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O-1

The epithelial-to-mesenchymal transition protein periostin is associated with higher tumour stage and grade in non-small cell lung cancer

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Background: The epithelial-to-mesenchymal transition (EMT) is vital for morphogenesis and has been implicated in cancer invasion. EMT of carcinoma cells can be defined by morphological trans-differentiation, accompanied by permanent cytosolic overexpression of mesenchymal proteins, which are normally expressed in the peritumoural stroma. We aimed for correlating the expression levels of the EMT indicator proteins periostin and vimentin with clinico-pathological parameters of non-small cell lung cancer (NSCLC). Method: 538 consecutive patients with surgically resected NSCLC were enrolled in the study and a high density tumour tissue micro-array, containing 2 cores per tumour, was constructed. Immunohistochemistry with antibodies against periostin and vimentin was performed and the protein expression levels in either stroma or cytoplasma were measured by semi-quantitative scores 0 to 3+. EMT was defined as cytoplasmic overexpression with score 2+ to 3+. Results: Of the 538 patients, 384 (71%) were men and 154 (29%) women; the median age being 64 years. Adenocarcinoma was diagnosed in 245 (46%), squamous cell carcinoma in 275 (51%) and adenosquamous carcinoma in 18 (3%) cases. EMT was observed in 30% (periostin) and 12.5% (vimentin) of all tumours, respectively. Overexpression of periostin in both stroma and cytoplasm was significantly associated with the squamous cell carcinoma subtype, a higher pT-stage, a higher histological tumour grade and an increased tumour size of >4 cm (p-values<0.05). Overexpression of stromal periostin only was associated with a higher pN-stage and a higher clinical stage (p-values<0.05). For vimentin, overexpression of cytoplasmic protein only was associated with a higher histological grade (p-value<0.05). Conclusion: To define EMT in NSCLC, the expression levels of the mesenchymal proteins periostin and vimentin have to be measured in both peritumoural stroma and carcinoma cell cytoplasm. Overexpression of periostin is closely associated with increasing TNM stage and grade.

O-2

Squamous cell carcinoma of the lung: polysomy of chromosome 7 and wild type of exon 19 and 21 were defined for the EGFR gene

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BACKGROUND: The use of tyrosine kinase inhibitors after first line chemotherapy, induced several studies to determine molecular characteristics in non-small-cell lung cancer to predict the response to those drugs.
The present study was delineated to clarify the status of *EGFR* gene by Fluorescence *in situ* Hibridization(FISH), Polimerase Chain Reaction (PCR) and Immunohistochemical protein expression in 60 cases of squamous cell carcinoma of the lung after surgical resection of tumours in stages IIb/IIIa.
METHOD: Representative sections were selected and submitted to tissue microarray construction for determination of EGFR protein expression by applying the antibody Mouse anti-EGFr, Clone 31G7 (Zimed Laboratories) and *EGFR* gene copy number was searched with LSI EGFR/CEP 7 probe (Vysis, Abbot Molecular). Also LP34, CK7, Chromogranin A and CD56 antibodies were applied to correctly define the squamous differentiation of each tumour. Genomic DNA was extracted from selected malignant cells by microdisection in 40 cases. PCR was performed to verify deletions in exon 19 and the pontual mutation in exon 21 of *EGFR* gene, assessed by capillary electrophoresis and restriction fragment length polymorphism (RFLP), respectively.
RESULTS: Immunohistochemical EGFR protein overexpression was identified in 35 cases, by the application of Hirsh scoring system. Increased gene copy number was observed in 32 cases by FISH, according to Cappuzzo method. Through capillary electrophoresis, the deletion in exon 19 of EGFR gene was detected in 3 cases; the exon 21 of *EGFR* was expressed in its wild type by RFLP, in all cases.
CONCLUSIONS: Our study concerning only cases of squamous cell carcinoma of the lung in surgical stages is the first to be done, to the best of our knowledge, after reviewing the published medical literature. In published studies performed in non-small-cell lung cancer in advanced stages, the cases of squamous cell carcinoma referred, expressed

higher levels of the parameters included in the present study. The results obtained showed that squamous cell carcinoma in surgical stages, independently of the presence of lymph nodes metastases, express EGFR protein and have high chromosome 7 polysomy in 50% of the cases and the gene remains without molecular alterations. These conclusions have to be correlated with follow-up of these patients to define potential recurrence related with the results obtained.

O-3

Inflammatory pseudotumour and myofibroblastic tumour of the respiratory tract: just one entity?

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Background. The nature and the cell of origin of lesions variously described as inflammatory pseudotumour (IP), pseudosarcomatous proliferation or inflammatory myofibroblastic tumour (IMT) are still debated. Most cases behave as benign, reactive lesions, but rarely may have an aggressive course. We analysed the clinicopathological and molecular features of a series of IMTs of the respiratory tract, with the aim to verify if they represent a single entity or a heterogeneous group of lesions.

Methods. Histological features of thirty-eight cases diagnosed as IMT or IP in various Institutions were reviewed. Immunohistochemistry was performed for the following antibodies: CAM 5.2, MNF116, CD34, CD21, CD35, beta-catenin, CD68, HHV8 and 6, smooth muscle actin, desmin, ALK, ki67, IgG4. Molecular investigations included in situ hybridization for EBV and DNA sequencing for c-kit, c-met, EGFR, PDGFR alpha and beta.

Results. Histologically, tumours were subdivided in two groups: type A (8 cases) included lesions with a predominance of inflammatory cells and no prominent spindle cell proliferation; type B (27 cases) included lesions composed of uniform proliferation of spindled cells, sometimes with myxoid features. Three cases had borderline features between type A and B and were classified as "type AB". All cases were negative for cytokeratins, HHV8, HHV6 and CD21 and beta-catenin. Cases from all subtypes were variously positive with muscular markers, CD34, CD68 and IgG4. ALK antibody stained only 4 cases of B type. No cases showed mutations in the genes studied.

Conclusions. Under the designation of IMT or IP are actually included different lesions. In the present series, we identify histologically two main groups: one which represents more likely true myofibroblastic tumoral proliferations, and a group which is represented by non-tumoural, reactive lesions. At moment, however, it appears that there are no immunohistochemical or molecular markers to distinguish the two types of lesions.

O-4

Expression of vascular endothelial growth factor (VEGF) and its receptors in lung carcinoids. A focus on neoangiogenesis.

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BACKGROUND: Angiogenesis is an essential process in the progression of different tumour types. Vascular endothelial growth factor (VEGF) is considered one of the most important angiogenic factor in *vivo*. Its expression and role in human lung carcinoid remains unclear. The aim of the present study was to investigate the expression of VEGF and its receptors VEGF-R1, 2 and 3. The expression was then correlated with tissue neoangiogenesis measured by endoglin (CD105). immunohistochemistry.

METHODS: Between 2003 and 2007, 45 patients (mean age±SD 46.45±15.66 yrs, range 16–75 yrs, M:F=1:1.7) underwent thoracic surgery for pulmonary carcinoid. In each case, immunohistochemical analysis of VEGF and its receptors (VEGF-R1, 2, and 3) and CD105 was performed on serial paraffin sections. VEGF was also evaluated by western blot analysis. Nonneoplastic lung tissues at distance from the primary tumor were also analyzed as controls.

VEGF was quantified in a semi quantitative manner (% of positive cells X intensity staining score, from 0 to 3, range 0 to 300). The micro vessel density (MVD) was assessed as the number of CD105 microvessel positive/mm^2.

RESULTS: All the cases were classified according to WHO as atypical (AC), 12 cases, and typical (TC), 33 cases. VEGF and its receptors VEGF-R1, 2 and 3 expression was mainly detected in the cytoplasm of tumor cells. AC had a higher expression of VEGF (mean±SD 112.28±87.1 vs 44.35± 84.52, p=.03) and a higher MVD (mean±SD 38±26.69 vs 14.1±21.87, p=.03) than TC. VEGF values were directly correlated with MVD (p=.02). No significant differences were found in the expression of VEGF-R 1, 2 and 3 between AC and TC.

CONCLUSIONS: Our data suggest that lung tumor cells are the most important source of VEGF in human lung carcinoids. Neoangiogenesis, which is more pronounced in AC than TC, seems to be highly influenced by VEGF.

O-5

Epidermal growth factor receptor expression in 334 non small cell lung carcinoma patients; prognosis and correlation between gene copy number and protein expression

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Background: Lung cancer is one of the most frequently occurring neoplastic disorders and overall survival is less than 15%. Some patients with advanced non-small cell lung cancer (NSCLC) may benefit from treatment with tyrosine kinase inhibitors directed against the epidermal growth factor receptor (EGFR). Patients can be identified by EGFR gene copy number determined by fluorescence in situ hybridising (FISH) and EGFR protein expression determined by immunohistochemistry (IHC). However, it is difficult to get enough tumour tissue from patients with advanced non small cell lung cancer (NSCLC) to construct high throughput tissue micro arrays (TMA). We studied 369 patients surgically treated for NSCLC during the time periods 1981–83 and 1995–97.
Materials and methods: A TMA with three cores from each case was constructed and evaluated for EGFR protein expression by IHC and FISH for gene copy number. It was scored with IHC as 0–3+ and with FISH as negative (non amplified) or positive (polysomy or amplified). Both tests were evaluable for 334 cases.
Results: *IHC*; 0+=132, 1+=63, 2+=67, 3+=2. *FISH*; non amplified=196, polysomy=115, amplified=23. All cores containing viable tumour tissue could be evaluated for both tests. The correlation between IHC and FISH was highly significant (p=0.0007). *Survival*; Kaplan-Meyer plots showed that EGFR IHC 1–3 was and adverse factor for survival (p=0.02) but not EGFR FISH (p=0.35).
Conclusion; This is one of the first studies to show a highly significant correlation between EGFR FISH and IHC. Also, EGFR IHC 1–3 was a negative prognostic factor for survival. The results suggest that testing for EGFR FISH and IHC should be performed before treating patient with EGFR tyrosine kinase inhibitors.

O-6

STAT1 expression is associated with EBV status in T/NK-cell lymphomas

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Background: T-cell and NK-cell lymphoma subtypes exhibit a high association with the Epstein-Barr virus (EBV). The role of cytotoxic T cells (CTL) and macrophages in the control of EBV infection is well established. Moreover, the signal transducer and activator of transcription 1 (STAT1) plays a pivotal role in the initiation and maintenance of certain cancers. The relevance of STAT1 expressing tumor associated macrophages (TAM) has been previously demonstrated in the outcome of follicular lymphoma. We examined the possible relationship between EBV status and the presence of these immune cells in these tumors.
Method: Thirty pathologic specimens with the diagnostic of anaplastic large cell lymphoma (ALCL, n=9), peripheral T-cell lymphoma, unspecified type (PTCLU, n=13), extranodal T-cell lymphoma, other type (ETCLO, n=6) and extranodal NK/T-cell lymphoma, nasal-type (NKTCL, n=2) were incorporated in tissue microarrays. EBV expression was detected by in situ hybridization (ISH) and infiltrated immune cells were detected by immunohistochemistry (IHC). The presence of CTL cells (TIA-1, Granzyme B), macrophages (CD68) and STAT1 into representative areas of the tumor was automatically quantified with the image analysis software Image-Pro Plus 5.0 implemented with appropriated algorithms.
Results: In total, 7 cases (22.6%) were detected EBV-positive: 5 PTCLU (38.46%) and 2 NKTCL (100%). All of ALCL and ETCLO were negative for EBV. The expression of STAT1 correlated with EBV expression (rho=0.489; p=0.011) and with the presence of CD68-positive macrophages (rho=0.616; p=0.001). The tumor tissue of EBV-positive cases presents a significant overexpression of STAT1 (mean 189.86±133.59 vs. 51.24±84.57; p=0.013) as compared to EBV-negative cases. No significant differences in the level of CTL and macrophages were observed in relation to the EBV status.
Conclusion: The association of STAT1 expression with the EBV status and the level of infiltrated macrophages suggest a possible role of STAT1-positive TAM in the immune response against T cell lymphoma.

O-7

Adult T-cell leukemia/lymphoma (ATL) presenting in the skin: clinicopathological and immunohistochemical features of 46 cases

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Background - Adult T-cell leukemia/lymphoma (ATL) is a severe and fatal form of HTLV-I-associated leukemia/lymphoma with frequent skin involvement. We describe the clinicopathologiasl and immunohistochemical findings of 46 cases with histologically-proven skin involvement and investigate whether there is any relationship between the deepness of dermo-hypodermal infiltration and CD8 positivity and median survival time (MST).

Methods - All cases were HTLV-I+ and HIV-, and had known follow-up. Search for HTLV-I proviral integration was performed in 19 cases.

Results - The skin lesions were always multiple and generally disseminated. Twenty-five cases were primary to the skin (smoldering and primary cutaneous tumoral), and 21 were secondary. In all 19 cases imonoclonal integration of the virus was demonstrated, including 9 cases that survived more than 5 years. Twenty-nine cases were histologically diagnosed as peripheral T-cell lymhoma, 16 as mycosis fungoides, and one as anaplastic large-cell lymphoma. Immunophenotype: CD3+, CD4+, CD5+, CD8-, CD45RO+, CD25+, CD7-, and CD20-, but 14 cases were CD8+. 17.4% of the patients are still alive with diesase (6 smoldering and 2 chronic). The overall MST was 21 months. The MST of the cases with superficial versus deep skin infiltration was 20 months and 22 months, respectively. The CD8+ and the CD8- cases had MSTs of 48 and 21 months, respectively.

Conclusions - There are no characteristic histological and immunohistochemical features that enable the pathologist to diagnosis ATL without knowing the results of serologic or molecular studies. No correlation was observed between the degree of skin infiltration and the MST. The MST was longer in CD8+ cases, but the difference between these and the CD8 negative cases was not statistically significant. The smoldering cases had a much longer MST (60 months) in relation to the primary cutaneous tumoral cases (20 months), confirming the importance of clearly defining these two types of primary ATL.

O-8

FOXP3 positive regulatory T-cells in cutaneous T-cell lymphomas

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Background: FOXP3 is a unique marker for CD4+ CD25+ regulatory T-cells (Tregs). In solid tumours, high numbers of Tregs are associated with a poor prognosis. Knowledge about the implications of Tregs for the behaviour of haematological malignancies is more limited.

Methods: Skin biopsies from 86 patients with either Mycosis Fungoides (MF) or cutaneous T-cell lymphoma (CTCL) unspecified and 27 patients with CD30 positive cutaneous lymphoproliferations (lymphomatoid papulosis (LyP) or primary cutaneous anaplastic large cell lymphoma (C-ALCL)) were analyzed for the expression of FOXP3 on tumour cells and tumour-infiltrating Tregs using immunohistology.

Results: Labelling of the neoplastic cells was seen in a case classified as an aggressive epidermotropic CD8+ cytotoxic CTCL and a case of C-ALCL. In the remaining cases, the neoplastic infiltrate was FOXP3-negative. By contrast, all biopsies showed varying numbers of strongly FOXP3+ tumour-infiltrating Tregs. MF with early or infiltrated plaques had significantly higher numbers of FOXP3+ Tregs than CTCL unspecified or advanced MF with tumours or transformation to large cell lymphoma. Similar results were obtained in 27 cases of primary cutaneous CD30-positive lymphoproliferations. In these conditions, the proportion of Tregs was significantly higher in LyP than in C-ALCL.

Conclusion: Our data indicate that the presence of FOXP3+ Tregs in CTCL is associated with disease stage and patient survival.

O-9

Primary MALT lymphoma of the thymus in a black patient

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Background. Thymic extranodal marginal zone B-cell lymphoma of mucosa associated lyhmphoid tissue has been included in the recently published WHO classification of tumors of the thymus. 42 cases have so far been reported in the world medical literature. A large percentage of patients are Asians (i.e. Japanese, Korean or Chinese), and many suffer either Sjögren's disease or Rheumatoid Arthritis. We

report a case occurring in a black patient with no history of autoimmune disease.

Method. A 47-year-old man from Angola with pneumococcal sepsis and meningitis underwent aortic valve replacement in January of 2005 because of severe regurgitation due to endocarditis. At the time of surgery, a biopsy was taken of a large, partly cystic anterior mediastinal mass that by imaging techniques had been interpreted as tuberculosis, lymphoma or cystic thymoma.

Results. The submitted 3.5 cm grayish mass consisted of lobules of lymphoid tissue with a vaguely nodular pattern, with interspersed sheets of plasma cells and rare epithelial cysts and Hassall's corpuscles. An extensive network of epithelial cells infiltrated by CD20-positive centrocytoid lymphocytes was disclosed by immunohistochemistry. The plasma cells were monoclonal (kappa) and expressed IgG and IgM. By PCR a clonal IgH rearrangement was demonstrated, in an oligoclonal T background. A RT-PCR for detection of the t(11;18) translocation was negative. The patient was treated with chemotherapy (CVP-Rituximab), with partial decrease in size of his residual mediastinal mass.

Conclusion. This is to our knowledge the first black patient with a MALT lymphoma of the thymus to be reported. The three proposed pathogenic pathways for this entity are: sistemic autoimmunity, spread from a primary gastric or parotideal MALT lymphoma, and aberrant paraneoplastic chemokine secretion from a coexisting micronodular thymoma. No associated autoimmune diseases or MALT lymphoma involving other organs have so far been detected in our patient.

O-10

A diagnostic challenge: Cystic carcinoma of the thyroid with papillary and squamous differentiation.

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BACKGROUND: It is well known that squamous metaplasia and dysplasia can occur in association with thyroid papillary carcinoma. However, the well-differentiated papillary thyroid carcinoma associated with squamous cell carcinoma denotes rare tumors of the thyroid and present highly difficult task for both, surgeon and pathologist. METHOD: We present a case of a 82 years old female with a painless left latero-cervical swelling, increased in size over the last 6-months. The ultrasonography revealed a large cystic mass, measuring 6 cm. Fine needle aspiration suggests a differential diagnosis of a squamous cell carcinoma, cystic variant, or a branchial cyst with atypia. RESULTS. The neck dissection involving the left lobe of the thyroid and cervical lymphnode was performed. The histopathological examination yielded two intermingled morphologically different patterns including conventional papillary thyroid carcinoma and poorly differentiated squamous cell carcinoma, cystic variant. Four lymphnodes out of 18, presented admixed metastases of both components.

