagnosis or sample inadequacy. Immunocytochemistry was performed on 2057 samples. For various membrane, cytoplasmic and nuclear antigens, adequate immunoreaction without background was obtained in samples stored in the medium overnight (1797) or over the weekend (260). Immunophenotyping by flow cytometry was performed in 1088 FNAB samples where up to 8 lymphoma antigens per sample were successfully determined when the samples were stored in the medium overnight. High quality flow cytometric DNA histograms were obtained from all 55 FNAB samples suspended in the short-term storage cell medium.

Conclusion The short-term storage cell medium that we have been using, allows effective and easy processing of FNAB samples for several ancillary diagnostic methods. It preserves cell morphology, various membrane, cytoplasmic and nuclear antigens as well as DNA content.

P-512

Fine needle aspiration cytology of ovarian lesions

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Introduction Fine needle aspiration cytology (FNAC) is an alternative method to surgery for evaluting the ovarian lesions. Despite some arguments for indications of FNAC, it can be used for diagnosis and management of ovarian masses. The aim of this study was to assess the diagnostic value of aspiration cytology in ovarian lesions.

Materials and methods Fine needle aspirations were performed in sixty-two ovarian masses between January 2000 and February 2003. Aspiration material was obtained from fresh tissues at the time of frozen section before dissection of specimens. The slides of aspirates were stained with May Grunwald-Giemsa and Papanicolaou. They were evaluated by a pathologist who was anaware of the gross findings and the histopathologic diagnosis. Cytologic diagnoses were classified as benign and malignant.

Results Only sufficient aspirates were included in the study. The average age of the women was 43 years (range 14-85 years). 62.9% of the cases were assessed as malignant and 30.6% of the cases were benign. Two serous neoplasms of low malignant potential were evaluated in malignant category. Two false positive and two false negative cases were determined. In our series the overall sensitivity and specifity were 90.4% and 95.1%, respectively. Beside this seven benign and 15 malignant lesions could be subclassified specifically.

Conclusion We studied on excised specimens but since ovarian masses are reachable by laparoscopy or ultrasonography guided aspiration can be performed safely, FNAC may be suitable for the diagnoses of these lesions. If multidisciplinary approach can be carried out for patients with ovarian lesions cytopathologic interpretation can provide optimum benefits.

P-513

Human papillomavirus detection in cytological material of cervix. Evaluation of hybrid capture hybridisation assay, PCR and PAP smear

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Introduction An improved understanding of the pathogenesis of cervical HPV-induced lesions has significantly changed the approach to diagnosis and treatment. The Pap smear is still the most effective cervical cancer screening test. However, molecular testing for the presence of high risk HPV-DNA increases the ability to identify women with an increased risk for cancer and true cancer precursors. The aim of the present study was to evaluate high risk HPV-DNA test systems in cervical cytological material and the correlation with Pap smear cytology.

Materials and methods We analysed 40 routinely processed cytological cervical specimen. Material was taken consecutively for Pap smear cytology and for Hybrid Capture (HC) hybridisation assay (Digene). The cytological report showed mild to severe dysplasia (7 cases were scored as PAP I/II). After performing the HC-assay, DNA was extracted from the same sample. HPV-DNA detection with conventional PCR using consensus primer (MY9/MY11), followed by subtyping of HPV 16 and 18 was carried out.

Results From the specimens showing dysplasia (83.3%), koilocytosis has been observed in 69% of Pap smear cytology. HPV-high risk positivity with the HC hybridisation assay was detected in 73% and 80% showed positivity for HPV-DNA content with conventional PCR. Subtyping of HPV-positive cases resulted in HPV-16 positivity in 65%, 12.5 were positive for HPV 18 and 18.5% showed a coexistence of HPV 16 and 18.

Conclusion Our results indicate that HPV-detection with highly sensitive and widely used molecular methods show a good concordance with cytological findings.

P-514

Fine needle aspiration findings of two different types of gastrointestinal stromal tumor

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Introduction Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract, derived

from interstitial cells of Cajal. The cytologic features of these neoplasms in fine needle aspiration (FNA) have been described in several articles. Aims: Cytologic and histologic features of two GIST cases one of which presented with liver metastasis and other with an intrathoracic mass mimicking lung carcinoma radiologically are presented comparatively.
Patients and results Sixty year-old male patient with a previous history of gastric GIST has had an FNA from a liver mass. In the cytologic examination, epithelioid cells with round-oval nuclei, large cytoplasms and indistint cytoplasmic borders, forming three dimentional groups with thin fibrovascular stroma were observed. The other patient was a seventy two year-old female who had an FNA from an intrathoracic mass. Branching fascicles of spindle cells with indistinct cytoplasmic borders in a necrotic background were observed in the cytologic examination. Mesenchymal origin was verified by vimentin immunopositivity. By detailed computerized tomographic evaluation, it was revealed that the mass originated from the gastroesophageal junction. Both tumors were resected and histologically examined. Cytologic and histologic findings correlated including immunocytochemical and immunohistochemical results which were positive CD117 and CD34, and negative S100 and smooth muscle aktin immunoreactions.
Conclusion As they share common histomorphological features with other types of mesenchymal tumors of the gastrointestinal tract, CD117 immunopositivity is the only diagnostic marker to differentiate these tumors from other tumors of mesenchymal origin. Cytologic diagnosis also requires detection of this marker in the neoplastic cells.

P-515

Peritoneal fluid cytology of sarcomas

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Material and methods Peritoneal fluid smears of 1179 female patients of all ages, were studied.
Results 229 cases were cytologically diagnosed as malignant (19,4%), the majority of which was characterized as adenocarcinomas. Histological diagnosis was available for 139 cases. Sarcomatous component was reported in only 7 cases, 4 cases were malignant mixed Müllerian tumors (MMMT), one was leiomyosarcoma, one rhabdomyosarcoma and one liposarcoma. The cytological findings in the equivalent 7 cases were « abnormal» neoplastic cells in isolation, with enlarged and hyperchromatic nuclei. Adenocarcinoma cells were identified in peritoneal fluid smears of 3 patients with MMMT.
Conclusion Cytological diagnosis of sarcoma is rare. Even when these «abnormal» cells are found, it is very difficult to give a definite diagnosis of sarcoma, based directly on the cytomorphological characteristics of peritoneal smears. However, such a possibility should be kept in mind by the cytopathologist to avoid missing the diagnosis.

P-516

Accuracy of cytologic diagnosis of primary malignant bone tumors and metastases in the bones

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Purpose The aim of this study was to show that imprint cytology is a valuable method in the evaluation of bone tumors as well as to determine its diagnostic accuracy in primary and secondary bone tumors.
Materials and methods From thirthy three surgical specimens of bone tumor imprints were made, air-dried and stained by May-Grunwald- Giemsa method. The cytologist gave detailed description of the smears, concluded whether the lesion was malignant and suggested a final diagnosis if it was possible. Results were compared with the corresponding histopathologic diagnoses.
Results Out of 19 cases with primary malignant bone tumors (chondrosarcoma 9, Ewing sarcoma 2, ameloblastoma 2, synoviosarcoma 2, malignant osteoclastoma 1, malignant fibrous histiocytoma 1, malignant schwannoma 1, leiomyosarcoma 1) the cytologist recognized malignancy in 18 of them and in 10 suggested the final diagnosis. In 14 patients with metastases (squamous cell carcinoma 11, anaplastic carcinoma 1, malignant melanoma 2), the cytologic and histopathologic diagnoses correlated in all cases.
Conclusion Cytologic analysis of the imprints of bone tumors is valuable as early orientation for the clinicians since the final histopathologic diagnosis needs time because of the decalcination procedure. The correlation of cytologic and histopathologic diagnoses was better for the secondary bone tumors because cytologist was more familiar with their morphology.

P-517

The value of combined fine needle aspiration and needle core biopsy in diagnostic assessment of pancreatic tumors

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Introduction Due to the anatomical location of the pancreas, it is often difficult to obtain sufficient diagnostic material from pancreatic lesions. The aim of this study is to present the results and our experience from CT guided fine needle aspiration (FNA) and needle core biopsy (NCB) from pancreatic tumors.
Materials and methods FNA using 22 Gauge needles and NCB using 16 and 18 Gauge needles were performed in 27 patients with pancreatic tumors. Both procedures were done under computerized tomography guidance, without complications. Twenty one tumors involved the body and 6 the tail of the pancreas

Results The diagnosed cases were 18 adenocarcinomas, 2 neuroendocrine tumors, 1 lymphoma and 2 pseudocysts. Diagnosis was reached in 21 cases by both FNA and NCB and in 2 cases by NCB only. 2 other cases were "diagnosed" as suspicious for malignancy by FNA only. Finally in 2 cases no diagnosis was reached by both methods, due to insufficient material.
Conclusion According to our experience, we believe that the combination of fine needle aspiration with needle core biopsy of pancreatic tumors under CT guidance improves the diagnostic accuracy.

P-518

Value of fine needle aspiration cytology in subtyping mucinous breast carcinoma

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Background Subtyping of mucinous breast carcinoma into pure and mixed type has prognostic implications. Aims of the study were to evaluate a) the usefulness of fine needle aspiration biopsy (FNAB) in differentiation between pure and mixed mucinous carcinomas, and b) to correlate key cytological features with survival.
Material and methods We reviewed FNAB of 98 women with mucinous breast carcinoma treated at the Institute of Oncology from 1983 to 1999. Key cytological features analysed were cellularity, amount of mucin, presence of capillaries, cell pattern, cytoplasmic characteristics and nuclear pleomorphism. Type of mucinous carcinoma was determined histologically after resection of the tumour. Association of cytological features with cancer specific survival was evaluated by univariate and multivariate analysis.
Results Histological analysis revealed 64 cases (65%) of pure and 34 cases of mixed mucinous carcinoma. Nuclear pleomorphism and amount of mucin in FNAB differed significantly between the two subtypes of mucinous carcinoma. Whereas cellularity of cytological samples, presence of capillaries, cell pattern and cytoplasmic characteristics were not related to prognosis, nuclear pleomorphism and absence of mucin in cytological samples were associated with poorer cancer specific survival. Furthermore, the absence of mucin in FNAB samples retained independent prognostic significance also in multivariate analysis.
Conclusions Higher grade of nuclear pleomorphism and small amount or even absence of mucin in FNAB favor the diagnosis of mixed mucinous cell carcinoma and are features associated with poorer prognosis.

P-519

Cardiac lipoma in a Mexican patient - multidisciplinary approach in a right atrium tumor

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Primary tumors of the heart are rare. Lipomas account for approximately 10% of all neoplasms of the heart and represent 14% of benign cardiac tumors. Symptoms may be produced by large lipomas, but more often the patients are asymptomatic and the diagnosis is made by chance. Recently, the ready availability of high-definition noninvasive imaging, such as echocardiography, computed tomography, and magnetic resonance image, has allowed easier diagnosis. Therefore, more cases have been reported. To date up to 70 cases of lipoma have been described. This case describes the multidisciplinary approach to the diagnosis of this rare entity with the use of open heart biopsy from right atrium, to sustain the correct diagnosis. After exhaustive internet-based search, this is the first case reported in Latin-American literature, a condition not previously informed from in vivo patient in Mexico.