CONCLUSION: Three differential diagnosis were considered: 1) primary papillary carcinoma of the thyroid, with squamous differentiation; 2) a collision tumor involving the papillary thyroid carcinoma and metastatic squamous cell carcinoma with unknown primary and 3) branchial cleft cyst cancerisation associated with papillary carcinoma on ectopic thyroid tissue. The metastatic origin was excluded by the absence of primary head and neck carcinoma. The branchial cleft cyst cancerisation was not retained because of the anatomical localization, absence of preexistent bronchial cyst or precancerous lesions in the cystic part of the tumor. Despite the advanced stage of the disease, the immunophenotype especially of the squamous component, with partial expression of TTF-1, thyroglobulin and p53 overexpression, was consistent with the diagnosis of a papillary thyroid carcinoma with squamous differentiation. This particular presentation of the thyroid carcinoma seems to have a poor prognosis with high recurrence rate and metastases over distant sites in about 20% of the cases.

O-11

Study of vascular invasion in thyroid carcinoma and its relation with histopathology of tumor

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Background: Papillary and follicular carcinoma are the two most common thyroidal malignancies. Recent data showed that histopathologic vascular invasion should be considered as a sign of higher tendency to hematogenic and lymphatic invasion. The aim of this study is determine the relation between microvascular invasion with type, stage, and grade of the papillary and follicular carcinoma and patients sex and age.

Method: This cross sectional study was performed on 100 patients (f=77, m=23)referred to shahid sadoughi university hospitals during 1991–2006. Pathologic vascular invasion was assessd with a new scoring system based on number of blood vessels invasion in microscopic fields. Score0=no blood vessel invasion,score 1=in one field seen, score 2=in 2–3 fields seen,scpre3=in 3–4 fields seen, score4=>4 fields invasion seen

Results:The increase of vascular invasion (score3,4)was seen in tumors with poor differentiation (pv=0.001)and

higher stage (tumor size and lymphnode involvement) (pv=0.03). Patients older than 45 years had higher scores (pv=0.036)The number of females was approximately three times more than males but males had a highest score(3,4)Largest tumors had the highest score.patients with lymph node involvement(15%) had highest scores (73.3%had score 3,4)(pv=0.02). There was no significant difference between vascular invasion and metastasis.(pv=0.169)and vascular invasion and type of tumor (pv=0.131) Conclusion: Higher scores of vascular invasion were seen in patients with higher stage and grade,in males and age over45 years. So this study suggest that vascular invasion should be considered as a prognostic factor in thyroid neoplasms. Additional studies need to approve it

O-12

In situ steroidogenesis in Differentiated Papillary Thyroid Carcinoma

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The influence of steroid hormones on the thyroid has been suggested in epidemiologic, case series and in vitro studies. Aim: Identify if aromatase and 17-betaHSD1 expression are associated with morphologic variables and prognosis in DPTC. Design: Analytical comparative study Material and Methods: In a cohort of similarly treated PTC (n=378), we select those patients with differentiated tumor >1 cm submitted to total thyroidectomy. Clinical charts and slides were reviewed. Tumor sections and paraffin blocks were selected to perform tissue arrays and immunohistochemistry for aromatase (Abcam ab18995, Cambridge MA), and 17-beta HSD1(Epitomics EP1682Y, Burlingame CA) was performed. A masked review of cytoplasmic expression was performed and a 10% cutoff was considered for positive cases with both antibodies. Recurrence development was the outcome. Descriptive statistics, Chi square and Fisher exact test were used when necessary. Results: 304 cases were studied, 93% were women, mainly premenopausic (68%) with a mean age of 40 and a 7 year follow up. Classic histotype was observed in 88% and stages I and II were identified in 70% of the cases. Six percent and 29% were positive for aromatase and 17-beta HSD1, respectively. In situ steroidogenesis did not correlate with recurrence, nevertheless positivity for one protein or both was associated with single tumor (p=0.022) and extrathyroid invasion (p=0.042). The percentage of positive cases for aromatase was significantly higher in men than in women (p<0.0001) and this difference was also observed in 17-beta HSD1 when more than 50% of the cells were stained. Conclusion: Single tumors with local invasion produce steroidogenic enzymes, without influence in prognosis at a mean follow up of 7 years. Additional studies including larger groups of recurrent cases or the aggressive variants of PTC should be conducted.

O-13

MERITS OF THE PMiT (PAPILLARY MICROTUMOR) TERMINOLOGY IN THE DEFINITION OF INDICENDAL PAPILLARY CARCINOMAS OF THE THYROID: EXPERIENCE OF FIFTY CASES

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Background: The term “papillary thyroid microcarcinoma (PTMC)” is generally employed to designate an incidental papillary carcinoma measuring 1 cm or less witch manifests extremely indolent behaviour. PTCMs are often detected by chance in thyroids removed for benign clinical nodules or widespread processes. There has been an exponential increase in the detection of PTCMs thanks to the recent improvement and application of ultrasonography in the management of thyroid lesions. However, no definitive treatment guideline has been developed to indicate how best to treat and manage these small tumours. For this reason the surgeon and patients may become alarmed when the pathologist reports the presence of PTMC and this may lead to re-operation, radical dissection of the neck or extensive irradiation, all of which are deemed unnecessary. On the other hand, the pathologist is concerned in defining as a “carcinoma” a lesions which he considers benign. For these reasons, Rosai et al proposed the term Papillary Microtumour of the thyroid (PMiT) during the 12th Annual Cancer Meeting in Porto (2003) and reported strict definition criteria for such entities.
Methods: Since 2003, we have adopted, the Porto proposal criteria for PMiT in agreement with clinicians and surgeons. Here we report a series of 50 consecutive cases designed as PMiT (during the interval from March 2003 to August 2007) collected and treated at Molinette Hospital, University of Turin.
Results: Patients (39F 11 M, median age 55.3 years) underwent to total thyrodectomy (47/50) or to lobectomy (3/50). No further treatment was performed. At histology, PMiT are often associated with benign nodules (19/50) or diffuse hyperplasic goitre (29/50), more rarely to parathyroid adenoma (1/50) and Thymoma B1 (1/50). All patients are alive and well after a median of 31.6 months follow-up (range from 5 to 57 months).
Conclusions: In our experience this terminology, is well accepted by both clinicians and patients, since it decreases the

danger of over-treatment, minimizes the psychological anxiety engendered by a diagnosis of carcinoma, and maintains the patient's eligibility for life insurance unalterated.

O-14

New isoforms of human periostin expressed in the thyroid gland

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Periostin is an extracellular matrix protein. Eight isoforms of human periostin (h-periostin) have been identified, which are generated by alternative splicing of the C-terminus. Recently, expression of h-periostin has been linked to carcinoma invasiveness and metastasis, but the role of each h-periostin isoform in the carcinoma invasiveness and metastasis are still little known. The expression and biological roles of h-periostin isoforms in the thyroid tissue and thyroid carcinogenesis have not been established. In the present study, we identified eight isoforms of h-periostin in thyroid tissue by RT-PCR and confirmed their identity by direct DNA sequence analysis. Four of these isoforms were unique to this study and have not been previously identified in any tissue. Especially three of them haven't been previously reported in any species. This is the first report that identified the other four isoforms in the thyroid gland, which have been reported in other human organs. Each of the isoforms was coexpressed in both thyroid carcinoma and matched non-neoplastic tissues. The results suggest that although the expression of these periostin isoforms is a common phenomenon in thyroid physiology, yet their roles in thyroid function and carcinogenesis still remain unknown.

O-15

TIME FOR CHANGE IN FIGO CLASSIFICATION OF MACROINVASIVE CERVICAL CARCINOMA (CX) – BASED ON HISTOPATHOLOGY

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Background: Limited knowledge exists about extranodal extension of the tumor outside the lymph node capsule, i.e. extracapsular spread (ECS) the prognostic impact of micrometastatic disease (i.e. metastatic deposits ≤0.2 cm = pN1mic) and for tumor size in FIGO-stage II tumors. *Methods:* 245 cases of FIGO stage II CX who received upfront surgery, were evaluated regarding tumor size. Pelvic nodes of 894 surgically treated CX FIGO IB to IIB were re-examined. In cases with lymph node involvement, the metastatic deposits were measured and extranodal spread of the metastatic deposits outside the lymph node capsule (ECS) was recognised.
Results: FIGO-stage II patients with bulky tumors (113/245) showed reduced 5-year overall survival (67.7% [95% CI: 58.2–74.8] vs. 49.5% [95% CI: 36.8–59.1]; p=0.0015). Patients with metastatic disease represented reduced recurrence free survival (RFS) time (pN1mic: 112.6+6.8 and pN1: 122.5+9.6 months), when compared to pN0 (190.9+3.0 months; p<0.0001). 5-year-overall survival (OS) was also decreased in metastatic disease (pN0: 86.6%, pN1mic: 63.8%, pN1: 48.2%; p<0.0001). The 5-year overall survival rate was significant lower in patients with ECS (33.5% [95% CI: 20.6% - 46.3%] vs. 60.5% [95% CI: 52.3% - 68.6%]; p<0.001). In multivariate analysis, tumor stage, pelvic lymph node involvement and maximal tumor size and ECS, were independent prognostic factors.
Conclusions: The results indicate that micrometastases and extracapsular spread (ECS) of pelvic lymph node metastases is of prognostic impact. A revised FIGO/TNM classification system for pelvic lymph node disease is recommended: pN1mic = micrometastatic disease (≤0.2 cm), pN1 = macrometastases; ECS 0 = lymph node involvement without extranodal spread of the metastatic deposits and ECS 1 = lymph node involvement with extranodal spread of the metastatic deposits as it is for FIGO stage II CX: stage IIA1 and stage IIB1 for tumors with <4 cm and IIA2 and IIB2 for tumors >4 cm (i.e. bulky disease).

O-16

Evaluation of cervical (pre) neoplastic lesions by in situ hybridization and immunohistochemistry: a tissue microarray study

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Background: Management of (pre) invasive human papillomavirus (HPV) induced cervical diseases depends on the histologic confirmation of the lesions. Misinterpretation of their morphologic criteria leads to a significant diagnostic disagreement. In addition, many benign changes mimic cervical preneoplastic lesions such as immature squamous metaplasia and atrophy. For these reasons, a continuous effort is still going on in order to discover surrogate markers which could help to reach an accurate definitive diagnosis. The aim of this study was to evaluate the usefulness of a panel of biomarkers in the characterization

of cervical intraepithelial neoplasia (CIN), invasive squamous cell carcinoma (SCC) and glandular lesions.
Methods: Archival biopsies of normal ecto- and endocervical tissues, squamous metaplasia, cervical intraepithelial neoplasia (CIN), squamous cell carcinoma (SCC), adenocarcinoma in situ (AIS) and adenocarcinoma (ADC) were retrieved to construct a tissue microarray (TMA). A panel of markers was tested on the TMA obtained slides by in situ hybridization (ISH) (HPV DNA) and immunohistochemistry (IHC) (p16, involucrin, Ki-67 and HPV L1 proteins).
Results: The sensitivity to detect high risk HPV DNA increased with lesion's severity. ISH signals suggesting integrated viral physical status predominated in CIN II/III, SCC and glandular (pre) neoplastic lesions. The p16 and Ki-67 protein expression increased from CIN I to CIN III and to infiltrative lesions. Involucrin positivity was better appreciated in well differentated diagnostic entities (ectocervix, mature metaplasia and CIN I). HPV L1 antibody detected the viral capsid protein in a low proportion of CIN I and II.
Conclusions: Using a panel of cervical biomarkers improves the final reporting of the various HPV induced epithelial lesions. Carefully constructed TMA with single spots of 1 mm diameter is a powerful search tool having a high reliability in representing the archival biopsies.

O-17

Androgen receptor expression in cervical carcinogenesis

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Background: If in vitro studies have demonstrated a potential interaction between HPV and androgen receptor (AR), their expression i vivo during cervical carcinogenesis remains unknown.
Methods: To clarify the issue, we have tested by immunohistochemistery the expression of AR in LSILs (CIN1) (n=30); HSILs (CIN2–3) (n=30) and invasive squamous cell carcinoma (ISCC) (n=13) linked to high risk HPVs (HR-HPVs). 30 cases of normal epithelium served as controls. A H-score system was used for quantification nd a H-score >50 was considered as positive.
Results: AR expression was observed in 100% of normal epithelium (30/30) and LSILs (30/30) but only in 63% of HSIL (19/30) and 23% of ISCC (3/13). A statistically difference ($p<0,05$) concerning this expression was found between normal epithelium and HSILs or ISCC, between LSILs and HSILs or ISCC and between HSILs and ISCC.
Conclusions: This is the first study study describing that the loss of AR expression is a frequent and common event in HSIL and ISCC resulting probably from complex interactions between HR-HPVs and these receptors. These data provide new insights concerning to potential role of androgen and AR in cervical carcinogenesis which should be confirmed in furthers studies.

O-18

PTEN AND KI-67 EXPRESSION IN NORMAL ENDOMETRIUM, ENDOMETRIAL HYPERPLASIA AND ENDOMETRIOID ADENOCARCINOMA

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PTEN is a tumor suppressor gene that inhibits cell proliferation by regulating intracellular signaling pathways. The aim of this study was to evaluate the expression of PTEN and Ki-67 in normal endometrium, endometrial hyperplasia and adenocarcinoma.
We studied PTEN and Ki-67 expression in 70 endometrial adenocarcinoma, 37 endometrial hyperplasia and 21 normal endometrium immunohistochemically. We evaluated the correlation between PTEN and Ki-67 expression and the most important prognostic parameters (tumor grade, myometrial invasion, lymphovascular invasion and stage) in carcinomas as well as the correlation between carcinomas, precancerous lesions and normal endometrium.
The expression of PTEN and Ki-67 index was the highest in the proliferative phase and PTEN –null glands were also seen in normal endometrium.
We found that the expression of PTEN in hyperplastic endometrium was significantly lower than normal endometrium and carcinoma ($x^2=11.90$, $p=0,003$). In accordance with this PTEN-null glands were significantly higher in hyperplastic endometrium ($x^2=16.55$, $p=0.00$). The difference in Ki-67 staining was not significant. When the subgroups of endometrial hyperplasia was evaluated PTEN-null gland ratio was significantly higher and PTEN staining ratio was significantly lower in hyperplasia without atypia ($x^2=7$, 48, $p=0,02$). No significant correlation in expression was found between subgroups of carcinoma, and the evaluated prognostic parameters. There was no correlation between PTEN and Ki-67 expression.
In conclusion our findings suggest that PTEN is not a distinctive factor in endometrial carcinogenesis but PTEN loss may be an important underlying mechanism for endometrial hyperplasia by causing susceptibility of cellular proliferation under the influence of estrogen.

O-58

Small Size Virtual Slides in Pathology

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BACKGROUND: Small Size Virtual Slide (SSVS) is a novel technique used by TEXCAN-II® software to make diagnosis on pathology slide images through an intranet or internet environment as these slides were seen under the microscope, with diagnostic quality, in a fast and reliable way, minimizing storage requirements.
MATERIAL & METHODS: SSVS technique was implemented in the TEXCAN-II® software. The system provide a online test where pathology samples were tested using a 5-degree scale of difficulty. Images corresponded to fluid, gynaecology smears and histological samples, captured and digitised using the TEXCAN-II®. JPEG2000 file format and JPIP server technology were chosen to optimise retrieving and transmission of virtual slides through any network.
RESULTS: The results showed coincidences of diagnosis at distance and under the microscope. Retrieving and transmission of images through intranet/internet were significantly faster than similar non-based JPEG2000/JPIP image sharing tools, keeping a optimum diagnostic quality of images.
CONCLUSION: The SSVS technique has been proved as a technique useful for delivering fast and accurate quality pathology images, offering a framework to digitise slides in relatively short time with a high compression rate, keeping the diagnostic quality of acquired images.
INDEX TERMS – Pathology, Telepathology, Telemedicine, Small Size Virtual Slide, TEXCAN -II®, JPEG 2000, JPIP server

O-59

Pathorama – atlas of virtual slides for various target groups

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ProjectDescription:
Histologic andcytologic preparations form an integral part of pathology education from basictraining to continuing education. At all levels of education the provision of adequatereference material poses a problem. In particular, from small biopsies andcytologic material only very few representative slides can be produced. Thisproblem can be circumvented by virtual slides i.e. fully digitized histologicand cytologic preparations that can be investigated microscopically over theinternet by an unlimited number of students.

Main objectivesof Pathorama at http://pathorama.ch/:

– Development of an open access online atlas with premiumquality histologic and cytologic reference slides for a visually orienteddiscipline.
– Provision of learning material for active and self-guidedlearning for a modern medical educationby allocating slides that can be interactively microscopied instead of static images.
– Pathorama has an integrative function for the institutionand for the society of pathologists and dermatologists by providing referencematerial for the application in different contexts (teaching, research, dailydiagnostics) in one location and readily accessible for all target groups.
– Modular technical concept allowing for the reuse of thecontents in different contexts and for a cost-saving support independent oftechnical experts.
– Integration of Pathorama slides in already existing awardwinning course modules at all levels of education.

Pathorama is used in three ways:

As a self-contained atlas:extensive metadata allow all users to find adequate slides.
As a supplement for existingmodules: our preexisting online pathology courses have been completed by virtualslides which allow the student to «microscope» each diagnosis.
As a foundation for new applications: for students, residents, and experts

O-60

Application programme for tumour bank authorisation in the Andalusian Network: The quality challenge

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Usually the creation of tumour banks (TBs) is an initiative of research-active centres seeking access to tumoral samples that have been conserved in excellent conditions. The situation differs in Andalusia because the Public Health System promotes the organisation of TBs as whatever other hospital service. The objectives are to improve treatment of oncological patients (especially their diagnosis) and promote research.
How may we set up a new service in a hospital that fulfils the requirements of treatment and academic interest, and that involves a range of professional disciplines and resources?
RESULTS
A programme has been designed for setting up and authorising TBs based on:

- Familiarisation of the centre's management team. Advantages of establishing the TB:
  - Improvement of diagnosis of oncological patients.
  - Promotion of research. Prospective availability of samples provided with the patients' permission.
  - Review of procedures for sending samples from sites of extraction to places of diagnosis.
  - Review of traceability of samples (identification, processing, storage, policy for obtaining samples, etc.).
- Election of person responsible for the TBs (Pathology)
- Analysis of each centre, specific adaptation of accredited guides for good practice. Policy for control of deviations.
- Familiarisation of all professionals involved.
- Trial period. Adjustment of procedures.
- External authorisation through auditing

In three years, of the 30 Andalusian hospitals, twelve are putting the process in place and four have already been authorised.

IMPROVEMENTS

- To increase institutional investment, allowing TBs to be organised more rapidly.
- To ensure information about the TB is available to professionals and patients. To improve the strategy of informed consent for obtaining permission to use samples: training of professionals, divulging policies for patients.
- Expansion of expectations for treatment (updating of molecular diagnostic hospital service).
- Development of scientific policy ensuring direct benefits to professionals.

O-61

Major discrepancy in clinical and autopsy cause of death: clinical doubt in only a minority of cases.

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Background: With progress in in vivo diagnostic methods, the role of autopsies in clinical medicine is sometimes questioned. We used autopsy request forms and reports to compare the postmortem findings with the clinicians' confidence in their cause of death (COD) diagnosis and with their judgment on the clinical relevance of the requested autopsies. Method: A consecutive series of 2414 clinical non-pediatric autopsies, performed between 2001 and 2006, was analyzed and compared with the autopsy request forms stating the clinicians' COD diagnosis and their rating of the clinical importance of the autopsy as minor, major or essential. Staff pathologists and a senior internist identified the cases with major discrepancies in COD diagnosis. Discrepancies were listed as class I or II, depending on whether or not pre-mortem knowledge of the correct diagnosis would have changed management with benefit in terms of life prolongation or cure.

Results: In 16% of autopsies, a major discrepancy was found between postmortem and clinical COD diagnosis. 61% of autopsies were judged of minor clinical importance, 30% of major importance and 9% essential. Major discrepancies were 14, 17 and 20% respectively. When doubt was expressed (12% of requests), major discrepancy was found in 26% (4% class I and 22% class II). In the large "no doubt" group, 14% showed major discrepancy (4% class I and 10% class II). Clinicians expressed doubt in 20% of discrepant cases.

Conclusion: Clinicians considered most autopsies of minor clinical importance. In 16% of autopsies major discrepancy was found between postmortem and clinical COD. Knowledge of correct diagnosis would have led to different treatment with clinical benefit for 4% of the patients. Clinicians had expressed no doubt on their diagnosis in 80% of discrepant cases. These data underscore the necessity for autopsies as a quality control tool in evidence-based medicine.

O-62

TMA-Match: automatic spot correspondence in TMA serial sections

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Background: Tissue Microarray (TMA) technology enables various high throughput RNA, gene and protein expression studies. For multiple studies on the same set of patients, TMAs are typically prepared from adjacent serial-sections of the 'recipient' paraffin block. During preparation, some tissue cores may fall off, making it a challenge to match spots across serial sections or relate them directly to clinical information, especially when a whole slide image is not available.

Method: We have developed automated tools that determine the spotcorrespondence between serial TMA slides and match the image numbers toabsolute coordinates (rows and columns), which in turn are matched toclinical information provided by pathologists. The tool relies entirely on the coordinates of the tissue spots obtained from the microscope and does not require the availability of a whole slide image. The whole slide image can be used when the spot coordinates are not available. We provide tools for visual validation as well.

Results: We used TMA-Match on ten TMA slides from a recipient tissue block originally consisting of 217 breast tissue cores from 55 patients. Six of the slides were conjugated with immuno-fluorescent (IF) dyes and examined under fluorescent microscopy. The remaining four slides were examined with bright field microscopy after Diaminobenzidine tetrahydrochloride (DAB) staining. The whole slide scan was not available for the DAB slides and one of the IF slides. Pathologists provided a TMA map for the block. All the spots were matched across all the serial sections with 100% accuracy. The TMAs were similarly matched with the TMA-map and hence clinical information without any error. The process of matching one TMA to another takes about 45 seconds on a Pentium M Windows PC. Visual images of the overlaid TMAs were generated for verification along with the spreadsheet file that lists the correspondences.

Conclusion: The ability to relate tissue spots in adjacent TMAs is very important for correlating multiple biomarker expressions in immuno histochemistry studies. TMA-Match is simple to use, and the required input is either the whole slide image or the relative x-y coordinates of the tissue spots on the slide. The coordinates can be easily obtained from the microscope. Spreadsheet files with the correspondences are automatically generated along with visual display of the correspondences for validation.

O-63

TMA-Q: A tissue quality assurance tool for sequentially multiplexed TMAs

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Background: Tissue Microarray (TMA) technology enables various high throughput RNA, gene and protein expression studies. Technologies to automatically quantify the expression of multiple proteins, genes or RNA on the same tissue by sequentially staining the tissues with dyes attached to biomarkers are the current focus of research in this area. One major challenge with the chemical-based sequential multiplexing approach is tissue folding and progressive tissue loss as the number of staining/bleaching steps increases. Also, the registration of corresponding images from sequential steps has to be verified before performing further analysis.

Methods: We have developed a workflow and quality assurance productivity tool that compares tissue images at any given step with the corresponding baseline images and computes metrics for registration failure, tissue folding or loss as well as tissue viability. This is automatically done for every step of a multiplexed study. The results are presented as a spreadsheet that can be further verified by a user. The quality assurance results are also illustrated graphically with annotated thumbnail images. The tool also allows the user to visualize all the tissue cores on a TMA from multiple sequential steps all at once and compare the results with the automatic evaluation.

Results: We conducted a number of experiments to quantify the performance of TMA-Q in speeding up the QA process. As shown with receiver-operating characteristic (ROC) curves, most of the image-to-image metrics evaluated perform well in identifying good quality tissues. For example, correlation coefficient (CC) gives up to 98.0% true positive rate at 4.9% false positive rate. Also, the tissues were automatically classified into Good Tissues, Partial Fold, and Tissue Loss and compared to manual labeling by an individual. About 94.4% of the images fall on the diagonal of the 3×3 matrix comparing the automated with manual annotation, indicating correct classification. This implies that the user will modify the spreadsheet output of TMA-Q in less that 6% of the images. It is interesting to note that no good tissue was classified as tissue loss (or vice versa) by the algorithm. The errors are in neighboring classes, some of the images of which are borderline cases.

Conclusion: A single TMA can have up to a few-hundred tissue spots and a multiplexed study may involve performing tens of sequential steps on the TMA. It is very crucial for the quality of each tissue spot at each step to be ascertained in order to avoid errors in consequent image analysis and tissue scoring. TMA-Q saves time in the quality assurance process by evaluating each tissue spot image based on registration accuracy, tissue quality, and tissue viability. Compared with examining the tissues one-at-a-time, the tool gives more than 15-fold productivity for validating a typical TMA.

O-64

RECURRENT LUPUS NEPHRITIS IN RENAL TRANSPLANT RECIPIENTS. A REVIEW OF 8 PATIENTS

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Kidney transplantation in Lupus Nephritis (LN) patients has become a successful treatment for this population. Recurrent LN (RLN) was previously considered rare, but its exact incidence is difficult to establish. We present a review of 8 renal transplant recipients secondary to LN followed for a long period, to establish our RLN rate.

PATIENTS AND METHODS. 8 women, mean age 40 years (y), graft from cadaveric donor (mean age 41 y).

First graft in 7 cases; second graft in 1 case. Mean Time on dialysis before transplantation, 48 months. Mean time to biopsy after transplantation, 28 m (7 d-90 m). Number of biopsied patients: 6, number of biopsies 12 (light microscopy LM and Immunofluorescence IF study). Mean follow-up: 71 m (15–126 m). Maintenance immunosupression: Ciclosporine A, Tacrolimus, or Mycophenolate mofetil and Prednisone. Outcome: graft survival is 100% at one year and 70% at 5 years.

RESULTS. LM study showed: Acute tubular necrosis (4/12), acute rejection (3/12), chronic rejection (2/12), CS-toxicity (4/12) and RLN (5/12). The 5 RLN biopsies belonged to 2 patients, with time to biopsy 11 m-60 m, and were graded as WHO stage 3 (4 cases) and stage 4 (1case). They showed extensive double contours and increased mesangial matrix, with semilunes in 4/5 cases. Compared to the non-RLN group, the only specific parameter was the presence of semilunes (0/7). IF showed glomerular mesangial and subendothelial deposits of IgG-IgA-C3 in 5/5 cases of RLN and in none case of the non-RLN group. Clinical outcome: C3-C4 in normal range in 8/8 patients, graft lost in 2 cases (1 chronic rejection, 1 RLN), proteinuria-increased Creatinine in 1 RLN.

CONCLUSIONS. RLN rate is 25% in our series, all of them as late RLN. Differential diagnosis with chronic transplant glomerulopathy is difficult and requires IF studies. RLN hastens in our series a bad prognosis, increasing the risk of graft lost.

O-65

Gastroesophageal reflux disease (GERD) is highly frequent in lung transplanted patients: the Padua experience

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Background: In lung transplanted recipients, gastroesophageal reflux disease (GERD) is associated with increased incidence of acute rejection, earlier onset of chronic rejection and higher mortality. We report the prevalence of GERD in all patients undergoing single (SLTx) or double (DLTx) lung transplantation from December 2006 to June 2007.

Methods: Sixteen patients (14 females, 2 males, mean age 49.5 yrs) were evaluated. Our protocol included: programmed spirometry, transbronchial biopsy and broncholaveolar lavage (BAL) for routine follow-up testing and lipid-laden macrophage index (LLMI) in the BAL for detection of GER-related lung aspiration, upper endoscopy, gastric emptying study, esophageal manometry and 24-hour pH-monitoring for detection of GER episodes. The median time to enter the scheduled protocol was 5 months from transplantation (range 2–84 mos).

Results: Gastric emptying was prolonged in almost all patients (12/16, 75%). GERD had a prevalence of 56% (9/16), documented at pH-monitoring. Oesophageal manometry revealed abnormal esophageal motility in 37% (6/16); endoscopy revealed the presence of esophagitis in 31% (5/16). LLMI suggestive for lung aspiration (cut-off value >85) was positive in 62% (5/8) patients with GERD and in 43% (3/7) patients without GERD. However, 2/3 patients with normal pH-assay showed moderate gastro-esophagitis at histology. In one case BAL collection was not available.

Conclusions: Our experience confirms the high frequency of GERD in lung transplanted patients. High values of LLMI are correlated with GERD, thus LLMI seems to be a sensitive diagnostic tool for lung aspiration.

PFP-1

Hybrid tumors in renal oncocytosis: the missing link between oncocytoma and chromophobe renal cell carcinoma?

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Background: The term "hybrid" tumor was proposed to describe tumors with morphologic features of both oncocytoma and chromophobe renal cell carcinoma. These tumors are composed of mixed histologic components in the same nodule, often showing transition features characterized by nuclear irregularity and perinuclear halos. The pathogenesis and genetic relationship between oncocytoma, hybrid tumor, and chromophobe renal cell carcinoma are uncertain.

Method: We analyzed the genetic pattern of hybrid tumors, oncocytomas and chromophobe renal cell carcinomas from three cases of renal oncocytosis comparing them with those found in 6 cases of multiple renal oncocytoma. Fluores-

cence in situ hybridization was performed to analyze chromosomes 1, 2, 6, 10, and 17 that are typically lost in chromophobe renal cell carcinoma and numerically normal in oncocytoma. X-chromosome inactivation analyses were also performed in women.
Results: The tumors studied from renal oncocytosis included 7 hybrid tumors, 3 oncocytomas and 1 chromophobe renal cell carcinoma. All three hybrid tumors from one oncocyotosis case showed gains of all five chromosomes tested, one hybrid tumor had gains of chromosomes 2 and 10; no numerical chromosomal alterations were detected in the other three hybrid tumors. In a third oncocytosis, three oncocytomas showed no loss or gain and the chromophobe renal cell carcinoma showed loss of chromosome 1, 6, 10 and 17. Twelve tumors from the patients with multiple oncocytomas showed no loss of any of the chromosomes; two other oncocytomas had loss of chromosome 1 only. A concordant pattern of nonrandom X-chromosome inactivation in the coexisting multiple lesions was seen in both cases of renal oncocytosis and multiple oncocytomas. **Conclusion**: Our data suggest that renal oncocytoma does not progress to chromophobe renal cell carcinoma or to hybrid tumor. These three renal neoplasms seem to be distinct and not related to each other.

PFP-2
PROGNOSTIC VALUE MICROMETASTASIS AND EXTRACAPSULAR SPREAD OF PELVIC LYMPH NODES IN CERVICAL CANCER PATIENTS
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Background: Limited knowledge exists about extranodal extension of the tumor outside the lymph node capsule, i.e. extracapsular spread (ECS) the prognostic impact of micrometastatic disease (i.e. metastatic deposits ≤0.2 cm = pN1mic) in cervical carcinoma (CX).
Methods: Pelvic nodes of 894 surgically treated CX FIGO IB to IIB were re-examined regarding metastatic disease. In cases with lymph node involvement, the metastatic deposits were measured using ocular micrometer and extranodal spread of the metastatic deposits outside the lymph node capsule (ECS) was recognised.
Results: 29.7% (266/894) patients represented with pelvic lymph node involvement. 22.1% of these patients showed micrometastases (pN1mic). Patients with metastatic disease represented reduced recurrence free survival (RFS) time (pN1mic: 112.6+6.8 and pN1: 122.5+9.6 months), when compared to patients without metastatic disease (pN0: 190.9+3.0 months; $p<0.0001$). 5-year-overall survival (OS) was also decreased in metastatic disease (pN0: 86.6%, pN1mic: 63.8%, pN1: 48.2%; $p<0.0001$).
ECS was seen in 30.9% of cases with metastatic disease. Occurrence of ECS showed a correlation to advanced stage disease ($p=0.02$), number of involved nodes ($p<0.001$) and the size of metastatic deposits ($p<0.01$). The 5-year recurrence free survival rate in patients with ECS was significant lower, compared to patients without ECS (59.75% [95% CI: 46.3% - 73.2%] versus 67.2% [95% CI: 58.9% - 75.5%]; ($p=0.04$). The 5-year overall survival rate was significant lower in patients with ECS (33.5% [95% CI: 20.6% - 46.3%] vs. 60.5% [95% CI: 52.3% - 68.6%]; $p<0.001$).
Conclusions: The results indicate that micrometastases and extracapsular spread (ECS) of pelvic lymph node metastases is of prognostic impact. A revised FIGO/TNM classification system for pelvic lymph node disease is recommended: pN1mic = micrometastatic disease (≤0.2 cm), pN1 = macrometastases; ECS 0 = lymph node involvement without extranodal spread of the metastatic deposits and ECS 1 = lymph node involvement with extranodal spread of the metastatic deposits.

PFP-3
VEGF and TSP-1 expression in basal/luminal subtype of breast carcinoma.
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Tumour progression depends, at least in part, on recruitment of new vessels or angiogenesis. This process depends on a balance between angiogenic and antiangiogenic factors. Vascular Endothelial Growth Factor (VEGF) is the main proangiogenic factor in breast cancer. Its secretion by tumour cells has shown prognostic relevance in most of the studies. Trombospondin-1 (TSP-1) is a well-known antiangiogenic factor that inhibits tumour growth and metastases in animals. However, its clinical relevance in breast cancer is equivocal. Some data suggest that TSP-1 can promote the increased expression of angiogenic factors by cancer cells reaching a greater propensity to metastasize successfully to distant organs. The aim of this study is to determine any association between TSP-1 and VEGF expression and to investigate their relation with the basal/luminal phenotype of breast cancer.
Method.- Paraffin-embedded tumour sections from 130 breast cancer patients were stained immunohistochemically for ER, PR, HER2, EGFR. Ck18, Ck19, Ck5,6, Ck14, TSP-1 and VEGF expression. Basal subtype was defined by

Ck5,6 and/or Ck14 positivity. Luminal subtype was defined by positivity of Ck18 and/or CK19.

Results.- A basal phenotype was found in 14% of the cases. ER and PR positivity were directly correlated with luminal subtype and inversely correlated with the basal one ($p<0.0001$). VEGF expression was considered as positive in 69 cases (53,5%). TSP-1 showed stromal stain in 78 cases (60%). An association between VEGF and luminal phenotype (Ck18) was found ($p=0,03$) while only 33% of the basal carcinomas showed VEGF positivity. TSP-1 stain was seen in most of the basal carcinomas (78,9%) although statistical signification was not reached ($p=0,06$). In addition, association between VEGF and TSP-1 was found.

Conclusions.- Our results suggest that luminal and basal subtype of breast carcinoma has different way of angiogenesis, with VEGF playing a relevant role mainly in luminal subtype. On the other hand, TSP-1 stromal expression is frequently found in basal carcinomas and its role in the angiogenic process must be investigated.

PFP-4

Clusterin expression in urothelial bladder tumors

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Background: Clusterin is a stress-associated cytoprotective protein, which is upregulated in many cancers and confers treatment resistance when overexpressed. To our knowledge, clusterin expression in urothelial tumors has been evaluated only in one study, but the relationship between the tumor grade and the clusterin expression has not been previously studied.

Method: We constructed tissue microarray (TMA) that included 153 urothelial tumors: 13 papillomas, 14 papillary urothelial neoplasms of low malignant potential (PUNLMP), 20 papillary non-invasive low-grade carcinomas and 106 high grade urothelial carcinomas (21 non-invasive, 21 with lamina propria invasion, 21 with muscularis propria invasion, 43 recurrent cases with muscularis propria invasion). The cases were selected from our institutional database. Immunostaining was performed using anti-goat clusterin antibody (Santa Cruz Biotechnology, Inc.). Clusterin expression was scored 0–3+ in the tumor cell cytoplasm, with a cut-off of >10% of positive cells.

Results: Clusterin was not expressed in benign or borderline urothelial tumors. Only one case (5%) of non-invasive low grade tumors showed clusterin expression. In contrast, 19% of non-invasive high grade tumors and 29% of all invasive high grade tumors, were positive, regardless of the depth of invasion. When the positive cases were grouped as low grade tumors vs. high grade tumors, there was a significant difference in the clusterin expression in high grade tumors ($p=0.0004$). No significant differences were found in the clusterin expression in relation to the depth of invasion (lamina propria vs. muscularis propria invasion) and when invasive recurrent and non-recurrent tumors were compared.

Conclusions: Clusterin expression correlated strongly with the urothelial tumor grade, which suggests that clusterin may play a role in the progression of the urothelial tumors. These findings may introduce a new target for nucleotide-based therapy and may lead toward better selection of patients who would benefit from additional aggressive chemotherapy.