P-520

Type I and III collagen synthesis and composition in the valve matrix in aortic valve stenosis

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Introduction Changes in the collagenous matrix may participate in the pathogenesis and progression of human aortic valve stenosis (AS). We investigated the expression, localization, rate of synthesis and composition of type I and III collagens in AS.
Methods Type I and III collagen mRNA expression and the immunohistochemical localization of the collagen antigens were studied from 36 AS and 2 normal aortic valves. The concentrations of various type I (PINP, PICP, ICTP and synthetic peptide SP4) and III (PIIINP and IIINTP) collagen antigens were measured by radioimmnoassays in soluble tissue extracts and trypsin solubilized calcified and noncalcified matrices of 11 AS samples, and 24 healthy aortic valves of different ages.
Results The synthesis of type I collagen, localized in the myo-fibro-blasts adjacent to calcified nodules, was 2-3 –fold higher in AS compared to controls. The proportion of collagen in total protein fraction was 90 % in the healthy valves, 50 % in the noncalcified matrix and 10 % in the calcified matrix of AS valves. In calcified valves the ICTP content was 6-fold higher than in the age matched controls and 2-fold higher than the young control group. In the controls, ICTP ($r=-0.908$, $p<0.001$) and IIINTP ($r=-0.753$, $p<0.001$) decreased with age, whereas SP4/ICTP ratio increased ($r=0.538$, $p<0.01$).
Conclusions Type I collagen synthesis was increased in AS. Localization of this collagen adjacent to calcified nodules and the profile of the C-telopeptide cross-linking indicate that the ectopic aortic valve calcification may have similarities to bone matrix mineralization.

P-521

A case report of sudden death in a patient recently diagnosed with cardiac hydatid cyst

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Introduction The cardiac hydatid cysts are rare cases, representing 0,5-2% from all human hydatid cysts. They are commonly located in the left or right ventricle and exceptionally in the interventricular septum. Because the symptoms and clinical signs of the cardiac hydatid cysts are non-specific, the diagnosis may be difficult.
Material and methods We report an intramyocardial hydatid cyst, diagnosed at Cardiology Center Iasi, with an early history of atypical chest pain, the death occuring through anaphylactic shock, secondary to the hydatid cyst endocavitary rupture. The study was made on necropsiy specimens, and the diagnosis was established by using routine morphological techniques.
Results The gross examination evidentiated an hydatid cyst of about 6 cm in diameter, communicating through a small orifice with the left ventricular cavity and through a slit-like fissure with the right ventricular chamber. The systemic embolism was revealed, only grossly, by the presence of small "hydatid daughters" at the level of the ascending aorta and the pulmonary embolism was confirmed only histologically, by the presence of anhiste membrane emboli in the peripheral pulmonary vessels.
Conclusions The study revealed the morphological aspect defining hydatid cyst, emphasising the necessity of an early diagnosis due to a high risk of death through complications.

P-522

A case report of arrhytmogenic right ventricular dysplasia – cause of sudden death

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Arrhytmogenic right ventricular dysplasia (ARVD) is a new form of cardiomyopathy probable more frequent than commonly reported. ARVD is a heart muscle disorder of unknown cause that is characterized clinically by an electrical instability associated with secondary ventricular arrhythmia and pathologically by fibro-fatty replacement of the right ventricular myocardium. It is a rare but important cause of sudden unexpected cardiac death in young, otherwise healthy persons, especially those that are exercise related. We report a case of cardiac sudden death in a 40 years old man without personal and familial cardiac history. The study was made on a necropsic biopsy specimens from the medico-legal laboratory. The diagnosis was made using routine histological techniques. Grossly, we revealed a fibro-fatty right and left ventricular wall myocardial replacement and a focal white thickening of the left ventricular endocardium. Histologically, we evidentiated a fibro-fatty myocardial replacement of the right and left ventricle suggesting an arrhythmogenic ventricular dysplasia of cardiomyopathic type and an endomyocardial fibrosis supported by the presence of a subendocardial fibrosis, affecting internal one third of the myocardium. There were no evidence of coronarian and valvular lesions and hypertensive cardiac changes, as well. The study revealed the morphological features defining a fibro-fatty myocardial replacement and an endomyocardial fibrosis evidentiating the case particular features, as well.

P-523

Assessment of diabetic micro- and macroangiopathies

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Introduction The objective of this study was to determine the potential value of digital thermography (DT) for the assessment of micro- and macroangiopathies of lower limbs in diabetes mellitus (DM).
Materials and methods We have studied 250 patients (pts) with DM (120 male). 100 pts had DM type I, 150 pts had DM type II, with age varying from 27 to 80 years. Thermograms were acquired using an original PC-interfaced thermograph, averaged and reformatted to 128x128 8-bit images. Analysis included filtering, background cutoff, obtaining x- and y-axis profiles and averaged temperature curves (ATC), determination of local maximums and minimums of the ATCs, definition of anatomic regions, building regions of interest (ROI).
Results Following thermogram patterns were found: 1. asymmetric unilateral hypothermia of 1st toe (in 35% of DM I cases; probably indicating microangiopathy); 2. bilateral symmetrical hypothermia of both feet at the level of distal phalanges (in 25% of DM I cases); 3. unilateral hypothermia of foot ('thermo-amputation'); 4. unilateral hyperthermia of foot and tibial region; 5. focal hyperthermic regions (diameter = 2..4cm; indicating potential subsequent ulceration). ATCs and symmetric left-right ROI comparisons helped distinguishing between macro- and microvascular disease, between neuro- and angiopathy in DM, evaluating therapy effectiveness. DT was most valuable in surgical cases, permitting (1) to locate the optimum level of amputation and (2) to detect early the inflammation.
Conclusion DT results correlate well with pathomorphology, and depend upon DM type, DM duration, and presence of local inflammation.

P-524

Immunomodulatory treatment improves myocardial histopathology and function in patients with biopsy-proven myocarditis

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Aim The aim of this study was to determine the impact of immunomodulatory treatment (interferon-α) on myocardial histo-pathology and contractility in patients with biopsy-proven myocarditis (MC).
Methods A total of 40 consecutive pts (24/40 male, mean age 40+10 years, baseline EF= 35.6+9.3%) underwent endomyocardial biopsy which established diagnosis (using modified Dallas classification) of MC (active MC in 15/40 pts, focal-active MC in 10/40 pts, borderline MC in 5/40 pts, and healing MC in 10/38 pts). All patients received interferon-α (SC application, 3 MIU, 3 time PW, for 3 months) on top of conventional triple therapy for heart failure. Rebiopsy was performed in all patients after the termination of interferon-α treatment, and both baseline EF and repeated biopsies were analyzed. Semiquantitatively we comapred the amount of cellular infiltrate, degenerative and/or necrotic changes, and interstitial fibrosis. Repeated biopsies were than classified as improved, no change and/or deteriorated, for each histopathological variable (by 2 pathologists, in a blinded manner).
Results By the end of interferon-α treatment EF improved significantly (40+11.9% vs. 35.6+9.3%), and no deaths were

registered. Repeated biopsy revealed ongoing MC in 3/40 pts, late-healing MC in 17/40 pts, focal-healing MC in 8/40 pts, healed MC in 5/40 pts, and dilated cardiomyopathy in 7/40 pts. Amount of cellular infiltrate, degenerative and/or necrotic changes, and fibrosis was reduced in 28, 23, and 1 pts, respectively, remained the same in 7, 8, and 18 pts, respectively, and increased in 5, 9, and 21 pts, respectively ($p<0.01$, between groups). The significant reduction was achived in the amount of cellular infiltrate and degenerative and/or necrotic changes, contrary to the amount of fibrosis. Applied immunomodulatory therapy made substancial reduction of inflammation with "relative" increase of fibrosis.
Conclusion Interferon-α, as main immunomodulatory agent, has favourable effects on myocardial histology and contractility in patients with biopsy-proven myocarditis.

P-525

Morphological changes in heart died of a heart attack of a myocardium

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Diseases CVS take conducting place among the most actual problems of modern medicine. From them it is possible to count the main thing IHD. IHD – the special form of pathology CVS including group of diseases which, according to definition the CART, are treated as follows: «Dysfunction sharp or chronic, arising owing to relative or absolute reduction of supply of a myocardium by arterial blood». Despite of significant number of the morphological researches devoted IHD, morphogenesis separate displays of it, in particular, AMI, and also mechanisms of indemnification and decompensation of hearts are investigated insufficiently. In connection with, the purpose of the present research definition morphofunctional conditions of various departments of heart was above-stated at diagnostics of an intimate ischemia.
Material and methods For achievement of an object in view researches of hearts died of a heart attack of a myocardium have been lead (have been carried out), and also have been investigated 32 morphometric parameters, such as: weight of a cardiac muscle, pure weight of heart, weight right and left ventricles, an index right and left ventricles, an index of blood supply of a myocardium. All received data have been processed statistically on personal computer with use of standard software package Windows Excel.
Results Summarizing macro- and the microscopic changes which have been found out by us in a cardiac muscle, it is possible to make the conclusion that the long reorganization occuring in a cardiac muscle in conditions increasing hypoxy, results in an exhaustion processes, to use of all possible resources cardiomyocytes and causes development decompensation.

P-526

The mucin genes in cardiac myxoma

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Introduction Cardiac myxoma, the most common primary tumor of the heart, has a varying clinical presentation and immunohistochemical profile. An abundant mucopolysaccharidic matrix exists, including mucin, within the cardiac myxoma. This study elucidates the clinical presentation and expression of mucin genes in cardiac myxoma.
Materials and methods From December 1976 to February, 2003, the retrospective study included 89 consecutive patients with cardiac myxoma underwent surgical excision. Detailed clinical parameters were reviewed as well. Mucin genes, Additionally, MUC1, MUC2 and MUC5AC, have been studied by immuno-histochemical method. The expression was scored from 0 to 3.
Results 52 (60%) women and 37 (40%) men with a mean age of 38 +/- 21 years comprised the control group. The presentation included asymptomatic (41%), dyspnea (35%), stroke (23%), chest pain (7%), fever (6%), syncope (5%) and tricuspid regurgitation (70%). We divided these patients into two groups; group A contained the 78 myxoma located in the left atrium, group B included three (3%) recurrent myxomas and 8 (9%) myxomas other than in left atrium. They did not differ with respect to many pathological scores, such as vascular proliferation, inflammation, cellularity, and hyaline. However, although less calcification and thrombosis occurred, more congestion was found in group B ($P < 0.0001$). Moreover, MUC1/5AC had a higher expression than MUC2 ($P < 0.005$). Interestingly, the group A displayed a stronger mucin expression than group B did.
Conclusion Preliminary results demonstrate invaluable and different pathological patterns, including mucin genes, between the myxoma coming from the left atrium and the other sites.
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P-527

Morphometric analysis of atherosclerotic changes in human coronary arteries adventitia

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Introduction Nowadays understanding of atherogenesis, Russell Ross' response-to-injury paradigm, considers atherosclerotic lesions as "a protective, inflammatory-fibroproliferative response" to harmful stimuli. Among these stimuli are the major risk factors, which contribute for atherosclerotic cardiovascular complications. Arterial blood flow impairment is associated with the progression of intimal atherosclerotic lesions. That is why most of the research work is concentrated upon intima, while adventitia is neglected. Several lines of evidence clearly show that adventitia is not an innocent bystander in atherogenesis. The aim of the present study is to evaluate morphologically adventitial changes in atherosclerotic arteries.
Materials and methods The cellular components and adventitial thickness are followed in 118 postmortem human coronary arteries.
Results Mononuclear cellular infiltration (lymphocytes, mast cells), neoangiogenesis and collagen overproduction are established in this part of adventitia, that corresponds to atherosclerotic lesion. Morphometric analysis reveals increased adventitia/media thickness ratio. The adventitial thickening represents an example of negative vascular remodeling, because of hampering the compensatory enlargement of the affected artery. On the other hand the cellular processes involved in adventitial thickening could be responsible for atherosclerotic lesion initiation.

Conclusion We need further arguments into the cell biology of atherosclerosis, viewed as a vascular healing process, involving also the adventitia, to provide new insights into atherogenesis and alternative therapeutical strategies respectively.

P-528

Study on postmortal autolytic changes of the myocardial blood vessels. Experimental study

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Introduction Modern forensic medicine practice has not yet been given the methods of investigation that would enable answering with sufficient accuracy to the question of death occurrence time. Present work is an attempt to make contribution to that very complex and significant problem in forensic medicine.
Materials and methods The investigation has been done on 112 Wister rats. The sacrifice was exerted by strangling. To respond on questions how both temperature of microenvironment and passed time influence on coronary arteries, the experimental animals were divided into 3 groups: I (10 C), II (20 C) and III (30 C). Every group had 8 subgroups, depending of passed time from the sacrifice, beginning of 1 hour, through 2, 4, 6, 12, 24, and 36 up to 72 hours. Control animals were autopsied and myocardial sections were taken immediately after sacrificed animals. Formaldehyde fixed and paraffin embedded blocks were cut and stained by HE, Van Gieson and Gomori methods.
Results In groups II and III almost all blood vessels of heart muscle had fragmented walls in the animals examined upon 72 hours after sacrifice. The vessels were almost normal in the group I also up to 72 hours after sacrifice. Basal membranes of blood vessels were observed upon 72 hours after sacrifice at the temperature of 10 C. Fragmented basal membranes in II and III group were found, in the form of spider's web residues. Total hemolysis of erythrocytes was upon 1 hour after sacrifice in III temperature group. However, contours of erythrocytes could be recognized upon 48 hours after sacrifice in I experimental group.
Conclusion Autolysis of myocardial vessels is influenced by two factors: time interval from the death and micro environmental temperature. The temperature exerted more profound influence on autolysis than time interval. The observed autolytic changes could be used in the expertise of the time of death occurrence.

P-529

Expression of tumor necrosis factor (TNF)-alpha and TNF-alpha receptors in human viral myocarditis

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Introduction Tumor necrosis factor (TNF)-alpha has been recognized as important physio-pathogenic factor in the initiation and continuation of inflammatory cardiomyopathies. Experimental and preliminary human studies have demonstrated that TNF-alpha plays a crucial role in enteroviral-induced myocarditis. The aim of this study was to investigate the expression of TNF-alpha and both its receptors (TNFRI and TNFRII) in the endomyocardial biopsies of patients (pts) with viral and non-viral myocarditis. Myocardial expression of TNF-alpha was then correlated with different clinical and pathologic findings.
Material and methods TNF-alpha expression was investigated in endomyocardial biopsies obtained from 38 pts with myocarditis and from 10 control subjects by using reverse transcriptase (RT)-polymerase chain reaction (PCR) and immunohistochemistry. Viral etiology was diagnosed by PCR in 20 cases. Immunohistochemistry was also used to analyse both TNF-alpha receptors (RI and II). Score 0-3 was used to grade morphological findings.
Results TNF-alpha mRNA and TNF-alpha protein was significantly more present in viral myocarditis than in non-viral myocarditis (16/20 vs 3/18, p=0.001). Remarkable immunostaining was observed for both receptors, particularly for TNFRI. Histological analysis revealed that myocardial necrosis (score 1.89 vs 1.15 p=0.01) and cellular infiltration (score 2.26 vs 1.78 p=0.05) were more prominent in TNF-alpha positive cases. Among TNF-alpha positive cases, TNF-alpha mRNAs were greater in pts with more impaired cardiac function.
Conclusion Our findings indicate that the expression of TNF-alpha plays an important role in the pathogenesis of viral myocarditis of any etiology and may influence severity of cardiac dysfunction. Cytokine effects are more strictly linked to over-expression of TNFRI.

P-530

Recurrence of viral myocarditis after cardiac transplantation: A retrospective study in a pediatric population

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Introduction Heart transplantation remains the only therapeutic option for pediatric patients (pts) with severe, end stage myocarditis. The aim of this study was to determine the presence of viral genome in native heart and the frequency of viral recurrence after heart transplant in pts with myocarditis.
Material and methods From March 1987 to November 2000, 36 heart transplants were performed in children (1 month to 18 years 24M/12F) at our Institution. Histology/immunohistochemistry was carried out in all native hearts; among inflammatory cardiomyopathy,4 cases were active myocarditis and 16 dilated cardiomyopathy/ chronic myocarditis. Foci of active myocarditis were also found in one case of hypertrophic/restrictive cardiomyopathy and one case of congenital heart disease respectively. Retrospective PCR (polymerase chain reaction) and sequencing analysis for detection of cardiotropic viral genomes was performed in 20 native hearts (55%) and in all serial post-transplant biopsies from viral PCR positive cases.
Results PCR amplified a viral product in 10 native hearts (50%). PCR analysis on postransplant serial biopsies disclosed the same viral infection in 5 of 10 (50%). Early enteroviral recurrent

myocarditis with identical genotype was demonstrated in 2 cases at 1 month after transplant. Fatal outcome occurred in 4/5 pts with viral recurrent myocarditis when prompt molecular diagnosis was not available; misdiagnosis of acute rejection in histological monitoring may have concurred in the fatal outcome.
Conclusion Viral myocarditis frequently relapses after heart transplantation in children and is a risk of fatal outcome. Molecular study in native hearts is mandatory to improve diagnostic accuracy allowing to a more strict surveillance after transplantation.

P-531

Sudden cardiac death induced by acute viral myocarditis

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Introduction Most commonly the sudden cardiac death (SCD) is defined as unexpected death from cardiac causes early after of without the onset of symptoms. Data suggested that SCD is a multifactorial catastrophe involving in most instances myocardial lesions that we confirm by this report.
Materials and methods Five cases were autopsied in Institute for Forensic Medicine because they have died from SCD. The mean age was 29,4 years, including one boy (13 years) and one girl (14 years). All specimens were fixed in 10 % formalin. Paraffin sections were cut and stained by: HE, Van Gieson and AB-PAS, pH=2,5.
Results Clinical symptoms lasted short time: fatigue, dyspnea, palpitations and precordial discomfort, accompanied by fever. Macroscopically, the hearts were normal or enlarged, with dilatation of chambers. The ventricular myocardium was typically flabby and mottled by either pale foci or minute hemorrhagic lesions. Histological finding: myocarditis was characterized by an interstitial mononuclear predominantly lymphocytic inflammatory infiltrate and by injury of focal necrosis to myocytes adjacent to the inflammatory cells; focal aggregates of macrophages and microhaemorrhages, often surrounding the conduction system, were also observed. Scattered incipient fibrosis inside the areas with disappeared myocytes was one of the features of myocarditis.
Conclusions The ultimate mechanism of death in viral myocarditis was fatal arrhythmia induced by inflammatory mononuclear infiltrates of the conduction system.

P-532

Surgical pathology of primary cardiac and pericardial tumors in the paediatric age

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Background Primary cardiac and pericardial tumors (CPT) are rare at all ages and even less common in infancy and adolescence. Aim of this study is to assess the prevalence and pathologic features of paediatric CPT consecutively examined in a single Institution.
Methods and results Among 170 surgically removed CPT collected at our Cardiovascular Pathology Unit in the time interval 1970-2002, 21 (12%) occurred in the paediatric age (<18 yrs). We retrospectively analysed them by histotype, age and clinical presentation, location, type of surgery and outcome. They were 11 male and 10 female pts, age ranging from 2 days to 18 years (median 4 months). The majority of CPT were diagnosed before the age of 1 year (76%), including 4 prenatal diagnosis by foetal echocardiography. In the remaining 17 pts, clinical presentation of CPT was congestive heart failure in 5, cardiac murmur in 5, systemic embolism in 2, and constitutional symptoms in 1, whereas four were completely asymptomatic and the mass was an incidental finding at echocardiography (3) or during cardiac surgery for congenital heart disease (1). PCT were located most frequently in the right ventricle (6), followed by right atrium (3), left atrium (4), left ventricle (3), tricuspid valve (2), pericardium (2) and ventricular septum (1). All had surgical resection except one who underwent cardiac transplantation. As far as histotype, myxoma was the most frequent (7), followed by rhabdomyoma (4), fibroma (3), angioma (3), pericardial teratoma (2) and hematic cyst (2). No recurrence was found at echo during follow-up and only one pt died three years after cardiac transplantation due to cerebral malignancy.
Conclusions With the improvement of cardiac imaging techniques, CPT have increasingly been detected even early during life. Despite the benign histotype, CPT carry the risk of heamodinamic, embolic and arrhythmic complications. Prompt surgical resection is indicated in all symptomatic pts and in those with tumour related obstruction. Among surgically resected primary CPT in the paediatric age rhabdomyoma is less frequent than expected since it often does not require surgical resection due to its potential spontaneous regression.

P-533

In vivo histologic diagnosis of primary malignant cardiac and pericardial tumors

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Background Primary cardiac and pericardial tumors (CPT) are rare and even less common are malignant ones. Aim of our study was to assess the prevalence and histotype of malignant forms among a consecutive series of primary CPT examined in a single Institution.
Methods and results In the time interval 1970-2002 among 170 consecutive pts who had an in vivo diagnosis of primary CPT, either by surgical pathology and/or endomyocardial biopsy investigation, 16 (9.5%) had a malignant form. They were 7 female and 9 male pts, age ranging from 36 to 80 yrs (mean 54±12). Besides gross and histologic examination, tumour histotype was defined by immunohistochemistry using a large panel of antibodies and by transmission electron microscopy. Primary malignant CPT comprised 6 extracardiac (3 pericardial mesothelioma and 3 pulmonary artery sarcoma) and 10 intracardiac masses. The latter group consisted of angiosarcoma of the right atrium +/-right ventricle (3), undifferentiated sarcoma of the left atrium (3), fibrosarcoma of the right atrium (1), malignant fibrous histiocytoma of the left atrium (1), B cell lymphoma of the right atrium (1) and leiomyosarcoma of the right ventricle (1). In four (40%) a preoperative histologic diagnosis was achieved by transvenous endomyocardial biopsy (all right-sided). Four malignant primary CPT, all located in the left atrium, had a preoperative misdiagnosis of myxoma. Surgical resection was

performed in all but two pts. All pts died with a maximum survival time of 24 months.
Conclusions Primary malignant CPT are rare and represent 9.5% of all primary CPT in our series. The prognosis is poor and generally measured in months independently from the tumour histotype and from surgical resection and chemotherapy. Endomyocardial biopsy is a safe procedure to achieve an in vivo diagnosis in the setting of right intracavitary masses.

P-534

Haemangioendothelioma retiforme. A case report

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Introduction Haemangioendethelioma retiforme is rare form of tumor which may have histological appearance and biological behavior of a low grade angiosarcoma. This is the reason why this form of tumor represents great diagnostic problem especially in small biopsies. We present the case of an 50- year old man with inherited gigantism and tumor masses in soft tissue at the right forearm and hand without changes on bone and joint tissue.
Materials and methods Gross and microscopic findings were noted. Histochemical stains with Masson trichrom, elastica, Perls` and PAS reaction and immunohistochemical analyses for CD 31, F VIII and a smooth muscle actine was performed in paraffin-embedded sections.
Discussion and conclusion Patient had several esthetic operations in last 20 years. Few of this subcutaneous lesions have undergone exulceration followed by bleeding in past 3 years, which was the reason for operation three times in a row. Diagnosis of retiform hemangioendothelioma was than established. One of the biopsies showed well differentiated angiosarcoma. Vascular malformations were evident in all biopsies. Inherited gigantism was probably the consequence of morphological changes in soft tissue related to the existence of vascular malformation. These were preexisting changes that led to the development of multiple focuses of retiform hemangio-endothelioma.