PFP-5

Gene expression profiling in gliomas and immunohistochemical evaluation of IGFBP-2

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Background. The differential behaviour of low and high grade gliomas and the possibility to develop novel selective agents that specifically target tumour-associated proteins in gliomas stimulate the research of molecules playing a role in glioma progression.

Method. In the present paper gene expression microarray using about 20,000 genes allowed the study of the gene expression profile in 39 glial neoplasias (28 glioblastomas (GBM) and 11 low grade gliomas, namely 4 oligodendrogliomas, 5 pilocytic astrocytomas (PA), 2 fibrillary astrocytomas (FA)).

Results. Unsupervised classification through hierarchical cluster analysis identified 4 groups of tumours: 2 groups mainly composed of low grade malignant tumours (all the 11 low grade gliomas and 4 GBM), the other 2 were constituted by GBM. 276 genes resulted informative in all the tests. Among them 15 genes were underexpressed in low grade gliomas, but overexpressed in GBM; on the contrary 27 genes were underexpressed in GBM, but overexpressed in low grade tumours. In all GBM there was overexpression of IGFBP-2, that on the contrary was underexpressed in all low grade tumours. This protein, that acts as carrier of IGFs, is synthesized in many tissues to inhibit or enhance IGF actions but it may also have ligand-indipendent activity. Furthermore it is also frequently

overexpressed in various malignant tumours outside the central nervous system. IGFBP-2 immunohistochemical staining was found to be positive in only one low-grade glioma (1/10=10%; 95%CI 0,3–44,5%), while it was found positive in 24 out of 27 GBMs (88,8%; 95%CI 69,7–95,1%).

Conclusion. Gene expression profiling and immunohistochemistry suggest that IGFBP-2 may play a role in glioma progression. IGFBP-2 appears to be a novel immunohistochemical marker of malignancy in glial tumours and probably is the basis for targeted chemotherapy.

PFP-6

Mammalian target of rapamycin (MTOR) signaling activation patterns in neuroendocrine tumors of the lung

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Background. Adjuvant therapeutic strategies in metastatic lung neuroendocrine tumors (NETs) are still incompletely settled and are mostly planned on the degree of differentiation of the tumor. Clinical trials testing the efficacy of mTOR inhibitors in lung NETs are ongoing, despite little is known about mTOR activation patterns in this spectrum of neoplasms.

Method. We analyzed the expression of phosphorylated-mTOR and of its major targets p70-S6K and 4EBP-1 by means of immunohistochemistry in a large series of 162 surgically resected lung NETs with complete clinical and follow up information available; the series included 53 typical carcinoids (TC, of which 16 with metastases), 50 atypical carcinoids (AC), 33 large cell neuroendocrine carcinomas (LCNEC) and 26 small cell carcinomas (SCC).

Results. Higher levels of mTOR were detected in well differentiated lung NETs (TC and AC), as compared to poorly differentiated LCNEC and SCC (Mann Whitney test $p=0.01$), whereas 4EBP-1 was more expressed in poorly differentiated tumors ($p=0.02$). A strong correlation between mTOR and p70-S6K expression was observed (Spearman test $p<0.0001$) whereas 4EBP-1 was unrelated to mTOR expression ($p=0.89$), possibly due to the fact that 4EBP-1 antibody does not recognized specifically the phosphorylated form. Low mTOR expression was correlated to the presence of lymph node metastases in AC tumor group (Chi-square test $p=0.018$) and to residual/recurrent disease and disease-related deaths in the poorly differentiated tumor group ($p=0.03$) However, mTOR did not correlate with adverse outcome both in well differentiated and poorly differentiated tumor groups at univariate survival analysis. 4EBP-1 and p70-S6K were both unrelated to specific clinical features.

Conclusion. Peculiar mTOR activation patterns are present within the spectrum of lung NETs, with possible clinico-prognostic implications. The definition of mTOR pathway activation profile in lung NETs is of potential interest to test its predictive value in patients treated with mTOR-targeted therapies.

PFP-7

Squamous cell carcinoma of the vulva: HPV detection and its correlation with p16INK4a and p53 overexpression.

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Objective To assess the correlation of HPV DNA detection with p16INK4a and p53 over expression in relation to histology classification of vulvar carcinomas.

Methods Paraffin blocks corresponding to vulvar cancers are being collected from pathology departments archives. HE slides are cut and reviewed by 2 pathologists. Histological classification includes conventional keratinizing, warty and basaloid squamous cell carcinoma, as well as other less common variants. HPV detection is done by SPF-10 PCR subsequently followed by DEIA and genotyping by $LiPA_{25}$ (version 1). Samples are tested at HPV laboratory at ICO (Barcelona, Spain). Immunohistochemical detection of p16INK4a (clone E6H4, provided by mtm laboratories) and p53 (clone BP53–12–1, Biogenex) staining is carried out using standard procedures and read blinded to HPV results.

Results Data are presented on 196 cases. HPV DNA was detected in 43 samples. All 23 HPV positive cases with warty/basaloid pattern showed p16 overexpression (sensitivity 100%). Among 155 non warty/basaloid carcinomas HPV was detected in 20 samples, of which 16 were p16 positive (sensitivity 80%). p53 was positive (>25%) in 93 (49,2%) cases, of which only 5 were HPV positive. In HPV negative cases, p53 was detected in 88 (59,4%).

Conclusion P16INK4a over expression strongly correlates with HPV detection in cancer of the vulva. In contrast, p53 positivity has a high negative predictive value (94.6%) for HPV detection, both in warty/basaloid carcinomas (90%) and in non warty/basaloid (95,2%). The combination of

p16INK4a and p53 staining appears useful to identify carcinomas related to HPV.

PFP-8

Different microRNA profile in hyalinizing trabecular tumours of the thyroid and papillary thyroid carcinomas

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Aims: There is much controversy about hyalinizing trabecular tumours of the thyroid (HTT) regarding their biological behaviour. Because of morphologic (nuclear features) and pathogenetic (Ret/PTC rearrangements) similarities with papillary thyroid carcinomas (PTC) they are considered to be a variant of PTC. Using a set of microRNA typically upregulated in PTC we compared the microRNA expression profile of PTC with those of HTT.
Methods: Paraffin-embedded tissue specimen of 20 patients with HTT were investigated for the expression of a set of microRNAs (miR-146b, -181b, -21, 222 and -221) using real-time quantitative stem-loop RT-PCR. Tissues of 10 multinodular goiter and 10 PTC served as control.
Results: miRNA expression profiles of PTC and HTT are completely different. PTC show an upregulation of all investigated types of microRNAs whereas HTT and nodular benign tissue disclose a similar regulation profile.
Conclusions: Although there are similarities between HTT and PTC in histopathology and some molecular pathologic features the different regulation of the investigated microRNAs is against the assumption of HTT being a variant of PTC probably faouring its classification as a benign tumour.

PFP-9

MUM1 translocations in primary cutaneous anaplastic large cell lymphoma

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BACKGROUND: Anaplastic large cell lymphoma (ALCL) is a heterogeneous entity composed of ALK+ and ALK- systemic types and a primary cutaneous (CUT) type. ALCL previously was reported to express the transcription factor MUM1. We recently observed a case of peripheral T-cell lymphoma, unspecified, with a translocation involving the MUM1 and T-cell receptor-alpha (TCRA) genes. We therefore studied a series of ALCLs for MUM1 translocations using fluorescent in situ hybridization (FISH).
METHODS: We evaluated 27 cases of ALCL, including 10 ALK+, 12 ALK-, and 5 CUT. Mean ages in the 3 groups were 31 y, 61 y, and 58 y, respectively. MUM1 staining was performed using immunohistochemistry; staining in tumor cells was scored as diffuse (>30% of tumor cells), partial (10–30%), or negative (<10%). FISH for MUM1 was performed using a home-brew, two-color breakapart probe. Data from G-banded karyotypes were reviewed when available.
RESULTS: MUM1 staining was positive in 8/10 ALK+ (80%, diffuse staining), 10/12 ALK- (83%; 8 diffuse, 2 partial), and 2/3 CUT (67%; diffuse). Overall, 20/25 cases were positive (80%). Two cases could not be scored. FISH showed a MUM1 translocation in 2/27 (7%); both were CUT cases and were diffusely MUM1 positive by staining. Additional FISH showed no evidence of TCRA or IgH as a translocation partner. Neither case had G-banded karyotyping available. Of 11 cases with available karyotypes, 3 were abnormal. Each showed multiple aberrations, but no anomalies involving the 6p25.2 MUM1 locus were seen.
CONCLUSIONS: Occasional cases of ALCL contain translocations involving the MUM1 gene locus. In this study, such cases were restricted to CUT ALCL. Further study is necessary to evaluate (1) the biologic significance of MUM1 expression in ALCL, and (2) the possibility that the presence of MUM1 translocations might be helpful in distinguishing primary cutaneous ALCL from systemic cases.

PFP-10

Caspase 8, survivin and XIAP expression in Ewing Sarcoma Family Tumors (ESFT)

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Background: ESFT are common pediatric bone and soft tissue tumors with only 60% cure rate, because of drug-resistant tumor cells with impaired apoptosis. TRAIL, a novel agent, which induces apoptosis by activating caspase 8 and circumventing the mitochondrial pathway, may offer alternative therapeutic possibilities. However, because the balance of the pro-apoptotic caspase 8 and the anti-apoptotic proteins survivin and XIAP may affect the response of ESFT cells to TRAIL, we studied the expression of these proteins in ESFT tissues and cell lines.
Method: Archival, paraffin-embedded ESFT tissues from the Laboratory of Pathology, NCI were studied by immunohistochemistry for the expression of XIAP (48 samples), caspase 8 (38 samples) and survivin (37 samples). An immunohistochemical score was generated by multiplying the intensity of staining (scored as 0, 1+, 2+, 3+) with the percentage of positive cells (0, 1=<10%, 2= 10–50%, 3=51–80%, 4=>80%). Low expression was

defined as score 1–3, medium as 4–6 and high as 7–12. We also examined the expression of these proteins by Western blotting in 15 ESFT cell lines (TC-32, TC-71, TC-248, TC-268, 5838, SK-N-MC, TC-300, TC-324, TC-389, TC-390, TC-392, TC-393, TC-394, TC-399, TC-400). Commercially available antibodies were used.

Results: Moderate to high expression of caspase 8 was seen in 92% and of XIAP in 70% of the specimens. Survivin was expressed in 54% of the specimens. In 24 cases, in which staining for all 3 proteins was available, over-expression of both survivin and XIAP co-existed in 58%. ESFT cell lines showed generally high expression of caspase 8 and XIAP. Survivin expression was variable, similarly to the immunohistochemical data in the tissues.

Conclusion: Our data demonstrate that ESFT express high caspase 8 and therefore, may be amenable to treatment with TRAIL. High XIAP and survivin expression suggests that they may serve as therapeutic targets in selected cases.

O-19

Paediatric Barrett's oesophagus: clinico- pathological features of a rare condition in this age group.

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Background: Barrett's oesophagus (BE) is a common condition in adults but is quite rare in children. We describe the clinical, endoscopic and histological features of all cases of BE diagnosed at our institution between 2000 and 2007.

Method: Information was collected with respect to age, symptoms, treatment, endoscopic and histological features (colonic-like, small bowel-like, cardia-like and a mixture) from patient case notes and pathology computer data base.

Results: Nine children (8 males and 1 female) with a median age of 11 (5 to 15) years were diagnosed with BE. In 8/9 the presenting features were typical of gastro-oesophageal reflux (GOR), 4/9 cases had symptoms of dysphagia (one had a signet ring cell adenocarcinoma of the stomach infiltrating the BE). Two cases had a co-existing Helicobacter Pylori gastritis. The 9 patients underwent 23 endoscopies. These showed features of GOR with various degrees of erythema and superficial ulceration. A stricture was noted in 3/9. Histology confirmed BE in 21/23 endoscopies with: a) colonic-like mucosa in 3/21, b) small bowel-like in 3/21, c) cardia –like in 11/21 and d) mixed in 4/21. None of the cases had fundic-type gastric mucosa. Intestinal metaplasia (IM) was present in all our cases of BE. Sialomucins were present in 21/21 and sulphomucins in 17/21 samples. Both mucins expressed more in columnar than in goblet cells.

The estimated prevalence of BE in the paediatric population of South Yorkshire (North of England) is 0.0018%, 0.6% in children referred for upper GI endoscopy and 9% in the children with reflux oesophagitis. All patients in whom subsequent biopsies were done had intestinal metaplasia. BE is an endoscopic diagnosis that requires histologic confirmation.

Conclusion: BE is rare in childhood, although it has become a less rare diagnosis in our institution in the last few years. Standardised documentation of endoscopic landmarks by the paediatric gastroenterologist and conveying that information to the pathologist is essential for an accurate diagnosis of BE.

O-20

THE USE OF MLPA FOR CYTOGENETIC DIAGNOSIS IN NEUROBLASTOMA TUMOURS

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Background: Neuroblastoma tumour cells show complex combinations of acquired genetic aberrations, including amplification of the *MYCN* oncogene, deletions of chromosome arms 1p and 11q, and gain of chromosome arm 17q. Up to date, many different methods have been used for the detection of deletions and gains including chromosome analysis, Southern blot, fluorescence in situ hybridization (FISH) and methaphasic-comparative genomic hybridization (mCGH). However, these methods have some limitations. Studies using array-CGH (aCGH) are necessary to map the breakpoints precisely. A new methodology called multiplex ligation–dependent probe amplification (MLPA) has been developed. This new approach is based on polymerase chain reaction (PCR) amplification of ligated probes hybridized to target sequences. Furthermore, the MLPA reaction is fast, relatively inexpensive, and easy to perform, and the equipment needed for MLPA analysis is present in most molecular laboratories. In this study we used MLPA technique to detect the genetic alterations in 100 patients with neuroblastoma.

Method: Specifically designed set of probes for testing for chromosomal abnormalities in neuroblastoma, SALSA MLPA Kit P251/P252/P253 (MRC-Holland) was performed in all patients. P251 probemix contains 38 probes for chromosomes 1, 3 and 11; P252 probemix contains 36 probes for chromosomes 2 and 17; P253 probemix contains 32 probes for chromosomes 4, 7, 9, 12 and 14.

Results: The results obtained by MLPA were validated with FISH (*MYCN* amplification; 1p loss), mCGH and aCGH, showing a high concordance. The genetic alterations were used to group neuroblastic tumour in: (a) *MYCN* amplification; (b) segmental chromosome imbalance; (c) entire chromosome imbalance.

Conclusion: The use of MLPA as routine technique will be very interesting procedure to detect the implication of the

common genetics alterations in neuroblastoma that will permit obtain important information for estimation of prognosis and therapy available for patients.
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O-21
Implications of high feto-placental weight ratio in appropriate for gestational age (AGA) infants

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Background: Feto-placental weight ratio (FPR) is a reliable indicator of fetal growth and uteroplacental function in small-for-gestational age (SGA) fetuses. However, a diagnosis of high risk appropriate-for-gestational age (AGA) infants is clinically difficult. We propose that an abnormally high FPR can reliably identify high risk AGA infants at any gestational age (GA).
Methods: Placental and fetal measurements were available from a group of 208 singleton intrauterine deaths (IUD), identified through an audit of perinatal post mortems, who died at 24–43/40. A second group of live births (LB) included 277 neonates born at 33–42/40 at two London hospitals and 50 neonates delivered at 30–36^{+6}/40, whose placentas were sent for histological examination. All placentas were fixed and trimmed prior to weighing. All cases were categorized into four groups according to the FPR percentiles by GA (<10th, 10–89th, 90–97th and >97th). The relationship between FPRs and survival was analysed using Chi-square test. Logistic regression was conducted to adjust for GA and birth weight (BW).
Results: The Chi-squared test showed that survival was significantly related to FPRs ($p<0.001$). Logistic regression demonstrated that after adjusting for GA and BW, FPR remained significant ($p<0.001$) with infants with >97th FPR 9.80 times more likely to die than infants with 10^{th}-89^{th} FPR ($p<0.001$, 95% CI 4.57–21.28). In infants with >97th FPR, 75.6% (n=34) were IUD and 24.40% (n=11) were LB. Of the IUD, 17.6% were less than 34^{+6}/40, 8.8% were between 35–36^{+6}/40, and 73.5% were older than 37/40. For each gestational age, 67%, 67%, and 76%, respectively, were AGA.
Conclusions: A substantial proportion of AGA fetuses can be reliably identified as at risk by measuring FPRs at any GA. This emphasises that placental and fetal weights are both important to identify AGA babies at risk, who may subsequently be rescued by an appropriate clinical intervention.

O-22
Congenital granular cell tumor in a newborn, a case report and literature review

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Congenital Epulis is a rare tumor of the newborn, also known as granular cell tumor of the newborn or congenital gingival granular cell tumour because of its histological features(this tumor arises from the mucosa of the gingival). Neumann first described CE in 1871. CE is usually benign tumor. Epulis is seen only in the newborn and is a different entity from other granular cell tumors. CE has a female predilection with 8:1 ratio. Epulis is most frequently located on the anterior maxillary alveolar ridge and usually occurs as a single mass although 10% cases occur as multiple. CE clinically appears as a pedunculated protuberant mass which may interfere with respiration or feeding. In cases with large lesions mechanical oral and nasal obstruction can impair fetal deglutition and neonatal respiratory efforts resulting in polyhydramnios prenatally or respiratory impairment postnatally. The exact histiogenesis is still uncertain, various theories of origin are epithelial, undifferentiated mesenchymal cells, pericytes, fibroblast, smooth muscle cells and nerve related cells. The recommended treatment is prompt surgical resection. Recurrences of the tumor and damage to future dentition have not been reported. Spontaneous regression of congenital epulis has been reported in a few cases. However, surgical excision is generally indicated due to interference with feeding or respiration. We report a female neonate with Congenital Epulis (multiple tumors in the anterior maxillary alveolar ridge) and review the relevant literature. The tumors was rejected by surgical excision. The intra- and postoperative course was uneventful. Follow-up for three months didn't show recurrence.
Key words: Congenital epulis, Congenital granular cell tumor, new born.