P-535

Primary intimal sarcoma of the aorta. Report of an autoptic case

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Introduction Primary sarcomas of the aorta are extremely rare neoplasms, described in occasional single case reports and antemortem diagnosis is difficult because of their polymorphous presentation. The tumours are classified into intimal and mural types according to their location and clinical behaviour. We describe a case of intimal sarcoma arisen in the thoracic aorta of a 62-years-old woman with multiple embolic abdominal metastases (spleen, kidney, adrenal).
Case report In november 2002 the patient was hospitalized for the embolic occlusion of the left popliteal artery. The recent clinical history revealed irritative cough, dispnea and fever. The laboratory tests reported anemia (hemoglobin: 8.3mg/dl) and the VES was 119. Electrolities, liver function tests and fibrinogen were within normal limits. The antiphospholipid antibody and lupus anticoagulant were negative. A CT-scan showed an intraluminal thrombus-like mass in the thoracic aorta and multiple geographic infarctions of the spleen suggestive for a primary aortic tumor. She died before undergoing to surgery, for left ventricular failure.
Results Post-mortem examination showed mild atherosclerosis and a mass with grayish-yellow, solid, rubbery and mixoid aspects, attached to the wall of the aorta, just after the origin of the left subclavian artery. Histologic sections disclosed a spindle-shaped sarcoma cells with pleomorphic nuclei in a myxoid stroma; necrosis was abundant. At immunohistochemistry tumor cells resulted positive for vimentin, actin and cytokeratins.
Conclusion The preoperative diagnosis of sarcoma of the aorta is possible but it should be supported with imaging techniques; the definitive diagnosis need to be confirmed histologically and immunohistochemically.

P-536

Arterial allografts cryopreservation antigenicity in pigs

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Background Coronary surgery requires vascular grafts. Donor fresh arterial grafts are useless because of rejection vasculopathy. Cryopreservation can be a good way for banking purposes and may limit rejection. The aim of this study was to assess the utility of arterial cryopreservation in increasing the quality of grafts, in an animal model in pigs.
Material and methods Small arteries, less than 3mm in diameters were implanted in pigs. Three groups were done: Group1(n=5): fresh autograft (AU) and fresh allograft (AL) one in each side; group 2 (n=5): Cryopreservated AU and cryopreservated AL; group 3 (n=5): fresh AL and cryopreservated AL. All animals got aspirin for three months and group 3 also cyclosporine A. The implanted grafts were embedded in paraffin and microscopically studied with hematoxilin/eosin and elastic stains for morphology and morphometrics. A basic immunohistochemical study with CD3 and MAC387 was also performed.
Results The patency rate of cryopreservated AL and AU was 100%, as it was in the fresh AU. The inflammatory reaction, evaluated by CD3 and MAC387 was present in all AL arteries, and absent in AU cases. In group 3, the infiltration was lower in cryopreservated, than in the fresh ones.
Conclusions In our series of pig's arteries, cryopreservation increases vascular patency and the inflammatory response is lower than in fresh arteries. This fact is independent of the immuno-supressive therapy.

P-537

Cytokeratin profile and neuroendocrine cells in the glandular component of cardiac myxoma

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Introduction Glandular cardiac myxomas are very rare tumors of uncertain histogenesis, displaying glandular structures within otherwise typical myxomas. Origin of glands has been attributed to epithelial differentiation of a cardiomyogenic precursor cell or to entrapped embryonal rests. We studied four cases of glandular myxomas (3 sporadic and one familial) to define the immunophenotypic profile of the glandular elements.
Materials and methods Histology and immunohistochemistry were performed on serial sections. Broad spectrum cytokeratin, cytokeratin 7 (CK7), cytokeratin 20 (CK20), CEA, EMA, chromogranin A, calretinin, thyroid transcription factor-1 (TTF-1), Ki-67 and p53 were investigated.
Results Three patients were males, aged 55, 23, 57, respectively and the fourth a 45 y.o. woman. The glands were either scattered within myxomatous stroma (3 cases) or in groups. In the latter case the glands featured villous projections, irregular profile, active inflammation or focal low grade dysplasia, and showed expression of acidic and neutral mucins (mostly sialomucins). Cytokeratin 7 and 20 co-expression was found, similarly to foregut derivatives. There were scattered chromogranin-positive neuroendocrine cells in one case and Ki-67 immunostaining showed a very low proliferative activity. There was no reactivity for TTF-1, calretinin or p53. None of the patients had adenocarcinoma or other neoplasm, either at diagnosis or at 1-19 years follow-up.
Conclusions Our findings indicate that glandular component of cardiac myxoma is morphologically heterogenous. The isolated glands suggest a possible divergent (epithelial) differentiation of myxoma cells, but other findings point to the possibility of entrapped foregut rests with mature neuroendocrine and mucous cell populations. Work partially supported by grants from the Italian Ministry of Education (Rome, to MP) and the Associazione Italiana per la Ricerca sul Cancro (Milan).

P-538

Infection and immune inflammation peculiarities in atherosclerosis

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Introduction During the last decade the basic postulate of atherosclerosis etiology and pathogenesis has changed. This review presents a spectrum of data regarding the link between infection and immune inflammation development in atherosclerosis.
Material and methods This study has been carried out on the human aorta, coronary heart vessels collected from 59 early performed autopsies and on the carotid arteries gathered during surgical vessels remodeling of 18 persons. The patients with cardiovascular insufficiency on atherosclerosis background from 31 to 59 years were taken into study group. Chlamydia Pneumoniae was detected by routine microscopy on Shtive fixed material, and by immunohistochemical reaction performed on cryophilic specimens marked with Fits antibodies. HSP-60 and TNF-α implication have been studied trough immunohistochemical reaction using monoclonal antibodies. This material was fixed in 4% formaldechyde solution.
Results Chlamydia Pneumoniae has been detected in endothelial cells, macrophages, smooth muscle cells of fatty streaks and fibrous plaques. Its detection incidence decreases in advanced arterial lesions. We also determined that Chlamydia Pneumoniae infection is capable to induce immune reaction against HSP-60 which can be considered like a target in autoimmune reaction. They produce an cytotoxic effect on the endothelial cells followed by permeability disturbances, faciliting the atherosclerotic lesions progress. Among the inflammation mediators in increased quantity TNF-α have been detected being localized on the surface and in depth of atherosclerotic plaque around the foam cells.
Conclusions Our outcomes demonstrate the toxic and infectious factors existence which can be considerated decisive in atherosclerosis pathogenesis. This data give us the reason to elaborate other diagnosis and treatment strategy.

P-539

Cardiac hemangioma

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Introduction Primary tumours of the heart are rare, the majority are benign, myxomas representing 50-80%. Hemangiomas are extremely rare, with an incidence of 0.8%-1.6%. They usually occur in adults, mostly are an incidental finding. We present a man with asymptomatic cavernous hemangioma of the left atrium.
Case report Transthoracic echocardiogram of a 46-year-old man with arterial hypertension, otherwise healthy, with normal ECG and chest X-ray, revealed a wide based isoechogenic tumour, measuring 3.1 x 2.2 cm, located in the moderately enlarged left atrium, attached to interatrial septum and anterior wall, adjacent to mitral valve annulus, sparing oval fossa. It showed minimal mobility but did not cause any obstruction of the blood flow or mitral regurgitation. Macroscopically, surgically removed tissue was spongy, partly haemorrhagic. Microscopically, there were cavernous venous structures, filled with blood and focally thrombosed. Focally, a few smaller capillary channels were found. There was no endothelial atypia and no papillary hyperplasia.
Discussion and conclusion Histologically, hemangioma of the heart has two major differential diagnostic possibilities. In case of a myxoid background, it is possible to be misdiagnosed as myxoma, but in hemangioma there are no myxoma cells and no ring structures. Grossly, opposite to myxomas, hemangiomas are rarely attached to the oval fossa. Papillary hyperplasia, which is reactive endothelial proliferation in hemangioma, may lead to misdiagnosis of angiosarcoma. Angiosarcoma can be excluded only when the whole tumour is examined and no marked endothelial atypia, cellular areas of spindle cells with poorly formed vascular channels, and necrosis are found. Compression, obstruction, effusion and bleeding are responsible for clinical picture. Symptoms depend also on the location, size and invasiveness. The natural history is unpredictable, for this reason surgical intervention is mandatory.

P-540

Limb-body wall complex (LBWC) versus amniotic band syndrome (ABS): a new pathological hypothesis

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Introduction The limb-body wall complex is a congenital syndrome, which includes limb defects, abdominoschisis associated with facial and neural tube malformations. Some authors believe that the limb-body wall disruption is separate from amniotic band syndrome. Others suggest that both belong to a wider spectrum, with a possible overlap between these two categories. In the present study, we describe a case of LBWC and based on our findings we suggest a new pathogenic mechanism.
Case report A 32 years-old Cap-Verdean female gave birth at 33 weeks to a pair of twins. One foetus showed multiple malformations incompatible with life. Physical examination showed partial lower extremity amputations. A large omphalocele was also noted. There was a large lumbosacral meningocele and the head showed micrognathia and high-arched palate. There was right hydronephrosis secondary to right ureteral obstruction and cloacal anomaly. The lungs were hypoplastic. The histology of the internal organs was characteristic for the developmental stage and cytogenetic testing revealed a normal male karyotype.
Discussion and conclusion We believe, that early amniotic bands may lead to vascular insults, proximally and distally by altered haemodynamics. The lower limbs were partly amputated as a result of the continued growth associated with altered vascular supply. The same mechanism may explain the other findings as well. Vascular disruption by virtue of altered haemodynamic state may give rise to thromboemboli, which may affect the growth of external/internal structures, distant from the site of disruption. However, this vascular lesion was masked by continued growth of the foetus. Therefore, the ABS may precede and may cause the LBWC.

P-541

Two cases of pleuropulmonary blastoma

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Pleuro-pulmonary blastoma (PPB) is a malignant tumour of childhood. The most common clinical sign is respiratory difficulty. PPB appears as a pulmonary and/or pleural-based mass and is characterized histologically by a primitive, variably mixed, blastematous and sarcomatous appearance. No metastases have been reported in neonatal cases. We describe two cases that presented PPB. The first was a term male infant of a G8P7 mother. The pregnancy was uneventful. After normal delivery, a male infant with severe respiratory distress was born. By ultrasound examination, a thoracic mass were identified. There was no response to the intensive care. The infant died at the age of 3.5 hours. At autopsy, a tumour of 212 g, measuring 10x8 cm was found in the left lung. In addition, there was a smaller mass, of 3 cm diameter in the mediastinum. Microscopically, the tumour masses consisted of PPB. The second case was a 3-year-old female with persistent fever and respiratory difficulties. In CT scan, the lower lobe of the left lung was replaced with a tumour mass. During operation, the tumour was seen to be infiltrating to the mediastinum and was partially resected, followed by intense chemotherapy. Histologically, the diagnosis of PPB was given. The child has shown no sign of the disease during follow-up of six months. The PPB is regarded as the pulmonary dysontogenetic analogue of Wilms' tumour, neuroblastoma, and hepatoblastoma. The first case showed its highly aggressive nature, with mediastinal metastases found at birth.

P-542

Causes of intrauterine foetal death associated with placental pathologic features

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Introduction Improvements in antenatal and intrapartum care have reduced perinatal mortality, but the rapid advances in intensive neonatal care have had a dramatic effect in reducing neonatal deaths so that stillbirths have become relatively more important. The aim of this study was to identify the relationship between placental lesions and stillbirth.
Materials and methods We analysed the causes of intrauterine foetal death after the 26th week of pregnancy during the years 1991-2002 at the Departments of obstetrics and gynaecology of the Clinical Hospital Osijek and General Hospital Karlovac. A complete autopsy was made on each stillborn, including investigation of the placenta, cord and foetal membranes. Histological specimens were studied from all internal organs of the foetus and from the placenta, cord and membranes. Haematoxylin and eosin stain is used in routine causes, PAS staining to assess thickening of the basement membrane and trichrome staining to assess stromal fibrosis of the villi when it was necessary.
Results There were 201 stillbirths who died before labour - 174 placentas of them were examined and 27 weren't. Abnormalities were found in 142 (81.61%) placentas and 'normal' placentas were 32 (18.39%). Autopsy findings in stillborn foetuses: asphixia 133 (66.17%), maceration only 57 (28.36%), major malformations 6 (2.99%), vitium cordis 2 (0.99%), anencephalia 2 (0.99%), others 1(0.50%). Placental pathologic findings: infections 26 (18.32%), single chronic and acute infarction 36 (25.35%), multiple chronic and acute infarctions 23 (16.20%), placental failure for other reasons 16 (11.27%), chronic ischemic change 12 (8.45%), infections associated with circulatory disturbances 13 (9.15%), retroplacental haematoma 9 (6.34%), massive subchorial thrombosis 2 (1.41%), chorangiosis 2 (1.41%), umbilical cord tumour 1 (0.70%), intervillous thrombus 1 (0.70%), chorionic cysts 1 (0.70%).
Conclusion The most of women with a stillborn infant were quite healthy and did not reveal any risk factors when assessed on clinical grounds. There were patients in whom evident foetal growth retardation had been overlooked at the maternal health centre before the death of the foetus. The importance of routine ultrasound screening for detection of unrecognised risk patients and placentas is emphasized.

P-543

T - lymphocyte subtypes in the diagnosis of celiac disease

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Introduction The objectives of this study was tot verify comparatively clinical age reported antecedents and the morphological aspect of the intestinal mucosa in patients sensitive to gluten, hospitalised at IOMC (Alfred Russescu Hospital) in the last 7 years. Celiac disease is an inflammation most frequently affecting the proximal small intestine, depending on the presence of gluten in the diet, whose pathogenesis seems to be immunological in nature.
Methods 107 cases were divided in three groups following clinical manifestations types at hospitalisation time: typical digestive, untypical digestive and extra digestive manifestations. Intestinal biopsies, made with Crosby probe, in children aged between 1.3 and 8 years (one single case was diagnosed as late as at the age of 15), regardless of gender. Then we analysed morphologically (HE usual and PAS histochemical staining) and immunohistochemically (lymphocytes B, T with possible subtypes).
Results The lesions counted according to the Marsh score were at the first biopsy: - 42 Marsh I infiltrating lesions (normal mucosa architecture, villi epithelium infiltrated with lymphocytes; lymphocytosis score: 30 lymphocytes /100 enterocytes); - 15 Marsh II infiltrating lesions (almost normal villi, enlarged crypts with immature epithelium and infiltrated with lymphocytes); - 17 Marsh IIIa destructive lesions (partial villi atrophy (VA) shortened, flattened villi, slight infiltration with intraepithelial lymphocytes and hyperplasic crypts); - 18 Marsh IIIb destructive lesions (subtotal VA clearly atrophied villi, still recognizable, widened hyperplasic crypts containing immature epithelial cells with a higher growth rate and influx of inflammatory cells); - 25 Marsh IIIc hyperplasic lesions (total VA completely absent villi, with severe atrophy, atrophied, hyperplastic glands and infiltrating lesions). The immunohistochemical tests have indicated the prevalence of T lymphocytes (UCHL1, CD3, CD4, CD8, gama-delta) both in the luminal epithelium with various degrees of aggression in lamina propria and also spread in stroma. B lymphocytes (L26) are distributed prevalently nodular in stroma.
Conclusion In conclusion, it is CD4 T cells that are present in particular in the control of the gluten immune response in patients with Marsh I and Marsh III lesions.

P-544

Clinical morphological analysis of early forms of polyarteritis nodosa

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Introduction Polyarteritis nodosa is a necrotic vasculitis of small and medium size arteries of not fully recognized aetiology. However, it occurs mostly in adults, it can also occur in children. The cutaneous form is one of the types of polyarteritis nodosa. It occurs in children very rarely. Polyarteritis nodosa is diagnosed on the basis of histopathologic results and clinical criteria established by the America College of Rheumatology. The aim of the study was to present 3 cases of polyarteritis nodosa in children.
Case reports Three cases of cutaneous polyarteritis nodosa in 16-year old girls and 1 case of general form of p.n. in a 14-year old boy At the beginning a tumour of pancreas head was suggested in the latter case. The final diagnoses in all cases were defined on the basis of histopathologic
Results The morphologic studies showed: skin and subcutaneous tissue specimens of 10mm diameter and 15mm thick were evaluated. Epidermis and corium were of normal structure. Abundant inflammatory infiltration consisting of neutral and acidophilic granulocytes, lymphocytes and plasmatic cells were observed on the boarder of corium and subcutaneous tissue. The infiltration was situated mainly in arterial walls and surrounding fibrous and fatty connective tissue. Different degrees of change advancement were observed in the walls of the vessels. Some changes were characteristic for an early stage of a disease and were demonstrated as swelling of the vessel wall and surrounding tissues (degeneration stage). In the oedematous wall fibrin concrement and only a few inflammatory cells were present. Other cells included abundant transmural inflammatory infiltrations (inflammatory stage). Vessel endothelium underwent desquamation and thrombi begun to appear in the arterial lumen. Only in few vessels, the process of considerable advancement of changes was observed – a repair process with consecutive fibrosis and thickening of arterial wall with considerable narrowing of its lumen. The described children were treated successfully with corticosteroid therapy and antistreptococcal prophylaxis with long-term remission.

P-545

Comparative morphologic analysis of the small intestine mucosa in a course of coeliac disease

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Introduction Microscopic diagnosis of oligobiopsy material taken during endoscopic examination plays an important role in diagnosing syndromes of absorption disorders, especially in case of celiac disease. The aim of the study was a morphologic evaluation of the small intestine mucosa and comparison of the used from many years Shiner/Shmerling classification with a new modified Marsh classification.
Materials and methods During the study, 500 bioptates if small intestine evaluated in the Chair and Department of Pathomorphology in Zabrze in the years 1998-2002 were analysed again. Out of the bioptates 344 were with I of villous atrophy, 88 with II, 64 with III and 4 with IV. Study material was from the paediatric hospitals in Zabrze and Bytom. During routine histopathologic diagnosing, villus atrophy was evaluated in the Shiner/ Shmerling scale. Presently, the Marsh scale is being introduced into histopathologic diagnosing. It takes into consideration not only features of villus atrophy and glandular crypts proliferation but also intraepithelial lymphocyte infiltrations. Another evaluation of villus atrophy was performed according to the modified Marsh classification.

Results The received results are presented in the modified Marsh classification: type 0 – 309 (61.8%), type 1 – 25 (5.0%), type 2 – 10 (2.0%), type 3a – 88 (17.6%), type 3b – 48 (9.6%) and type 3c – 20 (4.0%). Our study allowed to compare two classifications used in the evaluation of the atrophic and proliferous processes and to select from the Shiner/Shmerling degree I a group of cases (type 1 and 2 according to Marsh), which had no equivalents in the so far used scale. Moreover, it was shown that the sub-groups of the type 3 of destructive changes did not correspond fully with the so far used Shiner's degrees II-IV. It enables fast comparison of the atrophy degree with the results received before the introduction of the new modified classification. Moreover, a routine application of precise descriptions of histopathologic results of small intestine mucosa in syndromes of absorption disorders facilitates retrospective selection, from the so far used Shiner's degree I, of the cases corresponding with Marshal types 1 and 2.
Conclusions Application of the modified Marshal classification for an evaluation of the small intestine mucosa in celiac disease enables accurate presentation of atrophic and proliferous changes including selection of type 1 and 2, which have no equivalents in the so far used Shiner/Shmerling classification.

P-546

Embryonal rhabdomyosarcoma: a case report

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Rhabdomyosarcoma is an aggressive malignant skeletal muscle neoplasm arising from embryonal mesenchyme. The head and neck region is the most common site for this tumour in children. Neonatal presentation of this tumour is rare. We present an autopsy case of a female newborn having a large tumour mass (11x9x7 cm) in the frontal region, covering the entire upper half of the face. Similar subcutaneous noduli with diameter 3,5 cm, 3 cm and 2 cm were found on the left cheek, and another 4 with diameter 3,5 cm, 2,5 cm, 2 cm and 2 cm on the right cheek. The histological examination showed a tumour composed of large spindle cells with myoblastic features and small round to elongated undifferentiated cells. Associated with the spindle cells is various numbers of large round or globular cells with hyperchromatic nuclei surrounded by acidophilic granular or homogeneous cytoplasm. Cross-striations were found in the spindle cells. Imunohistochemically tumour cells were positive for desmin and vimentin. The final diagnosis was embryonal rhabdomyosarcoma. Immunohistochemical staining helped to make a proper diagnosis.

P-547

Effects of Sildenafile on ipsilateral testis damage following testicular torsion in rats

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Introduction Testicular torsion results in damage of the gonad and it is a surgical emergency. Testicular torsion evokes infertility as a result of ischemia. The aims of this study were to determine the effects of a vasodilatator agent Sildenafile on the testicular damage following testicular torsion.
Materials and methods Forty-two rats included to the study were divided into 7 groups: group 1; control, group 2; Sham, group 3: torsion (detorsion is performed 2 hours after torsion and orchiectomy is done at the 4th hour), group 4; torsion, detorsion and Sildenafile (1 mg/kg), group 5; torsion and Sildenafile (2 mg/kg), group 6; Sildenafile (1 mg/kg) without torsion, group 7; Sildenafile (2 mg/kg) without torsion. Testicular specimens were examined histopathologically for Johnsen tubular score and tubular diameter. The differences of these parameters between groups were evaluated statistically.
Results Johnsen tubular score and tubular diameters were fond to be significantly different in control and torsion groups ($p<0.05$). Johnsen tubular score and tubular diameter were significantly higher in the torsion groups which were given Sildenafile, and these parameters were again higher in the group which have taken Sildenafile at a higher dose ($p<0.05$).
Conclusion It is concluded that, by inhibiting cyclic guanosin monophosphate specific phosphodiesterase and resulting in vasodialatation, Sildenafile reduces the testicular damage caused by torsion (ischemia).

P-548

Expression of Bcl-x (S/L) in Wilms tumour

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Introduction Apoptotic cell death represents an important mechanism for the precise regulation of cell numbers in normal tissues. Various apoptosis-associated regulatory proteins may contribute to the rate of apoptosis in neoplasia. The present study analyses the inter-relationship between the expression of the pro-apoptotic Bcl-x (S/L) protein and histological type and stage of Wilms tumour.
Materials and methods Bcl-x (S/L) expression was investigated using an indirect immunoperoxidase technique, applying antibodies to Bcl-x (S/L) protein. Correlation of semi quantitatively scored Bcl-x (S/L) levels with histological type and tumour stage was performed for 28 cases of Wilms tumours (23 classical and 5 anaplastic).
Results The expression of Bcl-x (S/L) protein was present in most cases of Wilms tumour (71%). Generally, Bcl-x (S/L) was expressed in the blastemal and epithelial components of Wilms tumour. In most cases, the expression of Bcl-x (S/L) was moderate. Blastemal Bcl-x (S/L) expression decreased with tumour stage. There was no correlation between histological type and expression of Bcl-x (S/L) protein. Diffuse expression of Bcl-x (S/L) protein was observed in 2 cases of anaplastic Wilms tumour. These two cases were stage I and of unfavourable histology. In 3 cases of Wilms tumour, no immunohistochemical staining was observed. So, these results cannot indicate whether absence of Bcl-x (S/L) could play a role in the development of anaplasia.
Conclusion According to clinical follow-up, the results of our morphologic and immunohistochemical analysis suggest that decrease of the blastemal Bcl-x (S/L) protein could be associated with the capacity of Wilms tumour for local and distant spread.