O-23
Umbilical Cord Stricture Is Not a Genetic Anomaly

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Background: Umbilical cord stricture (UCS) at the fetal insertion is a poorly understood cause of fetal demise. Little is known about its epidemiology and pathophysiology. It has been traditionally considered a sporadic entity with a

very low risk of recurrence in subsequent pregnancies. However, a genetic cause for UCS has been proposed, based on two recent reports on recurrent cases.
Methods: We reviewed 130 cases of UCS located at the fetal insertion, diagnosed among 2,067 fetal and infantile autopsies we performed during the last 10 years, focusing on 2 recurrent episodes and 16 cases occurring in multiple pregnancies. Zygosity was analyzed by placental histology and/or a panel of 12 microsatellite markers to classify twins as monozygotic (8) or dizygotic (8).
Results: UCS was found in 6.2% of the autopsies. All cases were macerated stillborn fetuses and 54.6% were ≤20 weeks of gestational age. There is not a higher incidence of UCS in multiple gestations. Among twins, males were affected more than females (2.2:1), although no statistical significance was found. Associated umbilical cord anomalies were present in 8/16 cases, consisting in hypocoiling (3 cases), hypercoiling (2), excessive length (1), vascular tortuosity (1) and single umbilical artery (1). The most frequent placental finding was obliterative endovasculopathy (6). All eight genetically identical twins were discordant for the anomaly and two dizygotic twins with UCS had co-twins that were also affected.
Conclusion: UCS is not a genetic condition and parents with a fetal demise due to UCS should be counseled as having a low-recurrence risk for subsequent pregnancies. Twinning should not be considered a risk factor for UCS.

O-24

Cardiac Stem Cells in human right ventricular remodelling after surgical palliation for Hypoplastic Left Heart Syndrome

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Introduction: The recognition that myocytes and vascular structures could be formed from primitive cells homed to myocardium from circulation or from resident cardiac stem cells was accompanied by the idea that increased cardiac mass in cardiac hypertrophy could result from a combination of myocytes hypertrophy and hyperplasia. Hypoplastic Left Heart Syndrome is a complex congenital heart disease, characterized by severe hypoplasia of the left-sided heart and right ventricular hypertrophy. Aim of the study was to identify the role of cardiac stem cells in the right hypertrophy remodelling in a group of patients affected by Hypoplastic Left Heart Syndrome.
Aim of the study was to identify the role of cardiac stem cells in right ventricular remodeling of patients affected by Hypoplastic Left Heart Syndrome after Norwood surgical procedures.
Methods: Eleven hearts of the affected patients (ages 9 to 365 days) were compared with age-matched controls. Samples taken from the inflow and outflow tracts of the right ventricle were evaluated at histology and immunohistochemistry for stem cells markers.
Results: we observed a recruitment of cardiac precursor stem cells (CPSCs) CD117 and CD105 positive, both in the inflow and the outflow tract in operated hearts compared to the controls (*CD117* inflow: controls 7.8±3.3, operated hearts 18.09± 15.85 cells/field, with p=0.06; outflow: controls 9±4.73, operated patients 17.81±13.61cells/field with p=0.01; *CD105* inflow: controls 1.3±0.9, operated patients 9.18±3.8 cells/field, p=0.07; outflow: controls 1.8±1.6, operated patients 6.2±3.1 cells/field, p=0.1). CPSCs seem to be mainly mobilized by acute injury (early death patients) rather than by chronic processes (late death patients) since a higher presence of putative cardiac stem cells (CD105) was detected in operated patients. The major fraction of CPSCs were found to be localized near collagen III. Rare cycling cardiac precursor cells, positive both for myosin and Ki67 markers were found.
Conclusions: During right ventricle hypertrophy we observed a recruitment of CPSCs, suggesting an active role of endothelial precursor progenitor cells in cardiac remodelling mainly involving the interstitial and endothelial tissues.

O-25

Synovial sarcoma: Presentation of seven cases reports with unnusual localization

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BACKGROUND: Synovial sarcoma is a rare soft tissue tumor that most often occurs in the extremities of young adults. On exceptional occasions it is also encountered in areas without any apparent relationship to synovial structures, as in the retroperitoneum, pleura, mediastinum, parapharyngeal region, or abdominal wall.
MATERIAL AND RESULTS: Our report included 7 patients who ranged in age from 12 to 57 years with neoplasm. Primary site were mandible articulation, larynx, wrist, arm, forearm, abdominal wall, and chin. Histological and immunohistochemically these tumors were classified into monophasic and biphasic variants of synovial sarcoma. Complete surgical excision was the treatment of choice. Chemotherapy was administer in 6 cases in the postoperative period and in one case preoperatively. Local recur-

rence was developed in two patient and distant metastasis in three. Four of the patients were alive and three free of the disease.
Synovial sarcoma is a rare soft tiisue neoplasm. Only 3% of these mesenchymal malignant tumors occur in the head and neck region and only 13 cases with endolaryngeal loalization have been reported so far. We report 6 new cases, three of them in the mandible articulation, larynx and chin. The clinical and pathologic feattures of these cases, as well as a practical approach to the diagnosis, is presented.
CONCLUSIONS: Synovial sarcoma needs a differential diagnosis with other neoplasms. More than 50% of the cases shows recurrence and have a high rate of distant metastases. Accurate diagnosis of these tumors requires adequate tissue sampling, immunohistochemical staining and characteristic chromosomal traslocations findings. The poor prognosis of this sarcoma justified a radical surgery wth postoperative neoadyuvant treatment.

O-26

Is beta-catenin Useful in Evaluating Surgical Resection Margins in Desmoid Tumors?

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Background: Immunostain of beta-cateninis reported useful in confirming a diagnosis of desmoid tumor, which isa locally aggressive spindle-cell neoplasm that requires negativesurgical margins to prevent recurrence. However, H&E sections aredifficult to be sure of a negative margin due to the infiltrativegrowth pattern and bland cytology. The use of beta-catenin staining to evaluate the adequacy of surgical margins in desmoid tumor has not been documented. We investigated beta-catenin staining in a series of desmoid tumors to determine if tumor cells with nuclear expression of -catenin were present at surgical resection margins previously diagnosed as free of tumor by H&E alone.
Design: Paraffin embedded sections from 48 cases of desmoid tumor were immunohistochemically stained with -catenin(1:100, clone CAT-5H, Novocastra). Sections were evaluated for nuclearand cytoplasmic staining. Eight cases of desmoid tumor previouslydiagnosed as having free resection margins by H&E were reviewed,and all sections (n=42) of these margins were immunostained with beta-catenin. These sections were evaluated for the presence of tumor cells (identified by nuclear beta-catenin staining) at the margins.
Result: Nuclear and cytoplasmic staining for beta-cateninwas seen in 46 of 48 cases (96%) of desmoid tumor. One case had onlycytoplasmic staining, and one case had no staining. Tumor cells withnuclear staining were seen infiltrating the inked margins in 5 of the 8cases (63%), represented by 12 out of the 42 margin sections, in whichwe reevaluated margins using beta-catenin immunostain.
Conclusion: Nuclear expression of beta-catenin is a frequent finding in desmoid tumors in our series. Beta-cateninimmunostaining enabled us to identify desmoid tumors with positivemargins that were previously diagnosed as negative by H&E. Therefore, the use of beta-cateninimmunostain may be helpful in detecting foci of tumor cells involvingthe margins, subsequently enabling optimal treatment of the patient.

O-27

Peculiarities of cryptococcosis combined with HIV infection

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Cryptococcosis (C) remains one of the most important complication of HIV-infection on the stage of AIDS. We observed C up to 14,9% as the immediate course of lethal outcomes. On the clinical material prevailed the lesions of the central nervous system; their diagnostics was based upon mycological culture and cytological investigation of CSF with revealing of typical capsulated fungi. In cases the early diagnostics of C allowed the effective treatment.
In the lethal cases C always combined with other infections, recently in most cases tuberculosis. We succeeded to evaluate C. lesions in meninges, brain, lungs, lymph nodes, kidneys, spleen, adrenals and heart. Practically always there was no exudative reactions in the tissues. C varied in seize and thickness of the capsule. Of particular interest was one our case with long term clinical course of HIV, C, and tuberculosis. The patient of 24 years received VAART therapy, fluconazol, amfotericin B etc, died after 6 years history of illness. At the autopsy there were found the hallmarks of HIV infection at stage C: candida pharyngitis, esophagitis; widespread C.: meningoencephalitis, nephritis, splenitis, hepatitis, tuberculosis of lungs with dissemination in kidneys, liver spleen. Some C. were revealed just in the focuses of caseous necrosis. We considered the leading role in the lethal outcome of C lesions of the CNS with numerous polymorph fungi in the histological slices. In order to study the peculiarities of the ultrastructure of the tissue forms of C we refixed for electronic microscopy according our modification specimens of brain and

meninges. The relative good storage of the tissue allowed evaluating the state of C We succeeded to reveal 4 different variants of their degradation, totally intact fungi were absent. Some of the C were phagocyted by macrophages and had undergone degradation. In the macrophages were observed also the signs of catabolic and anabolic processes at once. The time and mechanisms of C degradation in the patients with HIV-infection has to be investigated specially.

O-28

Clinico-pathological discrepancies in the diagnosis of causes of maternal mortality in sub-Saharan Africa

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Background: Maternal mortality in sub-Saharan Africa continues to be a major public-health problem insufficiently recognized. Extreme differences in maternal mortality rates between developed and developing countries indicate that most of these deaths are preventable. Diagnostic errors may play an important role in this burden.

Methods: We carried out a retrospective analysis of clinico-pathologic correlation, using necropsy as the gold standard for diagnosis. All maternal autopsies during the period from October 2002 to December 2004 at the "Maputo Central Hospital, Mozambique were included and major diagnostic discrepancies, those involving the cause of death, were analyzed.

Results: Major diagnostic errors were present in 56 (40.3%) cases. A high rate of false negative diagnoses was observed for infectious diseases which showed sensitivities under 50%: HIV/AIDS related conditions (33.3%), pyogenous bronchopneumonia (35.3%), pyogenous meningitis (40.0%) and puerperal septicemia (50.0%). Eclampsia, was the main source of false positive diagnoses, showing a low predictive positive value (42.9%).

Conclusion: Clinico-pathological discrepancies were very frequent. They may have a significant impact on maternal mortality in sub-Saharan Africa. Increasing clinical awareness of the impact of obstetric and non-obstetric infections with their inclusion in the differential diagnosis, together with a thorough evaluation of cases clinically thought to be eclampsia could have a significant impact on the reduction of maternal mortality.

O-29

Comparative study of HER2/neu, CD34 AND SMA expression in benign, In situ and invasive ductal breast carcinoma.

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Background: Tumor-stromal interaction plays an important role in tumor invasion and metastasis of breast cancer. The purpose of this study was to evaluate HER2/neu expression in relation with CD34 and smooth muscle actin (SMA) expression in breast fibroblasts.

Methods: Immunohistochemistry for HER2/neu (HERCEPTEST), CD34 and SMA was performed on 159 cases including 10 normal, 20 cases of ductal carcinoma in situ (DCIS) and 129 infiltrating ductal carcinomas. The relation between staining pattern and histopathological features was recorded as positive, negative, or reduced.

Results: Mean age was 52±11 years. 50% were clinical stage II and III. Fibroblasts around normal duct–lobule units were CD34 positive and mainly SMA negative. In contrast, fibroblasts around invasive carcinoma were CD34 negative and SMA positive. In DCIS, loss of CD34 was significantly more frequent in high grade tumours than in low or intermediate grade ones. Her2/neu was seen in 25% of infiltrating ductal carcinomas and 60% of DCIS. There was no correlation between Her2/neu and SMA expression (Rho>0.05).

Conclusions: These results show that loss of CD34 is strongly related to the malignant phenotype, in both pre-invasive and invasive disease. SMA positive myofibroblasts were strongly associated with invasive ductal carcinoma. HER2/neu expression in DCIS and invasive ductal carcinoma showed similar findings compared with otherx studies. Loss of CD34 probably is not related with HER2/neu expression. The functional relevance of altered CD34 and SMA expression is unclear but focal changes implicate local signaling mechanisms probably of epithelial origin, independent of HER2/neu expression.

O-30

Prognostic significance of Aquaporin 1 (AQP1) in human breast cancer

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Background Aquaporin1 (AQP1) is a water channel protein widely expressed in endothelial and epithelial cells

in different organs, where it facilitates water flux across cell membranes. In the human female breast, AQP1 is expressed in endothelial and myoepithelial cells, but its function in resting, lactating and malignant breast tissues is largely unknown. There is increasing evidence that AQP1 is also expressed in high-grade tumour cells of different origin and it may play a role in tumour angiogenesis, cell migration and metastasis.

Methods The aim of this study was to assess for the first time the immunohistochemical expression of AQP1 in a well-characterised cohort of 203 invasive breast carcinomas with long-term follow up to unravel possible associations with patient and tumour characteristics and outcome data.

Results AQP1 expression was found in 5.4% of cases, all belonging to the group of basal-like carcinomas. Accordingly, significant correlations of AQP1 expression were found with high tumour grade (P=0.049), medullary-like histology, absence of oestrogen receptors (ER), "triple-negativity" for ER, progesterone receptor (PR) and HER-2, cytokeratin 14 (Ck 14) and smooth muscle actin expression (SMA) (P<0.000 for each). In univariate analysis, AQP1 was highly significantly associated with poor prognosis (P<0.000). Multivariate analysis showed that AQP1 expression has an independent value in predicting outcome if stratified by age, tumour size, lymph node status, histological grade and ER status (P=0.002).

Conclusions Our results suggest that AQP1 expression is a novel characteristic feature of a particularly aggressive subgroup of basal-like breast carcinomas, which are poor responders to standard chemotherapy. Such patients need new treatment options.

O-31
Quantitative Digital Image Analysis of Estrogen Receptor Immunostaining As a Model for Morphological Screening of Nuclear Immunomarkers

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Background: The application of tissue microarrays (TMAs) to histopathology has dramatically increased the productivity and reproducibility of the immunohistochemical analysis of biomarkers, and required the development of analytical tools for the rapid and accurate assessment of new biomarkers. The aim of this study was to evaluate the clinical utility of automatic analysis of immunomarkers with nuclear staining pattern.

Methods: We used TMAs containing 288 breast carcinomas and estrogen receptor (ER) immunostaining. The TMA slides were digitalized by ARIOL imaging system and scored manually by a pathologist using clinically validated guidelines, considering staining intensity (scores 0–3) and proportion (scores 0–5), resulting in a combined score 0–8. The images were digitally edited by the pathologist and tumor cells selected, where possible. Any images without tumor cells were excluded from the further analysis. The original and edited images were then scored automatically by both ARIOL (Applied Imaging Inc.) and ImageJ (NIH) software. The prognostic significance of all scores was measured by Kaplan-Meier analysis. Receiver-Operator Curve (ROC) analysis was used to measure the sensitivity and specificity of automatic analysis. Kappa-statistics were used to measure agreement between automatic and manual scoring systems.

Results: The automatic scoring of ER (produced by either method) was of prognostic significance in Kaplan-Meier analysis ($p<0.001$). Using original images and manual scores as gold standard in ROC analysis (optimal cut-off of 4–5% positive nuclei) the ARIOL showed 85% sensitivity and 95% specificity compared to 80% sensitivity and 85% specificity for ImageJ scoring (areas under ROC curve 0.94 and 0.88 accordingly). The ARIOL performance improved for edited images (ROC area 0.96) showing 97% specificity, and had no effect on sensitivity. Kappa statistics showed substantial agreement between manual and automatic scoring (Kappa 0.6–0.7, $p<0.001$).

Conclusion: Automated digital image analysis of nuclear immunomarkers is a robust high-throughput screening tool with potential clinical application.

O-32
Evaluation of the axillary clearance rest material, Telemark Hospital-preliminary observation

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Sentinel lymph node (sln) examination in the breast cancer patients is well established and performed in all cases of invasive, microinvasive carcinoma and DCIS grade 3 diagnosed patients. A positive result of sln by frozen section, imprint cytology or routine histology causes the axillary node sampling, and the histologic diagnosis has prognostic and therapeutic consequences.

In our study we have spared the rests of axillary clearance material and performed a "second look" cut up,especially searching for not previously found lymph nodes. New sections have been taken and also sections from all negatively reported lymph node remnants-were stained by AE1/AE3 cytokeratin. Our preliminary observation consists of 150 cases In the group of 31 "new lymph nodes" we found 8 micrometastases, while in negatively reported rest material 11 micrometastase have been detected.

Our short study shows, that there are differences in detected lymph nodes in axillary sampling between cut up performing pathologists. The reexamination and a use of simple cytokeratin stain increase the number of detected positive lymph nodes, what may contribute to the better assesment of prognosis and therapy.

O-33

Frequent exon 20 PIK3CA mutations in Her-2 positive breast carcinomas

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PIK3 mutations are mainly located in exons 9 and 20 and may be important events in breast cancer (BC) progression.. We studied the these mutations in aggressive tumours with Her-2 over-expression or triple-negative BC (negative for ER, PR and Her-2). Forty-nine cases of breast carcinomas with negativity for oestrogen receptors (ER) and progesterone receptors (PR) were collected: 20 with Her-2 over-expression and 29 with triple negative (TN) BC. Clinicopathological data were reviewed and DNA was extracted from frozen tumour or paraffin embedded tissues using standard procedures. PIK3 mutations were assessed by PCR amplification and subsequent sequencing analysis for exons 9 and 20. Five exon 20 PIK3CA mutations were identified among the 20 Her-2 positive BC, whereas one exon 9 PI3K mutation appeared in TN BC (p=0.03). No correlation was found with prognosis. Over-expression of p110α was related with exon 20 PI3KCA mutations (p=0.001), whereas no correlation was found with PTEN loss, Akt over-expression, or other pathological features. Although exon 9 and 20 PI3K mutations are rare in TN, PI3KCA mutations in exon 20 may be relevant in Her2 BC.

TN BC (p=0.03). No correlation was found with prognosis. Over-expression of p110α was related with exon 20 PI3KCA mutations (p=0.001), whereas no correlation was found with PTEN loss, Akt over-expression, or other pathological features. Although exon 9 and 20 PI3K mutations are rare in TN, PI3KCA mutations in exon 20 may be relevant in Her2 BC.