P-549

Expression of alpha, beta and gama catenins in Wilms tumour

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Background Loss of catenins is suggested to promote tumour invasion and distant metastasis in tumour development. The aim of this study was to determine the expression of catenins in Wilms tumour and their relationship with histological type and stage of Wilms tumour.
Materials and methods Catenins expression was investigated using an indirect immunoperoxidase technique, applying antibodies to alpha, beta and gama catenins. Correlation of semiquantitatively scored adhesion molecule levels with histological type and tumour stage was performed on 28 cases of Wilms tumours (23 classical and 5 anaplastic).
Results Beta and gama catenins were present in most cases (89% and 75% respectively) and were usually coexpressed. Anaplastic type of Wilms tumour displayed higher beta catenin and lower gama catenin level in comparison with other types. Nuclear positivity, in each case, was found to be very strong, but was usually present only in 5 % of tumour cells. Beta and gama catenin expression was not correlated with stage of Wilms tumour. Alpha catenin was present in 35% cases of Wilms tumour. Stage III displayed higher alpha-catenin levels than stage I/II, suggesting that impairment of their function might exist without actual loss from tumour cells.
Conclusion Our results suggest that the level of catenin expression is associated with histological type of Wilms tumour, regardless of tumour stage. Observed nuclear expression of beta catenin indicates that the activation of wnt-signaling pathway is crucial step in Wilms tumour genesis.

P-550

Comparison of apoptosis and Fas, Fas-L and Bcl-2 expression in Wilms' tumor

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Background Apoptosis is a complex process involving many genes that play role as antiapoptotic or apoptotic factors. It controls normal homeostasis of tissues. Expression of these genes vary from one type of tumour to another type. The aim of this study is to evaluate the expression of Bcl-2, Fas and Fas-L and apoptosis in Wilms' tumour (WT).
Method Thirty paraffin embedded tissue specimens were included. Immunohistochemical study was performed for Bcl-2, Fas and Fas-L and in-situ end-labelling technique (ISEL) for apoptosis.
Results The expression of these proteins varied among the tumours. There was no statistically significant correlation between the expressions of Bcl-2, Fas, Fas-L and. apoptotic index.
Conclusion Apoptosis is an important mechanism in the growth of the tumours. We suggest that not only Bcl-2, Fas and Fas-L play role as regulators of apoptosis in WT but also other apoptotic and antiapoptotic genes involve this process. Further investigations are necessary to understand the molecular mechanism of the progression of WT.

P-551

Uncommon tumours of the neuraxis in childhood

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Case 1 A 3 year-old girl presented with seizures and weakness in the left part of de body of 1-year duration. CT scan showed a mass that filled the right ventricle, with a strong mass effect, peritumoural oedema, midline shifting and slight ventricular enlargement. A partial excision was carried out and the pathological diagnosis was that of choroid plexus carcinoma. The patient died six months later due to adjuvant therapy side effects. In the last WHO classififcation of brain tumours, grade III choroid plexus carcinoma is defined as a choroid plexus tumour showing features of malignancy: nuclear pleomorphism, frequent mitosis, high nuclear/cytoplasmic ratio, increased cellular, density, blurring of the papillary pattern, poorly structured sheets of tumour cells, necrotic areas, and often diffuse brain invasion. All those tumours classically displaying a papillary pattern must be considered in the differential diagnosis.
Case 2 A 5 month-old child presented with respiratory distress since 3 months old. On RX and CT scan, a tumour in the left hemithorax was seen, with right shifting of the mediastinum. The tumour was partially removed. Pathological diagnosis was that of spinal canal invasion by a soft tissue lipoblastoma. Eight months after surgery the tumour relapsed, infiltrating the epidural spinal canal from C5 to D8. The tumour was fully excised by means of microsurgery techniques, without neurological deficits. This case is an example of invasion of the vertebral canal by a soft tissue tumour previously removed. Lipoblastoma is a rare fatty tumour occurring in children in the extremities or less commonly in the trunk or retroperitoneum. The tumour is benign but may recur. Grossly, lipoblastoma showed a distinct lobulated pattern of yellow-grey myxoid tissue, histologically composed of highly vascular fat in a myxoid stroma resembling embryonic white fat. Differential diagnosis, based on clinical-pathological grounds, include fibrolipoma and myxoid liposarcoma.
Case 3 A 8 year-old boy presented with partial complex seizures for 1 year duration. NMR showed a discrete hypodense cortical-subcortical lesion in the left frontal lobe. Neither oedema nor mass affect was detected on CT scan. The tumour was completely removed by surgery and the pathological diagnosis was that of dysembryoplastic neuroepithelial tumor. This is an intracortical grade I tumour composed of oligodendrocyte-like cells. Macroscopically, is a nodular outward expansion of the superficial cortex. Histologically, a low-power view of a perpendicular section of the cortical surface shows the characteristic pattern of multiple intracortical nodules and internodular tissue, both containing mucin. Also seen, is a specific glioneuronal component of vertical columns of neu-

rites transversing the cortex, sheathed by oligodendrocyte-like cells accompanied by mucin and with 'floating neurons' (cortical ganglion cells seen in individual small pools of mucin).
Case 4 A 14 year-old boy presented with diplopia and proptosis. The NMR showed a hypodense, diffuse lesion in the left temporal and parietal lobes that produced a slight collapse of the left lateral ventricle. Two weeks later the patient developed symptoms of time and space cognitive disturbances, language difficulty and seizures. A biopsy was done and the pathological diagnosis was that of gliomatosis cerebri. This is a rare primary brain tumour characterized by a diffuse proliferation of neoplastic glial cells that typically involve multiple brain areas with preservation of brain structures. The diagnosis depends on both histologic and radiologic features. On NMR there is a uni or bilateral diffuse involvement of the white matter extending to the cortex with poor mass effect. Microscopically, there is a proliferation of monomorphous astrocytes, grade II or III, infiltrating the brain parenchyma freely. Some undifferentiated cells show 'naked' nuclei while others look like 'rod' structures. In cases of low cellularity and cytologically bland astrocytes, it may be extremely difficult to make the differential diagnosis with reactive gliosis. GC is associated with a poor prognosis.

P-552

Spleen depletion in neonatal sepsis and chorioamnionitis

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Introduction autopsy studies in adult patients who had died of sepsis revealed a profound, progressive, apoptosis-induced loss of cells of the adaptive immune system (Hotchkiss et al, New Engl J Med, 2003; 348:138-150). In neonatal sepsis and chorioamnionitis, thymus modifications and shrinkage have been exhaustively described (Toti et al, Hum Pathology 2000; 31:1121-1128), while morphology of the spleen has not. The aim of this study was to describe the morphology of the spleen in neonates with early onset sepsis and with chorioamnionitis.
Material and methods the study population has been described previously (Toti et al, Hum Pathology 2000; 31:1121-1128). Ten preterm or full-term neonates who died because of proven sepsis within 48 hours after birth (mean gestational age±SD, 29.2±6.2 weeks); 20 foetuses spontaneously aborted (22±7.4 weeks) because of extensive ascending chorioamnionitis. Controls included 10 foetuses with induced termination of pregnancy (20±2.4 weeks) and 10 babies who died suddenly in the perinatal period, without evidence of chorioamnionitis. Spleen cell populations were studied by means of immunohistochemistry.
Results Early onset sepsis in the neonates occurred with severe spleen depletion, involving both B and T lymphocytes. In particular, CD20+, CD45R0+ and CD4+ cells were dramatically diminished in the spleen of neonates with sepsis, in comparison to controls. Foetuses with chorioamnionitis also showed spleen cell depletion.
Conclusion These results indicate that preterm and term neonates show an inflammatory reaction similar to that of adult patients, and that chorioamnionitis is associated with an unspecific inflammatory response comparable to that of sepsis.

P-553

Familial hemophagocytic lymphohistiocytosis with CNS involvement - autopsy case presentation

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Introduction Hemophagocytic lymphohistiocytosis (HLH) is a disorder of an immunological disturbance with proliferation of cells of the mononuclear phagocyte system, with the histiocyte as a central cell. It includes two different conditions: primary or familial HLH, an autosomal recessive disorder in which most patients develop the disease very early in life, and secondary HLH associated with neoplastic, autoimmune and a variety of infectious diseases, occurring as sporadic cases throughout life period. Clinically the disease is characterised by the occurrence of a hemophagocytic syndrome of prolonged fever and splenomegaly with laboratory signs of cytopenias, lipid, coagulation and liver abnormalities. Hemophagocytosis is readily identified in bone marrow, spleen or involved lymph nodes. Central nervous system spread of the disease may be present early, but is frequently seen later during the course of the disease. The aim of this study was to presentat an autopsy case of a 13 months old boy.
Case report Signs of HLH were present right after birth. He was treated by the treatment protocol HLH-94. The disease later progressed with CNS involvement. A complementary bone marrow donor was not found in time.
Materials and methods Tissue samples collected at the autopsy were proceeded with standard histological and immunohistochemical methods.
Results Severe bleeding in the right lung with other signs of coagulopathy were found as the main cause of death. Hepatosplenomegaly was a prominent feature. We detected hemophagocytosis in the bone marrow, liver and central nervous system.
Conclusions The diagnosis of HLH must be based on clinical, laboratory and cyto/histological findings. The pathogenesis of familial HLH is still not completely understood.

P-554

Evaluation of goblet cells in small intestine epithelium in celiac disease in children

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Introduction A changeable number of goblet cells in intestine epithelium is rarely a matter of consideration among other morphological factors of small intestine mucous membrane atrophy in celiac disease. It is also not evaluated in the current classifications of goblet cell condition in celiac disease. The aim of the study was to evaluate a number of goblet cells in small intestine epithelium with reference to the degree of atrophy in celiac disease.

Material and method Morphological analysis of 80 bioptates of small intestine mucous membrane, taken for a routine histopathologic diagnosis from patients aged from 0 to 18 years, was performed. The patients were diagnosed and treated in paediatric university hospitals in Zabrze and Bytom in the years 2001-2002. Specimens were stained with haematoxylin and eosin (H.E.) and alcian blue-PAS (AB-PAS). Villous atrophy was evaluated according to the modified Marshal scale.
Results The following types were defined: type 0 – 33 cases, type 1 – 8 cases, type 2 – 6 cases, type 3a – 9 cases, type 3b – 21 cases, type 3c – 3 cases. Then, a number of goblet cells per 100 enterocytes of small intestine epithelium of each oligobioptate were calculated. The received results were evaluated statistically with U Mann-Whitney test. The average number of goblet cells in small intestine epithelium was calculated in relation to the degree of atrophy with the modified Marsh scale: type 0 –8.08 (+/- 3.79), type 1 – 9.41 (+/- 4.04), type 2 – 10.48 +/- 1.59), type 3a – 11.55 (+/- 2.94), type 3b – 13.20 (+/- 3.52), type 3c – 17.53 (+/- 0,92). The analysis used significant relations between an average number of goblet cells in small intestine epithelium and a degree of mucous membrane atrophy in celiac disease. Moreover, a correlation according to Rang Spearmann test showed a coefficient $R=0.5790$ for $p<0.001$.
Conclusions Significant relation between a number of goblet cells in epithelium and a degree of small intestine mucous atrophy in celiac disease. It allows an introduction of a new objective quantitive parameter in the evaluation of atrophic lesions.