O-34

Nature and prognostic signification of ckit gene expression changes in breast cancer

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Background: The c-kit gene encodes a transmembrane tyrosine kinase growth factor receptor involved in the development of certain neoplasms. Conflicting results concerning its expression have been reported in malignant breast lesions. Using immunohistochemistry approaches, c-kit expression seems to be lost in most breast neoplasms. However, an increase of c-kit expression has been associated with aggressiveness and poor outcome. The objective of this study was to better characterize the c-kit gene expression within the spectrum of breast ductal carcinoma.
Method: One hundred and twenty eight randomly selected cases of invasive ductal carcinoma containing normal and neoplastic areas were obtained from the local Tumor Bank. The expression of c-kit was analyzed by immunohistochemistry and mRNA expression was evaluated by real time polymerase chain reaction (QPCR). C-kit mRNA was selectively quantified in normal and neoplastic breast epithelial cells using cytokeratin 18 expression as internal reference. We also correlated the findings with tumor size, WHO grade, proliferative activity by Ki67 expression, lymph node metastasis, and expression of HER-2, estrogen receptor (ER)-α, and progesterone receptor (PR).
Results: Using immunohistochemistry and QPCR, protein and mRNA expression of c-kit was high in normal mammary gland. Analyzing tumor regions the different methodologies gave somewhat different results. Protein expression was lost in most invasive breast cancer (89,84%) and was only maintained in few tumors with high tumor size (≥2 cm), high proliferative activity, and estrogen receptor expression. However, mRNA expression maintained a normal index in many neoplasms (51,72%) and the decrease of expression on neoplastic cells was statistically associated to high tumor size and high WHO grade.
Conclusion: These results suggest that the c-kit pathway plays an important role in the maintenance of normal mammary epithelium and that malignant transformation and progression is accompanied by its loss.

O-35

Impact of American Society of Clinical Oncology/ College of American Pathologists guideline recommendations on HER2 testing in breast cancer

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Background: Testing for HER2 gene amplification by fluorescent in-situ hybridization (FISH) and protein expression by immunohistochemistry (IHC) plays a critical role in management of patients with invasive breast cancer. The new 2007 guidelines by ASCO/CAP define a HER2 IHC staining of 3+ as uniform intense membrane staining in >30% of invasive tumor cells as compared to previously defined >10% strong staining. We set out to study the

impact of this change on the results of HER2 IHC testing and its correlation with FISH results.
Method: One hundred forty cases of invasive breast carcinoma with IHC of 3+, scored according to the HercepTest interpretation guidelines of strong membrane staining of >10% cells, were retrieved from the archive of Mayo Clinic Rochester. The HER2 IHC slides were re-reviewed and results recorded as percentage of 3+, 2+, 1+, and 0 staining. HER2 FISH analyses were performed in tumors with 11%-30% of IHC 3+ staining.
Results: Of the 140 cases, 14 cases (10%) showed intense membrane staining in 11–30% of the invasive tumor cells and would have been scored as 2+ according to the new ASCO/CAP guidelines. Of the 14 cases, 5 cases were negative for HER2 gene amplification by FISH (HER2/CEP17 ratio of <1.8), 2 cases were equivocal (ratio of 1.8–2.2), 6 cases were positive (ratio >2.2), and 1 case showed dramatic intratumoral heterogeneity with high level amplification (ratio of 12.2) in the IHC 3+ area and no amplification (ratio of 1.0) in the IHC 1+/2+ areas.
Conclusion: The new ASCO/CAP guidelines down-scored 10% of tumors from 3+ positive to 2+ equivocal in this study, and half of these tumors are positive for HER2 gene amplification. Clinical study is needed to determine whether the new guidelines would be better at predicting response to anti-HER2 therapy.

O-36

Basal- and non-Basal Phenotypes in Triple-Negative Breast Cancers

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Background: Triple negative breast cancers are characterized by lack of expression of oestrogen, and progesterone hormone receptors, and lack of HER2 overexpression. Frequently they are associated with a basal phenotype, but there is a distinct subgroup of "quadruple-negative" cancers which not basal-like, and are yet ill defined yet. Therefore, in the present study, we have analyzed the expression of basal and other markers the triple negative breast cancers.
Methods: 158 triple-negative invasive breast carcinoma were selected from the archives and used for construction of tissue microarrays. A panel of immunohistochemical markers, which included ER, PgR, HER2, CK5/6, CK14, CK18, EGFR, p53, c-KIT, MKI-67, bcl-2, and p16 was used to further characterize these tumours.
Results: 102 tumours (66%) showed a basal phenotype by being positive for either CK5/6 or CK14 using a cutoff value of 10%. The non-basal triple-negative cancers differed from the carcinomas with a basal phenotype by having a lower proliferative rate ($p=0.04$), and were less frequently CD117 positive (14% vs. 32%, $p=0.01$) and less frequently overexpressed p16 (31% vs. 52%, $p=0.01$). No differences were seen for bcl-2 (9% vs. 10%) and p53 overexpression (37% vs. 36%).
Conclusions: Non basal-like triple-negative breast cancers differ from basal-like triple-negative breast cancers in several aspects, and have a lower malignant phenotype. Therefore, this subgroup of triple-negative breast cancers is important to distinguish from the basal-like triple-negative breast cancers.

O-37

Role of intraoperative imprint cytology for evaluation of surgical margins in breast cancer: a prospective controlled study about 400 lumpectomy

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Objective: The aim of this study is to assess the efficacy of intraoperative imprint cytology (IIC) in conservative surgery for early stage of breast cancer.
Materials and methods: This is a prospective controlled study led at the Comprehensive Cancer Center of Rennes, France. We reviewed a total of 736 patients who underwent lumpectomy for unifocal breast cancer from January 2004 to 31st December 2005. 400 procedures were finally included. After a standardised surgical procedure, the lumpectomy specimens were intraoperatively oriented by he surgeon and were submitted fresh to pathology for cytologic assessment. The IIC consisted of touching the four faces of interest (superior, inferior, and laterals) onto the glass slide.
Results: We evaluated 1665 separate margins. The correlation between IIC and permanent sections was 91.5%. Sensitivity was 88.6%, and specificity was 92.2%. Positive predictive value was 73.6%, and negative predictive value was and 97%. The analysis of the false positives, show significantly ($p<0.001$), that the lesions of fibrocystic mastopathy were more frequently identified in the lumpectomy. The rate of false positives was significantly increased when the technique used more than four apposition slides ($p=0.0019$). The ductal carcinoma in situ was statistically greater represented among the false negatives ($p<0.005$), above all when its size was ≥30 mm ($p<0.005$). The intraoperative reexcisions not requested by the pathologist were tumour free in 93.9% of cases; conversely there was 71.2% residual tumour when the pathologist requested these reexcisions. The global rate of postoperative reexcisions was 13.25% and IIC avoided a second intervention for 47 patients.
Conclusion: Intraoperative imprint cytology is a rapid, simple, reliable and cost-effective method to analyse the margin status

of lumpectomy in patient undergoing breast conservative surgery. This technique allows a survey of the entire surface area of the lumpectomy specimen. This method is enables to reduce the number of second operative procedures.

Keywords: Imprint cytology, Margins, Breast cancer, Lumpectomy

O-38

Luminal and basal phenotypes in salivary duct carcinomas

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Background Salivary duct carcinoma (SDC) is a morphological look-alike to ductal breast carcinoma. Molecular studies have identified four subgroups of breast carcinomas, oestrogen receptor (ER) positive, luminal cell, basal cell and non-specific types. Luminal carcinomas are characterized by HER-2 amplification, basal cell type by cytokeratin 5 positivity and epidermal growth factor receptor (EGF-R) overexpression. SDC are not associated with ER expression whilst HER-2 receptor overexpression is frequent. A group of non-HER-2 amplified SDC exists. Thus, the question emerged whether there is a basal type of SDC.

Method 28 SDC were available for analysis. The cases where either *de novo* or carcinomas ex plemorphic adenoma with SDC morphology.

CK5, p63 and EGF-R were immunohistochemically stained. Cases with >10% cytoplasmatic (CK5), nuclear (p63) and membranous staining (EGF-R) were considered positive.

Fluorescence in situ hybridization for HER-2 was performed. A ratio (HER-2:centromere) of ≥2.2 was considered amplified, high (not countable) HER-2 signal were considered as clusters. HER-2 data has previosly been reported.

Results HER-2 clusters were found in 14 tumours, two tumours were heterogeneous with non-amplified areas. One case showed low-level amplification.

18 SDC showed basal phenotype, 17 of these with EGF-R overexpression. Nine were HER-2 negative, 5 showed HER-2 gene clusters while the three remaining cases were heterogenous or low level concerning HER-2.

CK5 and/or p63 positivity occurred in 9 of the tumours with basal phenotype, two of the cases showed HER-2 gene amplification.

Discussion Luminal and basal phenotypes occurs in SDC. There is a group of tumours with both HER-2/luminal as well as EGF-R/basal associated molecular events in SDC. Emerging interests in molecular targeted therapies highlights the need to characterize tumours concerning several oncogenetic pathways. In the case of SDC, these results indicate that targeted therapies for both EGF-R and HER-2 need to be evaluated.

O-39

ESR1 hypermethylation in prostatic adenocarcinoma correlates with high Gleason score

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Background: Abnormal DNA methylation of the gene promoter regions is linked to numerous cancers through silencing the expression of tumor suppressor and other regulatory genes. It refers to covalent bonding of a methyl group to the 5′-cytosine of the dinucleotide, CpG. Aberrant promoter methylations have been reported for several genes in prostate carcinoma. We have evaluated the methylation status of estrogen receptor alpha gene (ESR1) and investigated its value in the disease prognosis.

Method: Sixty eight radical prostatectomies from clinically localized prostate cancer patients were included in the study. Preoperative and post-operative data were obtained from the hospital charts. Slides were reviewed to note prognostically important pathological parameters. Paraffin blocks were used to isolate tumor DNA from each case. The presence of ESR1 CpG island hypermethylation was determined by real-time methylation specific polymerase chain reaction.

Results: Gleason score of the tumors ranged from 6 to 9 while pathological stage ranged from T2N0Mx to T3BN1. ESR1 gene was found methylated in 11 cases. Unfavorable prognostic features were more frequent in radical prostatectomies with ESR1 hypermethylation. The rates of extraprostatic extension, seminal vesicle invasion, lympovascular permeation, PSA relapse, Gleason score ≥7 and pT ≥3a were 68.4%, 22.8%, 14%, 31.4%, 73.6% and 68.4% respectively in hypermethylated group in comparison to 72.7%, 45.5%, 27.3%, 40%, 90.9% and 72.8% in non-methylated group. The correlation between presence of hypermethylated gene and high Gleason score was statistically significant ($p=0.028$).

Conclusion: ESR1 hypermethylation is rare in prostate carcinoma, seen in 16% of cases. Our findings suggest that the patients with methylated ESR1 are more likely to present with adverse prognostic features. ESR1 silencing may play a role in the progression of the disease.

O-40

Invasion front: Cadherin-shift and tumorproteases in prostatic cancer

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Background: *I*ntratumoral heterogenity in prostatic cancer (PC) leads to different architectural classifications. Studies of the expression of cellmembrane-adhesion-molecules and

proteases considered only the Gleason-Score of the tumor but not the localisation of different cadherin- expressing cells in respect of centrally located tumorcells versus cells of the invasionfront and epithelial-mesenchymal transition zone (EMT), being probably more important for its biological behaviour.

Method 60 EMT-zones of PC were analysed in respect to their expression of E-cadherin(Biologo,Dr.Schultheiss, Kronshagen,Klon 5H9), N-cadherin (Biozol,Eching, GeneTex; IgG,polyclonal), β-catenin (Dianova,Hamburg; rabbit polyclonal) and hepsin (Cayman Chemical; IgG) in comparison to adjacent nontumor epithelia and carcinomacells in the central parts.

Results *E-Cadherin:* Membranous expression in non-neoplastic glands. Low expression in tumor-cell-membranes Gleason-Pattern 3 and 4; good expression in peripheral parts of cribriform areas and in small tubular and trabecular parts of the tumor. Loss of E-cadherin in the inasionfront and EMTs.

N-Cadherin: No N-cadherin in non-neoplastic glands; high cyoplasmic and membranos expression in tumorcells of EMTs corresponding to the loss of E-Cadherin (E-cadherin: N-cadherin-shift).

β-Catenin: Membranous localisation in non-neoplastic glands; membranous and granular intracytopamic deposits in tumor-center; intranuclear β-catenin in cells of the invasionfront.

Hepsin: low expression in non-tumor glands; mediocre expression in centrally localised carcinomacells; very high expression in epithelia of invasionfront and EMTs.

Conclusion: Tumorcells in EMTs show extensive remodelling of shape and function: shift of intercellular connections (cadherins), nuclear translocation of β-catenin, inducing wnt-pathway, abundance of serin-protease (hepsin), fostering invasion by dissolvin collagens and dissolution of tight cell-adhesions in the invasion front influencing the tumor's biology and architecture.

O-41
CORRELATION BETWEEN BIOPSY and PROSTATECTOMY - PREDICTIVE FACTORS

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Background: The increase in detection of the PSA has taken to a rising number of prostate biopsies (bx) that plays an important role at the time of making therapeutics decisions. We have tried to correlate biopsy and prostatectomy (p) that allows us to predict the neoplasia stage.

Materials and Methods Radical prostatectomy was performed in 77 patients between 2005–2006. We have 48 biopsies, 17 of them with information of Gleason(G). The age average is 66.5. We have reviewed in bx as well as in p: PSA measurement before and after the treatment, Gleason (G), extraprostatic extension, perineural invasion, volum in %, pT and surgical margins status (irregular/regular, focal/extensive).

Results The score correlation between bx and p is of 58% rising to 85% for G=7. In 1,5% there is an overgrading and infragrading in the 30%. PSA before p was between 12.5 and 4.4 ng/ml. We have found bilaterally in 15 bx, and 100% is confirmed in p. All of the p had bx with intraprostatic cancer. The majority of the cases (46/77) are T2C, 3/77 of them had previous treatment and one case was not valuable by fragmentation. The rest is distributed in T3A, B, T2A, B. The resection margins were not valuables in 2, affected in 21, and free in the 53. 13/21 cases have been submitted to RDT treatment. Only one of the cases that have PSA measurement post-treatment, has detectable PSA.

In the biopsies, 8 cases had perineural invasion. 4/8 turned out to be T2C, and 4/8T3A-B had affected margins. Of those without perineural invasion, 71% were T2C and only 5% T3A, and 20% had affected margins. There are not significant differences with theGleason.

Conclusions It is significantly important to evaluate a series of parameters in the biopsy, because they will be decisive by the time of making a suitable treatment, and to establish a reliable prediction. The perineural invasion biopsy is a factor that predicts an advanced local stage. In our series, the resection of margins status has no influence in the short time prognostic (two years). It is necessary longer pursuit to take out conclusions. Pathologist accurate valuation of the biopsy is vital, so that a strict professional discipline is very important at the time of biopsy grading.

O-42
PIK3CA MUTATIONS IN PROSTATE CANCER

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Background: An important goal in cancer research is the identification of genes involved in tumor initiation and progression. One of thecrucial genes is PI3K, specifically its catalytic subunit p110α, also known as PIK3Ca, has been found to be mutated in different tumor types such as colon, breast and bladder cancer. There have been no reports of studies on this gene in prostate cancer. The aim of our project is to analyze the frequency of mutation of this oncogene in a well-defined group of prostate tumors.

Methods: A total of 35 cases with prostate cancer were selected from the files of the Department of Pathology (Hospital del Mar, Barcelona). DNA was extracted from formalin-fixed, paraffin-embedded tissue in which representative tumor areas were selected and manually microdissected. 5 consecutive 10 μm sections were deparafinized and DNA was extracted using Dneasy Tissue Kit (Qiagen GmbH, Hilden, Germany). We studied the exons 9 and 20, in which most of the reported mutations have been discovered. Mutational analysis was performed by direct sequencing from the purified PCR product.
Results: Only one change was found in a total of 35 radical prostatectomy cases containing clinically significant tumors, 15 with a Gleason grade ≤6; 14 with a Gleason Score of 7 and 6 with a Gleason Score ≥8). The only change occurred in intron 9 (IVS9+91) producing a transition C-T in different tumor areas of the same patient.
Conclusion: Our findings indicate that PIK3Ca mutations could be an uncommon abnormality in prostate cancer, but further analysis in larger series of patients must be carried out.
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O-43
DISTINCT CELLULAR EXPRESSION PATTERNS OF NEUTRAL ENDOPEPTIDASE (CD10) IN PROSTATE CANCER PREDICT DIVERGING CLINICAL COURSES

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Background: Prostate cancer is a heterogeneous disease with variable clinical courses. Individual prediction of outcome is relevant to select the appropriate therapies and avoid overtreatment. CD10, a cell surface bound endopeptidase, has been suggested as a potential molecular marker for prostate cancer.
Methods: 3261 prostate cancer patients treated homogeneously by radical prostatectomy were evaluated using tissue microarray (TMA) technology. Immunohistochemically, the cellular domain (membranous, membranous-cytoplasmatic, cytoplasmatic only) of CD10 expression was analyzed and correlated with various clinical and histopathological and immunohistochemical tumor features.
Results: CD10 expression was present in 62.2% of the cancer samples and occurred preferentially in higher Gleason pattern. CD10 expression positively correlated with adverse tumor features like elevated preoperative PSA, higher Gleason score and advanced stage ($p>0.0001$ each). Survival analyses showed that PSA recurrence was significantly associated with CD10 expression intensity and also staining pattern. Outcome significantly declined with increasing staining intensity ($p<0.0001$) and from negative over apical, apical-cytoplasmatic to exclusively cytoplasmatic CD10 expression ($p<0.0001$). In multivariate analysis CD10 expression was an independent predictor for PSA failure.