P-555

Immunohistohemical evaluation of mast cells activity in small intestine mucosa in children in the course of celiac disease

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Introduction Microscopic evaluation plays an important role in diagnosing small intestine inflammatory diseases. Defining a degree of villous and brush border atrophy, enterocytes changes, elongation of glandular crypts allows a correct evaluation of treatment clinical effects and child's body response to the applied selective diet. Development of immunomorphological studies contributed to confirmation of the autoimmunological basis of several diseases and of the influence of exogenous factors on their development. Due to the new approach, evaluation of the changeable character of inflammatory infiltrations including mast cells and marking activity of enteroendocrinal cells have become very important diagnostic elements. The aim of the study is to examine a changeable intensity of infiltrations from mast cells of small intestine mucosa in children in the course of celiac disease.
Material and method Oligobioptates of small intestine mucosa taken in endoscopy from 500 patients (age 0-18 years) at the Department of Pathomorphology in Zabrze in the years 1998-2002. The patients were diagnosed and/or hospitalised in paediatric university hospitals in Zabrze and Bytom. A degree of villus atrophy was evaluated according to the modified Marsh classification. Tissue material in paraffin cubes from the routine oligobiopsy of the patients with celiac disease was qualified for immunohistochemical study. Immunohistochemical reactions revealed a presence of brown dyed mass cells in intestine mucosa. The cells were evaluated by counting their number in 5 high power fields (magnification 400x) in each oligobioptate. The received results were calculated statistically with Mann-Whitney U test.
Results Mean number of mass cells in intestine mucosa was calculated in relation to the degree of villus atrophy according to the modified Marsh classification: type 0 (status normalis) – 7.55 (+/- 2.23), type 1 – 13.59 (+/-5.20), type 2 – 17.62 (+/-1.94), type 3a – 20.82 (+/-6.10), type 3b – 27.94 (+/-4.59), type 3c – 34.31 (+/- 3.61). The analysis showed significant relation between a number of mass cells and a degree of mucosa atrophy in a course of celiac disease. Moreover, a correlation after the Rang Spearman test showed a coefficient $R=0.8151$ for $p<0.000001$.
Conclusions Significant relation between a number of mass cells and a degree of small intestine mucosa atrophy in a course of celiac disease was shown. Defining (marking) a number of mass cells in small intestine mucosa in a course of celiac disease allows an introduction of quantitive method of atrophic changes evaluation as a new objective parameter, especially in cases difficult to diagnose.

P-556

Morphological analysis of tissue reactions in mandible after dental implants of the pressed COCrMo alloy

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Introduction Production of materials for dental implants is of great social importance and is related to the progress in material technology. Main directions of development in this discipline concern an accurate choice of implant material in order to fix an implant in a bone tissue firmly and to decrease tissue reactions. New direction of research on implant materials is powder metallurgy. In comparison with the so far used technologies, it has many advantages, such as homogenous fine grained material structure, possibility to make composites including biologically active implants, and also ability to overgrow the implant with bone tissue. Aim of the study was morphological analysis of healing of dental alveolus canals filled with metal implants. Evaluation of tissue reactions in bones and surrounding soft tissue from several types of metal implants.
Material and method The experimental study was performed on 56 mixed breed rabbits of 2600-3200g weight. After premedication with atropine with relanium, surgical procedure was performed in each animal. One of the incisors was extracted and after the canal was formed, metal implant was inserted. The first group (D1) consisted of animals whose dental alveolus canals were filled with cylinder of 6x3 mm made of pressed Co-Cr-Mo alloy covered with calcium triphosphate. The second group (D2) consisted of animals whose dental alveolus was filled with implants of cylindrical shape of a size 6x3 mm made of pressed Co-Cr-Mo alloy. The third group (D3) consisted of animals whose dental alveolus canal were filled with a titanium screw of a size 3x1 mm. The control group (K) consisted of animals whose dental alveolus canal was left only with blood clot. The postoperative wounds were sutured with dexon thread. The clinical and observations and histopathological

examinations were performed after 3, 7 and 21 days and 6, 12, 24 and 52 weeks.
Results Fibrous connective tissue reconstructing dental ligament system was found to be created around metal implant in the three experimental groups. A reconstruction process of the bone tissue and its maturation in the form of calcification around the implant was the fastest in the group D1 (Co-Cr-Mo + TCP), next in the group D2 around the implant with a pure pressed Co-Cr-Mo alloy and the group D3 with a titanium screw. However, deposition of calcium salt in the mature bone tissue occurred at the fastest in the group D2. The reactions caused by corrosion of the implants were not observed.
Conclusions The process of bone tissue reconstruction was the most active at the presence of CoCrMo alloy covered with calcium triphosphate (group D1). The fastest maturing of the bone tissue manifested as its mineralization was observed in groups D1 and D2. The metallic implants did not influence negatively the healing process of the post-operative wounds.

P-557

Morphological evaluation of bone defects filled with deproteinized bovine bone

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Introduction Correct reconstruction of bone defects is a serious problem in orthopedics and in maxillo-facial surgery. Hence, numerous experiments and clinical studies on a usage of natural and artificial materials being a scaffold enhancing a reconstruction of bone tissue (i.e. carbon fibre, hydroxyapatyte granulate). Deproteinized bovine bone is a new material. It was elaborated in at the Mineralogy Faculty, Mining and Steel Academy in Kraków. The technology of natural animal bone tissue enables its use in filling bone defects, accelerates reconstruction of bone tissue and minimizes unfavorable tissue reactions through total immersion into the reconstructed bone. It gives no incorrect immunological response of the body to the implanted xenogenic tissue. The aim of the study is to evaluate healing processes of the defects made in rat femoral bones and filled with deproteinized bovine bone and to compared it with spontaneous healing.
Material and method The experiment was conducted on 84 Wistar rats divided into 2 groups (42 rats per group). The surgically made bone defects were filled with the study material. The animals, whose defects filled with blood clots spontaneously, made up a control group. The healing process in bone tissue was examined clinically and morphologically after 1, 3, 7, 14, 28 and 56 days after the experiment.
Results In histopathological estimation, the healing speed of the defect in femoral bone filled with blood clot (control group) was compared to the healing of the defect filled with deproteinized bovine bone (study group). No inflammatory reaction to the implant was observed. Moreover, acceleration of the healing of the defect filled with deproteinized bovine tissue was observed. Bovine bone was, in the study group, totally immersed in the body's bone tissue, i.e. was surrounded by the arisen on its surface sponge bone. In the last periods of the observation, on the borders of the observed synostoses no osteoblast cell activity was observed and normal bone marrow was found between osseous trabeculae.
Conclusions Osseous wounds filled with deproteinized bovine bone heal without any inflammatory complications. No negative systemic reactions proves that the applied material was properly prepared for implantation. Histopathologic studies showed that deproteinized bovine bone accelerates regeneration process of a damaged bone tissue.

P-558

Withdrawn

P-559

The effect of specific inhibitors of caspases on apoptosis of thymocytes induced with paclitaxel

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Although caspases are involved in molecular mechanisms of the execution phase of apoptosis, the precise mechanisms of their involvement in paclitaxel induced apoptosis are still not identified. The aim of this study was to investigate and compare the effects of inhibitors of caspases (50 mMol/L) on apoptosis of rat thymocytes induced by paclitaxel (10 mMol/L) and colchicine (2 mMol/L). The effect of z VAD and specific inhibitors of caspases (caspase 1, 3 and 9) after 4, 8 and 12 hours of incubation of thymocytes with paclitaxel and colchicine were analyzed. After incubation cells were fixed, processed for LM and EM, and the percentage of apoptotic cells (apoptotic index) was determined. General inhibitor of caspases zVAD and inhibitor of caspase 9 led to a significant decrease of apoptotic index after 8 hours of incubation with paclitaxel (from 29.03% to 19.76% and 14.23%). Inhibitor of caspase 3 and caspase 1 lowered the apoptotic index after 12 hours incubation with paclitaxel from 64.54% to 26.36% and 44.23% respectively. Inhibitor of caspase 1 did not affect the apoptotic index in controls, while the other used inhibitors reduced it. Inhibitors of caspase 1, 3 and 9 and zVAD had similar effects on apoptosis of thymocytes induced by colchicine. These results show that apoptosis induced by paclitaxel is caspase dependent, and that it, due to the effect of inhibitor of caspase 9, probably involves mitochondria. Caspase 1 activity might also be involved in mechanisms of induction of apoptosis of thymocytes by paclitaxel.

P-560

Effects of concurrent administration of lithium and gentamicin on the histoembryogenesis of rat fetus during the developing periods

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This study was planned to investigate the effects of gentamicin and

littium on rat histoembryogenesis and to determine the effects, if any, of lithium as a potent gentamicin inhibitor. The rats were exposed subcutaneously to 110-mg/kg gentamicin on days 6-15 of gestation. Lithium administered orally at doses 1200 mg/l on days 6-15 of gestation. Fetuses were collected on day 21 and compared with those of the untreated, and saline treated control as well as with those of the gentamicin and lithium treated groups. All control and cases embryos groups were killed and their tissues were obtained. Then prepared for paraffin block, sectioning, H-E staining and light microscopic studies. We fond that gentamicin caused growth retardation versus lithium which dosn't did so. Lithium has a state like control but no wary exactly. Furthermore, concurrent administration of gentamicin and lithium caused regularity in histoembryogenesis cycle and the normal growth beside the lithium or gentamicin. Of course the recent result is not exactly comparable to those of controls.

P-561

Immunohistological and pharmacological studies of anti-inflammatory drugs in experimental Schistosomiasis

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Introduction Schistosomiasis mansoni infection increases the level of prostaglandins and leukotrienes that are secreted mainly by macrophages within the granulomas. These mediators have a pronounced role in mediation and modulation of inflammation. Most non steroidal anti-inflammatory drugs (NSAIDs) act mainly by inhibiting the cyclooxygenase pathway of arachidonic acid metabolism, thereby diverting it towards the lipoxygenase pathway. These leukotrienes are important in maintenance of acute and chronic inflammatory condition.
Methods In present study, we used mofebutazone (cylcooxygenase and lipoxygenase inhibitor) and tenoxicam (cylcooxygenase inhibitor) either alone or in combination with praziquantel (antibilharzial drug) in mice infected with Schistosomiasis mansoni. We study their effects on liver pathology as PCNA (proliferating cell nuclear antigen), granuloma diameters, percent of degenerated ova and type of granuloma after 10&14 weeks after infection.
Results shown that infection increased the percent of positive nuclei in granuloma and in hepatocytes to be about 72% & 82% at 10 weeks and these percentages were reduced to be about 37% & 43% at 14 weeks after infection respectively. Treatment with mofebutazone and tenoxicam produced a reduction in PCNA to be about 33% & 40% in granuloma and 36% & 44% in hepatocytes at 8 weeks and more reduction occurred after 14 weeks after infection. Also, mofebutazone tended to produce more protection to isolated hepatocytes (% viability of hepatocyte) than tenoxicam in Schistosomiasis mansoni infected mice. Combined therapy with praziquantel produced more reduction in PCNA and more protection to hepatocytes. It is evident from the present study that, mofebutazone when combined with praziquantel has shown good tolerability more than tenoxicam which was associated with an improvement in PCNA, granuloma diameter, % viability of isolated hepatocytes, liver function tests, a fact that would seen to advocate the use of mofebutazone in bilharzial patient.