O-44
The role of allelic imbalance at 8p in cases of atypical adenomatous hyperplasia of the prostate and its implications on prostatic carcinogenesis

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Background: The putative precursor for prostate cancer is Prostatic Intraepithelial Neoplasia (PIN), that shares morphological, functional and genetical features with intermediate-grade Prostate Cancer (PCA). However, little is known about the origin of low-grade PCA. Atypical adenomatous hyperplasia (AAH-adenosis) has been proposed as such preneoplastic lesion. The goal of this study is to investigate the role of Allelic Imbalance (AI) at 8p -a common feature of PCA- in AAH, trying to define different populations of AAH indicating a possible transition to PCA.
Method: We studied 40 formalin-fixed, paraffin-embedded samples from simple prostatectomies which contained 42 foci of AAH, coexisting with NH and transition zone well-differentiated PCA. Stereological studies were performed determining mean nuclear volume, volume fractions of stroma and basal cells and relative number of nucleoli in acinar cells. Immunohistochemical demonstration of p21, p27 and MIB-1 was made and corresponding labelling indices were determined. Also they were tested for AI at four different loci of 8p (D8S133, D8S261, D8S254 and DS258) using standard methods. Statistical analysis was done using Student t test to compare cases with or without AI.
Results: 33 cases were informative. Of them, 13 cases showed AI at different loci of 8p. When stereological and immunophenotypical features of these cases were compared with Normal (N) cases, an increase in the relative number of nucleoli (N: 0.85%, AI: 1.85%, $p>0.001$) and p21LI (N: 9.13%, AI:13.66%, $p=0.006$) and a decrease in p27LI

(N:45.72%, AI:31.55%, N.S.) was noted, The rest of parameters showed similar values for N and AI groups.
Conclusion: AI at 8p seems to be related with morphological and functional changes in a subset of AAH, more similar to those noted in previous studies for well differentiated PCA. This group should be more likely to be the immediate precursor of PCA of the transition zone of the prostate.

O-45

Clear-cell papillary renal cell carcinoma: a distinct histopathological and molecular genetic entity

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Background: Tumors composed of cells with clear cytoplasm arranged in papillary patterns have been recently described in kidneys with end-stage renal disease.
Method: We investigate the cytogenetic and immunohistochemical phenotypes of papillary tumors entirely composed of clear cells showing the same peculiar features previously described in tumors arising from kidney with end-stage renal disease but arising also in kidneys unaffected by end-stage renal disease. Interphase fluorescence in situ hybridization was performed with centromeric probes for chromosomes 7, 17 and Y, to analyze the typical chromosomal alterations seen in papillary renal cell carcinoma. We also tested deletion of 3p usually seen in clear cell renal cell carcinoma using centromeric probe for chromosome 3 and subtelomeric probe for 3p25.
Results: Seven tumors from 5 patients (age range: 53 to 64 years, mean: 60 years; 3 male and 2 female) were identified. Only one patient had end stage renal disease. All 7 tumors (ranging from 4 to 50 mm in diameter) were stage pT1. None showed recurrence or metastasis after a mean follow-up of 24 months. Six tumors showed no chromosomal losses or gains of chromosomes 7, 17 or Y; one case had gains of chromosome 17. Chromosome 3p deletion was not detected in any case. Cytokeratin 7 and CA-IX were strongly positive in all cases. CD10 immunoreactivity was negative in six tumors and focal in one case, Alpha-methylacyl-CoA-racemase and TFE3 were negative.
Conclusion: Clear-cell papillary renal cell carcinoma may arise in otherwise normal kidneys as well as kidneys with end-stage renal disease. This tumor has immunophenotypic and genetic profiles distinct from those of either classic papillary or clear cell renal cell carcinomas.

O-46

FLUORESCENCE IN SITU HYBRIDIZATION, FLOW CYTOMETRIC AND KARYOTYPING ANALYSES IN THE DIFFERENTIAL DIAGNOSIS BETWEEN RENAL ONCOCYTOMA AND CHROMOPHOBE RENAL CELL CARCINOMA.

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Background: Cytogenetic analysis usually reveals low number of chromosomes 1, 2, 6, 10 and 17 in chromophobe renal cell carcinoma and a normal numerical complement of chromosomes in renal oncocytoma. However, different chromosomal patterns have been rarely reported in both renal cell neoplasms.
Method: We investigated 23 renal cell neoplasms (11 chromophobe renal cell carcinomas, 12 renal oncocytomas)by metaphase karyotyping and interphase FISH for chromosomes 1, 2, 6, 10 and 17 and flow cytometric analyses on tissue sections.
Results: FISH showed losses of two or more chromosomes in 10 chromophobe renal cell carcinomas (91%) and gains of multiple chromosomes in one (9%). Six (50%) renal oncocytomas were totally disomic, five (42%)showed one chromosomal loss (chromosome 1 in 3 cases), one case (8%) two losses. Among 9 chromophobe renal cell carcinomas with available istograms 6 (67%)showed aneuploid stemlines whereas the three remaining and 8/9 (89%) renal oncocytomas were diploid.
Karyotypically, 3 hromophobe renal cell carcinomas (33%) were hypodiploid, 3 (33%) were polydiploid, one (11%) was diploid. Nine out of 12 (75%) renal oncocytomas were diploid, one showed -Y (8%), one 47XX (8%), one multiple different clones (9%). All chromophobe renal carcinomas which failed to grow and 2/3 (75%) showing gains by metaphase analyses displayed multiple chromosomal losses by FISH. Eight renal oncocytomas with normal DNA content and those three with additional chromosomal abnormalities (91%) by karyotyping showed normal complement of chromosomes by FISH.
Conclusion: 1) chromophobe renal carcinomas usually display multiple chromosomal losses by FISH analysis in spite of a different spectrum found by karyotyping and flow

cytometric analyses; 2) chromophobe renal carcinomas that fail to grow in culture are characterized by chromosomal losses in FISH; 3) renal oncocytomas usually show a normal DNA content by both interphase and metaphase analyses.

O-47
ROLE OF POLYSOMY 17 IN TRANSITIONAL CELL CARCINOMA OF BLADDER (TCC). IMMUNOHISTOCHEMICAL STUDY OF HER2/neu EXPRESSION AND FISH ANALYSIS OF c-erbB-2 GENE AND CHROMOSOME 17.

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Background: We investigate HER2-neu gene and chromosome 17 alterations by FISH analysis and HER2/neu overexpression in transitional cell carcinoma of urinary bladder correlating with tumor grade and stage.
Methods: Sixty-three cases of TCC retrieved from the files of two departments of Pathology were studied for chromosome 17 aberrations and HER2-neu amplification by FISH and for HER2/neu overexpression by immunohistochemical analysis. Five tumors were low grade (G1), 29 tumors were intermediate grade (G2) and 29 tumors were high grade (G3); 32 tumors had stage T1, 18 had stage T1 and 13 were T2.
Results: We found polysomy of chromosome 17 in 37/63 (58.7%) TCC with average chromosome copy number > 2.26; increased number of HER2/neu gene copy (cut-off > 2.00) was observed in 42/63 (66.7%) of tumors. HER2-neu amplification occurred in 4/63 (6.3%) of tumors. Immunohistochemically 38/63 (60.3%) TCC overexpressed HER2/neu. All tumors with polysomy showed simultaneously increase of HER2/neu gene copy number whose 34/37 (92%) with protein overexpression. A significant correlation between polysomy of chromosome 17 and tumor stage ($p=0.0003$) and tumor grade ($p<0,0001$) was found: none of low grade tumors(G1), 8/29 (27.6%) of intermediate grade tumors (G2), and 29/29 (100%) of high grade tumors (G3) showed polysomy of chromosome 17; in 22/50 (44%) of non-invasive tumors (8/32 Ta and 14/18 T1) and 13/13 (100%) of deep invasive tumors (T2) polysomy 17 was observed. Moreover we found that 7 superficial tumors (1 Ta and 6 T1) showed high polysomy with average of chromosome 17 copy number ≥ 3.76 as observed in all invasive tumors (T2).
Conclusions: Our study suggests that HER2/neu gene amplification in invasive and high grade TCC is a rare event. However polysomy of chromosome 17 is fairly frequent, and it is correlated with tumor stage and grade, providing important information on tumor progression. Further work is needed to directly study these issues.

O-48
EXTRATESTICULAR EXTENSION IN GERM CELL TUMORS

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Background;Extratesticular extension (ETE) in germ cell tumors (GCT) is defined as tumor penetration through tunica albuginea. Depending on the site of invasion tumor may involve various extratesticular structures including tunica vaginalis (TV), hilar soft tissue, epididymis or spermatic cord. We investigated the frequency and morphological patterns of ETE in GCT with special focus to involvement of hilar structures including the rete testis. Method:We reviewed all 289 primary testicular GCT (209 seminoma and 80 nonseminoma) at our center between October 1999, and December 2006. Pathology slides and reports were reviewed. When ETE was identified, extension of tumor into the following structures; TV, epididymis, hilar soft tissue and spermatic cord were assessed. Results:ETE was identified in 58 (20%) patients which represented 16% (34/209) of seminomas and 30% (24/80) of nonseminomatous GCT. 97% of all ETE was through the hilum. Direct invasion of the rete testis was identified in 83% (48/58) of the cases. Hilar soft tissue was involved 62% (36/58) and epididymis was involved in 27% (16/58) of cases. Spermatic cord involvement was identified in 17% (10/58) of ETE cases, one of which showed tumor in lymphovascular spaces only. In four cases where hilar involvement were extensive, reliable distinction of paratesticular soft tissue involvement from a true spermatic cord invasion was difficult. TV was involved only in 2 cases (0.6% of total GCT and 3% of ETE cases). Conclusion: ETE is more frequent in nonseminomatous GCT. TV invasion is rare in GCT and the least common type of ETE. Most common form of ETE is through the hilum into the hilar soft tissue via direct invasion of the rete testis. Since there is no established anatomical landmark present between paratesticular soft tissues and spermatic cord pathological staging in a subset of patients can be problematic.

O-49

EVALUATION OF MSPATH SCORE COMPARED TO REVISED BETHESDA GUIDELINES FOR DETECTING LYNCH SYNDROME IN A CONSECUTIVE SERIES OF 225 COLORECTAL CARCINOMAS

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Introduction: The Bethesda guidelines (BG) for Lynch syndrome were developed to preselect patients for microsatellite instability (MSI) testing before germline mutation screening. Among the 5 revised criteria, 1 focuses on particular pathology features. MsPath score (MsPS), based on site of the tumor, age at diagnosis and pathology features was recently shown to predict high-MSI (MSI-H) tumors in patients diagnosed before age 60 years. Our aim was to compare this score with the BG for detecting Lynch syndrome in unselected patients.

Methods: A consecutive series of 225 resected colorectal invasive adenocarcinomas from 214 patients was collected in our institution. All tumors were reviewed by a senior pathologist. Familial history, pathology features, site and age at diagnosis were obtained. MSI analysis was performed for all tumors. Germline mutations in the mismatch repair genes pointed out by immunohistochemistry were searched for MSI-H tumor patients.

Results: Ninety (42%) patients met the BG. MsPS was ≥1 in 105 patients (49%). Twenty-one patients (9.8%) had MSI-H tumors. Germline testing identified 8 mutations (3.7%) (MSH2, n=5; MLH1, n=2; MSH6, n=1). The BG failed to identify 2 of the 8 probands. All patients with Lynch syndrome had MsPS ≥2.3. The sensitivity and the specificity of the BG in combination with MSI were 75% and 98.5%, versus 100% and 54% for MsPS ≥1.

Conclusion: In our study, the MsPS identified all the patients with germline mutation, while a quarter of them were missed by the BG. Furthermore, MsPS increased only slightly the number of patients who should be tested for MSI (49% versus 42% for the BG). These data suggest the MsPS could replace the BG for preselecting patients for MSI testing. Further studies in larger series are needed to confirm these data and to determine a MsPS value which would increase specificity with no decreasing sensitivity.

O-50

Prognostic Value of Vessel Location in Angioinvasive Colorectal Cancer

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Background: Lymphatic and venous invasion represent established prognostic parameters in colorectal cancer. Whether the prognostic significance of these parameters is related to intra- and/or extramural vessel location remains to be elucidated.

Method: 381 patients (215 men, 166 women; ratio 1.3:1) with colorectal cancer operated upon between 1992 and 2000 were randomly selected for analysis. Mean and median age of patients was 69.9 and 68.5 years, respectively. Patient records and histopathological slides were re-evaluated with respect to tumour stage, tumour grade and presence of intra- and/or extramural lymphatic and/or venous invasion. Follow-up data were available from 92% of patients (median time of follow-up 61 months).

Results: Lymphatic invasion was observed in 126/381 (33%) patients and was significantly associated with tumour stage ($p<0.001$) and grade ($p<0.001$). Intramural (L1a), extramural (L1b) as well as both intra- and extramural lymphatic invasion (L1ab) was seen in 44%, 6% and 50% of cases. Venous invasion was observed in 87/381 (23%) patients and was significantly associated with tumour stage ($p<0.001$) and grade ($p<0.001$). Intramural (V1a), extramural (V1b) as well as both intra- and extramural venous invasion (V1ab) was seen in 16%, 62% and 22% of cases. Presence of extramural lymphatic (L1b, L1ab) and venous (V1b, V1ab) invasion was significantly associated with high tumour stage ($p<0.001$) and grade ($p<0.001$). In addition, extramural lymphatic invasion (compared with intramural invasion) proved to be a significant prognostic variable in both univariate ($p<0.001$) and multivariate ($p=0.01$) analysis, whereas presence of extramural venous invasion (compared with intramural invasion) demonstrated prognostic significance only in univariate analysis ($p=0.02$).

Conclusion: Patient outcome was significantly associated with presence of lymphatic and/or venous invasion. With respect to lymphatic invasion, extramural lymphatic permeation proved to be a new independent prognosticator (compared with intramural lymphatic permeation). Its presence should thus be recorded during routine pathological work-up of colorectal cancer specimens.

O-51

Prognostic Value of pT Subclassification in Colorectal Cancer

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Background: Tumour stage is the most important prognostic parameter in colorectal cancer. Our study aimed to assess the prognostic significance of a pT2 and pT3 subclassification.

Method: 381 patients with colorectal cancer operated between 1992 and 2000 were randomly selected for analysis. PT2 classification was subdivided as follows: pT2a with infiltration of the inner circumferential and pT2b with infiltration of the outer longitudinal layer of the muscularis propria. pT3 classification was subdivided by measuring the maximal tumour invasion beyond the muscularis propria: pT3a ≤1 mm, pT3b >1–5 mm, pT3c >5–15 mm and pT3d >15 mm. Follow-up data were available from 92% of patients, mean and median time of follow-up being 64 and 61 months, respectively.

Results: pT1 classification was noted in 28 (7%), pT2a in 37 (10%), pT2b in 22 (9%), pT3a in 49 (13%), pT3b in 83 (22%), pT3c in 53 (14%), pT3d in 33 (9%) and pT4 in 65 (17%) patients. 168 (44%) patients presented with nodal metastasis at time of diagnosis. 4/37 pT2a (11%) and 10/33 (30%) pT2b ($p=0.07$) as well as 12/49 (24%) pT3a, 32/83 (39%) pT3b, 36/53 (68%) pT3c and 23/33 (70%) pT3 tumours ($p<0.001$) showed nodal disease. Regarding patient outcome, no significant difference was detected between patients with pT2a or pT2b tumours. The pT3 subclassification, however, proved useful in predicting disease progression: 11/47 (23%) pT3a, 23/75 (31%) pT3b, 29/49 (59%) pT3c and 14/24 (58%) pT3d cases developed progressive disease ($p<0.001$; log-rank test). These data were confirmed in a Cox's multivariate analysis using the 5 mm cut-off level (pT3a/3b vs. pT3c/3d; $p<0.01$).

Conclusion: pT3 subclassification proved feasible in our patients, a cut-off level of 5 mm maximal tumour invasion beyond the outer border of the muscularis propria is recommended. Although the proposed pT2 subclassification tended to correlate with nodal status, no prognostic significance was observed.

O-52

Serrated adenomas and their relationship to colorectal carcinoma: Histopathological reevaluation of colorectal epithelial lesions diagnosed before the WHO (2000) classification

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Objectives:

1. To revise previously established diagnoses of colorectal epithelial lesions according to the WHO 2000 classification with special emphasis on serrated adenomas.
2. To characterize the frequency of focal CA arising from or CA coexisting with benign epithelial lesions depending on sex, age, localization and size.

Materials and methods: During the period of 1981–1994 in the Department of Gastroenterology of Medical Center for Postgraduate Education 24 000 consecutive endoscopic examinations of GIT were performed. Sigmoido- or colonoscopy revealed 2285 colorectal epithelial polyps and tumors in 1753 patients (mean age 59,9). There were 1544 adenomas: 1146 tubular (TA), 359 tubulo-villous (TVA), 39 villous (VA); furthermore there were 5 transitional polyps (TP), 128 hyperplastic polyps (HP) and 487 carcinomas (CA) (50,15%; 15,71%; 1,71%; 0,22%; 5,60%; 18,69%, respectively). The patients with familial adenomatous polyposis (FAP) were excluded from the study.

In 2007 the previously established diagnoses of all colorectal epithelial lesions were verified microscopically to disclose serrated adenomas (SA) according to the WHO 2000 classification. Multivariate logistical regression statistical analysis was applied.

Results: 128 of 2285 epithelial lesions were reclassified (5,6%). Most of them were SA (121 cases), which constituted 7,27% of all adenomas. 81 of 204 formerly diagnosed HP (39,7%) were reclassified as SA but 2 HP (0,98%) as TP. Focal CA arising from benign epithelial lesions was found in 60 cases. There were 7/46 focal CA in VA (15,22%), 28/387 in TVA (7,24%), 23/1169 in TA (1,97%) and 2/128 HP after revision (1,67%), however none CA was found in SA. CA coexisting with benign polyps of any kind in other localizations was found in 44 of 1753 patients (2.51%). Surprisingly, it was most common in patients with SA (5/57=8.8%) in contrast to lower frequency in groups with TVA (14/358=3,91%), TA (28/759=3,69%), VA (1/39=2,56%), and quite similar to the

latter one in HP (2/100=2,00%). HP and SA were generally small (≤5 mm), in contrast to large (>10 mm) TVA, VA and CA. Adenomas were significantly more frequent in older people, in the sigmoid colon and rectum. Most differences were statistically significant.
Conclusions: Introduction of WHO 2000 classification caused reevaluation of essential part of previous HP into SA. HP, formerly thought to be harmless, may have malignant potential, which was similar to TA in our material. All adenomas and HP may coexist with CA, however SA most frequently.