P-562

Patterns of injury in car and motorcycle accidents

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Introduction The role of the forensic pathologist in road-traffic accidents is to detect atypical injuries that could suggest an alternative cause of death, demonstrate natural diseases capable of creating sudden incapacity, and to collect samples for toxicological examinations. The main objective of this work is to study the different patterns of injuries in car and motorcycle fatalities.
Material and methods We have studied the autopsy reports of 34 deaths related to road traffic accidents (25 involving cars and 9 motorcycles). A complete autopsy was performed in every case including toxicological investigation.
Results The most common cause of death was head injury, responsible for 52% and 66.6% of deaths in car and motorcycle accidents, respectively. Cranial base fractures were more commonly found in passengers rather than in drivers, both for cars and motorcycles (40% vs. 30% and 33.3% vs. 50%, respectively). The distribution of cranial vault fractures was similar with percentages of 15 and 12.6 for car and motorcycle drivers, versus 40 and 50 per cent for the respective passengers. Nevertheless, brain injury was even more frequent in car accidents, accounting for 60% of the cases, and in motorcycle fatalities, where every single case showed some degree of brain injury.
Conclusion Our results confirm the importance of head injury in road-traffic accidents, especially when motorcycles are involved, being the most common cause of death. Although all motorcycle victims wore a helmet at the time of the accident, it did not seem to provide an effective protection, probably because of the high speeds involved. The incidence of cranial fractures and brain injury is higher for passengers than for drivers, probably due to the lack of attention to the traffic of these latter, and the fact that drivers are usually holding the handlebar or wheel at the moment of the accident.

P-563

Heat-induced epitope retrieval in immunohistochemistry

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Introduction Heat-induced epitope retrieval (HIER) has dramatically changed the applicability of immunohistochemistry in routine pathology. HIER has increased the number of primary antibodies useful on formalin-fixed paraffin-embedded material and in general improved the histochemical staining. The aim of this study was to compare various buffers for HIER on a broad range of primary antibodies.
Materials and methods HIER using four different buffers (citrate pH6; Tris/EDTA pH9; DakoCytomation Target Retrieval Solution, High pH, code No. S 3307 and DakoCytomation Target Retrieval Solution pH6, code No S 1700) for pre-treatment of formalin-fixed paraffin-embedded material has been compared using more than 120 different primary antibodies and the EnVision+TM visualisation system.

Results HIER with citrate buffer retrieve epitopes recognised by the majority of the tested antibodies. However, in several cases the epitopes could not be detected using this method but were retrieved using Tris/EDTA and/or Target Retrieval Solution, code No. S 1700. Also retrieval by means of the Target Retrieval Solution, High pH can be used however, the morphology quite often was partly destroyed. Furthermore, some antibodies could only be used after enzymatic pre-treatment whereas HIER abolished the histochemical staining.
Conclusion Although HIER using citrate pH6 buffer is a standardised and widely used technique, it is in numerous cases sub-optimal or even obliterate the immunohistochemical staining. In general more epitopes can be retrieved using Tris/EDTA pH9 or DakoCytomation Target Retrieval Solution.

P-564

Bone morphology and calcemia in ovariectomised (OVX) Wistar rats

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Postmenopausal oestrogen deficiency results in bone loss (osteoporosis) in humans and experimental animals. Wistar rats provide a useful experimental model of post-menopausal osteoporosis. To demonstrate the histomorphometric and histological changes of bone 4 weeks after bilateral ovariectomy in rats and to investigate the impacts of 2 different hormone replacement therapies on the bone. Specimens of proximal femur were embedded undecacified for histomorphometric analysis and of distal femoral metaphysis were procured for pathologic examinations. Three weeks after OVX the femoral BMD, mean cortical thickness decreased significantly while the number of osteoclasts increased significantly as compared with sham group. The trabeculae became thinner and irregular which changed the bone microstructure in three dimensions. After treatment with 2 different preparations, the above parameters restored to various extents to the sham operation levels. Bilateral ovariectomy induced increased osteoclast activity and bone turnover, therefore caused accelerated bone loss. Treatment with combined sex hormones preparation could inhibit bone absorption and stimulate bone formation. This investigation was supported by MSTD of Srbia.

P-565

Vilnius pathological anatomy in the first half of the 19th century

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The beginnings of pathological anatomy as a separate medical subject were connected in the area of the Republic of Poland and Lithuania with the Vilnius University. The first post mortem was done in Vilnius by a priest, Stefan Bisio only in 1770 year. At the end of the 18th century, Jakub Briotet, a surgeon and anatomist, created a modest anatomical surgery (study). In 1804, Jan Piotr Frank and his son Józef arrived to Vilnius and became professors of the University. Jan Piotr Frank became the Head of the Teaching Hospital and Józef Frank the Head of the Chair of Pathology. In November 1804, he started lecturing for students, which he recalled in his "Memoirs": Several days after my father, I also started a course with a dissertation "De meliori pathologiam tradendi modo" In this speech I put the main stress on a significance of the explanations at the patient's bed-side and of a good knowledge of pathological anatomy… When in 1805, Józef Frank became the Head of the Teaching Hospital, he founded the first anatomo-pathological study (surgery). Most of the samples kept in spirit in the Frank's study were eaten by the starving French soldiers during the retreat of Napoleon's army. Besides Frank's study at the Vilnius University at the Chair of Anatomy, there still existed the study founded by Briotet. In 1808, the Emperor Alexander I destined the ruins of the Spaska's Orthodox Church for an anatomical theater. After seven-year redecorations, the building was opened in 1815. Besides the theatre itself, dissection room and Veterinary Institute, it also included a zoological, veterinary and anatomical museum. The growing anatomical museum had, in 1841, 2881 preparations including 1239 anatomo-pathological preparations. After closing down the Vilnius University and foundation of the Academy of Medicine and Surgery, pathological anatomy was made an individual subject of study. The first lecturer of pathological anatomy in Vilnius was Ludwik Siewruk. He held the classes till 1840 and then he took charge of the Chair of Anatomy at the Moscow University. His successor was Jan Leonow who continued his work until the tsar's authorities closed down the Academy of Medicine and Surgery in 1842. After the Academy had been closed down, all the exhibits of the Vilnius anatomical museum, including anatomopathological preparations were moved to the anatomical museum of Kiev University. Few of them remained in the Vilnius Medical Society.

P-566

Detection of cardiac mast cells in rats poisoned by T-toxin

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Introduction T-2 toxin, a mycotoxin produced by Fusarium fungi, is a well-known cardiotoxin that cause myocardial injury, capillary damage, haemorrhage and focal polymorphonuclear cell infiltration. Mast cell (MC) is one of the the earliest multifunctional effector cells of the immune system and important constituent of microvasulature in the heart. Aim: We wanted to investigate appearance and localisation of mast cells in the heart of T-2 toxin-poisoned rats.
Materials and methods Adult female Wistar rats were poisoned with a single injection of 1 LD-50 of T-2 toxin (0.18 mg/kg sc). Rats were divided into two groups: (1) control group and (2) T-2 toxin group. T-2 toxin was produced in laboratory conditions from Fusarium sporotrichoides fungi. Animals were sacrificed after the end of the day 1, 3, 5 and 7 of the study. Cardiac MCs were counted in whole visual fields, magnified by 40-fold on paraffin section stained with haematoxylin and eosin (HE) and Giemsa methods.
Results In the heart of rats treated with T-2 toxin, blood vessels were congested with thickned walls, and a large number of mononuclear cells was present nearby. 24 hours after administration of

T-2 toxin, a solitary hypergranular MCs were situated on the external wall of blood vessels. However, on days 3, 5 and 7 the number of perivascular MCs was increased. More than 50 percent of these MCs showed degranulation.
Conclusion Our results suggest that perivascular localisation and later degranulation of MCs may play an importantant role during the acute reaction in the heart of rats poisoned by T-2 toxin.

P-567

Effects of bromadiolone on rodent liver: pathohistological findings

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Introduction Bromadiolone is one of the most commonly used rodenticides. This coumarin anticoagulant blocks multiple steps in the coagulation cascade. It is absorbed through the gastrointestinal tract and accumulated in the liver, mainly. The aim of this study was to investigate pathohistological alterations in the liver after oral application of bromadiolone in rodents.
Materials and methods Adult Wistar rats and Swiss mice, of both sexes, were fed with a ready-to-use baits of low bromadiolone concentration (0.005%), for first three consecutive days. Animals were sacrificed every day during the one week period, starting with 24h after the beginning of the experiment. Liver paraffin sections were stained by haematoxillin and eosin (HE) method.
Results The pathological alterations detected in all animals ranged from diffuse degeneration to a focal necrosis of hepatocytes and massive circulatory changes. Parenchymal degeneration, diffuse oedema and hyperemia were predominant in the liver of animals sacrificed on the first three days of experiment. Dissolution of cytoplasm with nuclear pleomorphism in round or ovoid hepatocytes were seen. Thickening of the blood vessels with necrosis of endothelial cells were particularly prominent from the fourth to seventh day of experiment. The most interesting finding is the presence of massive hemorrhages with a focal mononuclear cell infiltrations. The majority of hepatocytes were irregular, with macronodular fat transformation of cytoplasm. These changes were the most intensive in male mice on the day 7 of the study.
Conclusion Multiple oral ingestion of bromadiolone induced prominent pathohistological alterations in rodent liver, especially mice.

P-568

Mobile telepathology - a new intringuing tool

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The mobility of information, patient data and images introduced by telemedicine/ telepathology systems designated a completely new viewpoint in pathology. Mostly two areas of diagnostic work are so far covered by telepathology service - second opinion/expert consultation and frozen section diagnosis. Frozen section diagnosis, is still a very important issue. Smaller hospitals, with no sufficient material for a resident pathologist often could benefit by introduction of telepathology. As in many of these hospitals surgery requiring frozen section diagnosis is not performed on a daily basis the telepathology system is not in full use, producing an unfavorable cost-benefit ratio. Also establishing a fully equipped frozen-sections lab yields additional costs and personnel cutting frozen sections only occasionally often produce suboptimal. In this the introduction of a mobile telepathology unit could solve some very important problems. The unit should include a fully equipped mobile frozen sections lab including a macro stand and a microscope with CCD TV color camera and a notebook (laptop) computer. The telepathology software used must be stabile and versatile like the Pharos/ISSA system (VAMSTECH, Croatia) used by us. By such a system and synchronization of operation schedules hospitals in a radius of up to 200 km around the referral center could be covered without exceeding standard working hours. The ISSA-server based system option alerting the consultant and referring pathologist by SMS that a consultation case is in his mailbox at the server ads additional time effectiveness.

P-569

A novel high throughput marker validation tool

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High throughput genetic and expression screening is generating many possible diagnostic markers and therapeutic targets for wide variety of clinical conditions. The rapid validation of these markers is a challenging process. Tissue microarrays can be used for the evaluation of many of these markers. However, they have drawbacks and unresolved issues, which are result of the sampling and sectioning problems. We introduce a novel microarray technique based on cell suspensions. Multiple slides can be made, all of which are equally representative of the initial samples. A manually operated mechanical device was designed that can deposit 60 distinct spots on the glass slide. Each spot of cells deposited in this manner may correspond to unique source. Controlling the number of cells per spot their uniformity and size can be achieved by modifying the viscosity of the cell solution or precisely regulating amount of fluid deposited. A fully automated analysis of quantitatively stained microarray samples has been performed to quantify the number of cells per spot, the size of spots, and how reproducible these parameters are. Cellular material used in the experimental arrays constructed to date has been acquired from cell lines and liquid prepared lung and cervical specimens. A variety of molecular markers can be evaluated, with minimal cost. FISH probes have been tested on cytology microarrays constructed from 20 different lung cell lines. Using cell suspensions generated by the dissociation of tissue would enable the creation of more representative samples for clinical marker/target validation or evaluation.

Author Index

The numbers are the abstract numbers; oral presentations (O), poster presentations (P)