O-53

Nuclear expression of the splice factor Tra2β in ductal epithelium adjacent to ductal pancreatic carcinomas predicts poor patient outcome

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Background: Alternative splicing represents an important regulatory mechanism of gene expression. Tra2β, a member of the extended family of serine-arginine rich (SR) splicing factors regulates alternative splicing of exons with C/A-rich enhancer sequences. Biological important genes (e.g. CD44, vegf, insulin receptor, BRCA2, SYK) are alternatively spliced and contain these C/A rich sequences. **Methods:** We investigaetd expression levels of Tra2β in a matched pair analysis of microdissected tumor samples and normal tissues in tissues of invasive ductal pancreatic cancer by qRT-PCR (n=6) and immunohistochemistry (IHC, n=58). **Results:** Tra2β transcript expression was twofold upregulated in ductal pancreatic cancer. IHC revealed high Tra2β expression in 36, and low Tra2β expression in 22 pancreatic carcinomas. Tra2β expression within the tumor tissue did not have impact on patient survival. Of the corresponding histological normal duct epithelia 24 revealed weak and 18 revealed strong nuclear expression of Tra2β. Of interest, high nuclear Tra2β expression in the corresponding normal ductal epithelia correlated significantly with poor patient survival. **Conclusion:** Our data support a biological relevance of Tra2β in ductal pancreatic cancer. Tra2β expreesion might have a potential as a marker of patient outcome in ductal pancreatic cancer. In addition, high Tra2β expression level possibly reflects changes in alternative splicing patterns in ductal pancreatic carcinogenesis.

O-54

Correlation of EGFR expression with clinicopathological features in Pancreatic Ductal Carcinomas

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Background: The Epidermal growth factor reseptor (EGFR) is a receptor tyrosine kinase of the ErbB family that is abnormally activated in many epithelial tumors. The effect of overexpression EGFR in pancreatic carcinoma is not clear. We examined the EGFR expression and its relation with clinicopathological in invasive ductal adenocarcinomas of pancreas.
Method: Using tissue microarray technology, 70 histologically confirmed pancreatic ductal adenocarcinomas were cored twice and re-embedded in paraffin blocks. TMA slides were immunostained with antibody for EGFR obtained commercially and evaluated by two pathologists on light microscopy.
Results: Five of cases had to be eliminated from the study group. The mean age was 61,8 and F/M was 24/41 (n=65). EGFR expression was seen in 30/65 cases (46,2%). There was significant negative correlation between EGFR expression and lymphatic vessel invasion (r:-,265; p: 0,03). The frequency of lymph node metastases was lower in EGFR positive group. Thirteen of cases were death of disease with mean time of 17 months. Survival time was shorter in cases who had blood vessel invasion and longer in cases who had EGFR expression but these did not reach statistical significance.
Conclusion: Immunohistochemical analysis of EGFR expression has useful information for biological behaviour, although this analyse does not demonstrate prognostic value in pancreatic ductal adenocarcinomas.

O-56

Correlation of Hepatitis B surface antigen (HBsAg) and Hepatitis B Core Antigen (HBcAg) immunohistochemistry on liver biopsy specimens and serum HBV DNA levels

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Hepatitis B core antigen (HBcAg) and hepatitis B surface antigen (HBsAg) expressions in the liver may reflect the status of the balance between viral replication and the host immune reaction.

Aim: We aimed to search the correlation between serum HBV DNA levels and the percentage of positive cells and the staining patterns of HBsAg and HBcAg on liver biopsy specimens (LBS).
Patients and Methods: 162 patients with chronic hepatitis B were included in the study (mean age: 43±10, M/F: 107/55). The activity of hepatitis on LBS was assessed by using Ishak's score. HBsAg and HBcAg immunohistochemistry was performed using an automated immunohistochemical stainer according to the manufacturer's guidelines (streptavidin-peroxidase protocol; BenchMark,Ventana, USA). The staining pattern (membranous, cytoplasmic, nuclear, mixed) and the percentage of positive cells were assessed semi quantitatively.
Results: Mean HBV DNA level was 5.49±1.64 log copies/ml. On LBS, 32 out of 162 cases (19%) were HBcAg(+) and 145 (89%) were HBsAg(+). There was a positive correlation between the serum HBV DNA levels and percentage of HBcAg ($r=0.593$; $p<0.0001$) and HBsAg ($r=0.568$; $p<0.0001$) positivity. In all cases with HBV DNA > 8 log copy/ml HBcAg was found to be positive. There was no HBcAg positivity in cases with HBV DNA < 4.2 log copy/ml. The immunostaining of HBcAg on LBS indicated that serum HBV DNA level is over 6.25 log copy/ml with 91% specificity and 81% sensitivity. When HBcAg is taken ≥0.01, specificity and sensitivity rose to 96.4% and %81 respectively. The correlation of HBcAg and HBsAg staining was statistically significant ($r=0.470$; $p<0.0001$). There was no relationship between the staining pattern of HBcAg (nuclear, nuclear dominant mixed, cytoplasmic dominant mixed) and serum HBV DNA level. Whereas, HBsAg staining pattern tended to shift from the membrane to the cytoplasm with higher HBV DNA levels ($r=0.356$; $p<0.0001$). HBeAg positivity was not related to HBsAg staining, but HBeAg ($p<0.0001$).
Conclusions: The HBcAg expression in the liver is highly related with HBV DNA level in the serum (100% in HBV DNA>8 log copies/ml; 0% in HBV DNA<4.2 log copies/ml). HBcAg immunostaining is a reliable method for the demonstration of viral replication.

O-57

Mesothelin and p53 expression in pancreatic adenocarcinoma

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Background: Mesothelin is an emerging marker for cancer diagnosis and target based therapy. In addition to mesothelial malignancies, mesothelin expression in malignancies from the ovary, and more recently from the pancreas has been demonstrated. The purpose of this study is to evaluate the expression of this monoclonal antibody in pancreatic carcinoma, chronic pancreatitis and compare it to the expression of p53 protein.
Methods: The authors reviewed 100 cases of pancreatic ductal carcinomas from the files of McMaster University Medical Centre. Representative blocks of tumour and adjacent non-neoplastic pancreas were selected for immunohistochemistry. Immunohistochemistry was performed using mesothelin monoclonal antibody (5B2, Nova Castra) and p53 protein (DO-7 Dako). Mesothelin staining of greater than 25% of tumour cells was considered to be strongly positive, less than 1% was considered negative, and the remaining cases were called focally positive. Intensity of staining was semi quantitatively graded on a scale of 1–3 (weak, moderate and strong) Up to 5% of p53 staining was considered negative.
Result: Mesothelin staining was seen in 90% of pancreatic ductal carcinomas, of which 78% of cases showed staining in 25% or more of the tumour cells. The staining intensity was graded as 2 in the majority of the positive cases. Cases of chronic pancreatitis showed only occasional focal positive staining in 5% of cases. The normal pancreatic tissue was negative in all cases. 75% of carcinomas expressed p53 in the range of 10–60%. Less than 2% of chronic pancreatitis cases were positive.
Conclusion: We conclude that mesothelin is a useful marker for the diagnosis of pancreatic ductal carcinoma and may be helpful in discriminating cases from chronic pancreatitis. However, p53 showed higher specificity but less sensitivity and the expression can be complementary

O-66

Myocardial Chromogranin A (GCA) protein expression in chronic heart failure is negatively regulated by left ventricular assist devices, but its plasma levels fail to reflect hypertrophy regression

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Aims: In chronic heart failure (CHF), among increased expression of natriuretic peptides (NPs), recently Chromogranin A (GGA) was found to be up-regulated and associated with disease severity. Significant decrease of both NPs and hypertrophy after mechanical ventricular suppoprt (LVAD) was demonstrated.

Methods and results: Prior to and after support, ANP-, BNP, CGA and NCAM/CD56 expression was investigated by immunohistochemistry and morphometrically quantified in 33 paired myocardial samples. In 40 patients, ANP-, BNP- and CGA plasma levels were evaluated. In CHF, ANP-, BNP- and CGA levels were increased compared to controls and decreased significantly after LVAD. However, CGA levels did not show significant intergroup differences after multiple comparisons (post-hoc testing) and no significant differences of CGA plasma levels were outlined after support. Colocalization of BNP and CGA was found before, but not after ventricular unloading. CGA and NCAM/CD56 were not correlated with cardiomyocyte diameters.
Conclusions: Similar to NPs, CGA is significantly decreased by LVAD. BNP and CGA are colocalized in CHF, but not after unloading. Neither CGA nor NCAM/CD56 are associated with cardiac hypertrophy. In contrast to NPs, changes in myocardial CGA expression are not reflected by plasma levels, thus CGA does not appear to be an approriate biomarker of hypertrophy regression after unloading.

O-67

Cell culture as a testing system for lipid-lowering substances

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In the large series of publications started by Chazov EI, Orekhov AN et al. in 1986 (The Lancet, 2:595) and continuing until today (e.g., several presentations at the ISA Symposium in Rome, 2006) it has repeatedly been reported that incubation of cultured macrophages, smooth muscle and other cells with the serum from patients suffering from coronary heart disease caused significant lipid accumulation in cytoplasm, whereas serum from healthy individuals had no effect on lipid content in cultured cells. The ability of serum to cause lipid infiltration in vitro was named atherogenicity; and the method has been used by the same group of researchers for testing anti-atherogenic drugs and food components. Extract from garlic added to the "atherogenic" serum significantly reduced lipid infiltration and proliferation of cultured smooth muscle cells in vitro. Interestingly, the same effect was observed ex vivo: blood serum taken two hours after oral administration of garlic "caused substantially less cholesterol accumulation in cultured cells". Numerous substances have been reported to be efficient against serum atherogenicity: dibutyryl cyclic AMP, calcium antagonists, lipoxygenase inhibitors, carbacyclin and many others. The method was applied for assessment of hormonal influences: glucagon, estrogens and testosterone reduced serum atherogenicity and suppressed proliferation of cultured cells. The authors claim to have developed a novel principle of direct anti-atherosclerotic therapy based on inhibition of cholesterol deposition in the arterial wall without giving any plausible explanation of mechanisms of its action directly upon the serum. According to the current knowledge, anti-atherogenic substances can act on lipid metabolism in the liver, on intestinal absorption of lipids or on the endothelial receptor- or gene-mediated mechanisms. All these targets are absent in the cell cultures used. Therefore, reliability of reported results and recommendations for praxis appears questionable.

O-68

Pathological study of primary cardiac and pericardial tumours in a specialist UK centre: surgical and autopsy series

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Background: Primary cardiac and pericardial tumours are rare with a prevalence of between 0.001 and 0.3%. Thus pathologists do not see them. Although uncommon, cardiac tumours represent an important cause of morbidity and mortality. Modern advances in cardiac imaging have increased the number of patients identified with a primary cardiac tumour in its early stage and also improved prognosis. At the Royal Brompton Hospital, London, which is a tertiary cardiac referral centre, we conducted a study to investigate the pathological features of primary cardiac and pericardial tumours and compared our findings to other centres.
Methods: All pathologic records at the Royal Brompton Hospital, between 1990 and 2007 were reviewed to identify patients with a confirmed diagnosis of primary cardiac tumours. A total of 94 patients with a histological diagnosis of primary cardiac and pericardial tumours were identified and formed the study population. Both surgical and autopsy specimens were included.
Results: The majority (n=67, 71.3%) of cases were benign cardiac tumours. Myxoma was the most common histologic type accounting for 28.7% of total tumours. Among cases with malignant tumours (n=27, 28.7%), unclassified sarcoma (n=11), leiomyosarcoma (n=5) and lymphoma (n=4) were the most common histologic types of primary malignant tumour. Primary cardiac tumours occurred more commonly on the left side of the heart (n=56, 59.6%), with the majority being benign tumours (n=43/56, 76.8%). When the tumours were present only in the right side of the heart, nearly half of them (8/20, 40.0%) were primary cardiac malignancies.
Conclusion: This study demonstrates a large spectrum of cardiac tumours (surgical and autopsy cases) seen in recent cardiologic practice and confirms that myxoma is

the most common primary cardiac tumour, but more fibroelastomas are being diagnosed. Moreover, the study shows that right sided tumours are more likely to be malignant.

Key words: heart, myxoma, sarcoma, leiomyosarcoma.

O-69

Carotid fibromuscular dysplasia: the main pathohistologic features

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Background Fibromuscular dysplasia (FMD) is a non-atherosclerotic, noninflamatory vascular disease that most commonly affects the renal and internal carotid arteries. The major task of this study is to determine the main pathohistologic features of carotid FMD.

Materials and Methods The biopsy material of carotid arteries of 211 patients being operated on account of carotid stenosis (CS) for six years (2001–2006) was investigated. The operating material was analysing macroscopically; pathohistological examination including H&E staining, resorcine-fuxin, picrofuxin, trichrome Masson.

Results The analyse shows, that FMD ranks as the second cause (15%) of CS after atherosclerosis (71%). Men and women are in nearly equal ratio. The age of patients varied from 18 to 69 years. In 12.5% carotid FMD was asymptomatyc, in others clinical presentations were variable. Most often carotid FMD was observed in the middle and in the upper parts of the internal carotid artery (95%), bilateral involvement – in 60% of cases. Amongst pathological deviation discovered in one third of cases, the most frequent were kinking (62%) and atherosclerotic plaques (21,8%). Pathohistologically the most frequent type of FMD was medial fibroplasia (65,6%), rarely – intimal fibroplasia (34,4%). Medial fibroplasia was characterized by fibrosis of the media, segmental hyperplasia of the intima, and adventitia was invariable. Under intimal fibroplasia the subendothelial collagen deposits were revealed with ruptures of internal elastic membranes, while media and adventitia were invariable.

Conclusions Finally, the investigation showed that FMD is not characterized by sexual and age-specific features; clinically it may be symptomless; pathohistologically it is displayed by medial and intimal fibroplasias. We are not yet in a position to explane ethiology and morphogenesis of FMD and further research is needed with larger groups of patients.

O-70

Cardiac Histiocytosis. A Clue To The Erdheim-Chester Disease.

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Background: Erdheim-Chester Disease (ECD) is an uncommon entity, whose etiology and pathophysiology still remain unclear. It may present multisystemic involvement and the diagnosis requires high level of suspicion. The authors report a case where apparently unspecific histiocytic infiltration of the heart pointed out the diagnosis.

Material and Methods: A 58 year-old male with complex cardiological clinical history, underlying multiple hospital admissions and therapeutic procedures since 1998, was refered to surgical treatment of severe mitral insufficiency. The marked thickning of both atrial walls - obstacle to the valvular correction - and a previous diagnosis of Langerhans Cell Histiocytosis in a tooth alveolus lesion, led to the hypothesis of a cardiac manifestation of the disease. Yet, during a second attempt of valvular approach, samples procured from right atrial wall and peri-aortic fat de novo "infiltration" were submitted to macroscopic and microscopic examination (including immunohistochemical characterization with a panel of markers).

Results: Macroscopically, the fragments were white-pinkish and elastic, measuring from 9 mm to 2.3 cm. Microscopy revealed "moderate to severe multifocal aggregates of histiocytic cells, lymphocytes, plasmocytes and other inflammatory cells in a background of fibrous tissue and residual cardiac muscle". The immunohistochemical profile of the histiocytic cells was CD68 +, lisozime +, alfa1-antiquimiotripsine + and S100 protein negative, among other negative markers.

Conclusion: Although apparently reactive, the density of the macrophagic/histiocytic infiltration suggested the possibility that it might represent Erdheim-Chester Disease, which was confirmed by examination of the skeleton (namely bone scintigraphy). This case not only highlights the possible histiocyte intra-mutation fenomena, allowing the coexistence of the two entities - Langherhans Cell Histiocytosis and Non-Langherhans Cell Histiocytosis (ECD) - in the same patient (1); but also emphasizes the Histopathology contribution for the diagnosis of such a specific entity, despite the cardiac microscopic unspecific features (2).

O-71
EFFECT OF SWIMMING ON MYOCARDIAL VASCULARIZATION IN SPONTANEOUS HYPERTENSIVE RATS

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Background: hypertensive cardiopathy caused by pressure overload is characterized by myocardiocyte hypertrophy, followed by microvascular rarefaction in the myocardium due to exacerbation of the vasoconstrictors responses. Moderate physical exercise, in the long run, can cause anatomical alterations that attenuate hypertension and its harmful effects. The aim was to describe the quantitative effects of physical training by swimming on the myocardial vascularization of spontaneous hypertensive rats (SHR). Methods: the male rats were divided into four groups: sedentary Wistar-Kioto (WKY C), trained WKY (WKY T), sedentary SHR (SHR C) and trained SHR (SHR T). The quantification of the vessels was done in myocardium of the left ventricle using HE, and the interative software "AxionVision 3.1". Fifity fields of each case were analyzed with ×40 magnification. Results: After the swimming protocol it was observed that there was no have significant difference in the number of vessels between the hypertensive groups and the WKY groups. Groups SHR presented a significantly bigger number of vessels than the WKY. The correlation between the weight of the heart and the number of vessels presented was negative, being significant only in SHR C. The correlation of arterial pressure and the number of vessels was not significant. Conclusion: According to the results, the swimming was not capable of stimulating increase of vessels, however it proved to not be harmful because it did not cause a reduction in the already existing number of vessels in the myocardium. Moreover, it can be inferred that the SHR has a bigger vascular proliferation capacity than the WKY perhaps as a mechanism of protection against hypertension.

O-72
Medianecrosis of aorta – Gsell-Erdheim syndrome: the main pathohistologic features

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Background Today a great part of progressively increasing amount of aortic dissection is associated with medianecrosis of aorta – Gsell-Erdheim syndrome. It may be explained by insufficient studying of morphologic features of aortic dissection and especially of nonatherosclerotic aneurysms of aorta.

Materials and methods 24 cases of Gsell-Erdheim syndrome during 2004–2006 were analyzed: 20 men (83,4%) and 4 women (16,6%) from 22 to 69 years. Aortic wall was studied at all parts. The pathohistologic examination included: H&E staining, resorcine-fuxin (Hart), picrofucxin (Veigert) trichrome Masson, alcian blue.

Results Irregular focal thickening of the intima; focal accumulations of alcian+ mucoid substances, single cystic cavities, focal irregular anucleated zones with disoriented fibers of the media were seen in the areas, distant to the rupture of the aortic wall. Medial lamina elastica interna was partially straightened, fragmented, with the zones of hyperelastosis and multiplication. Adventitial edema, rarely focal sclerosis and perivascular sclerosis of vasa vasorum were typical.

Signs of total medial disorganization were seen near the area of rupture: focal accumulations of mucoid substances, ribbon-like anucleated zones, chaotic orientation of smooth muscle cells with severe degeneration; irregular sclerosis. Elastic fibers showed focal hyperelastosis, fragmentation. Areas of severe elastrolyses and zones filled by amorphous masses with altered tinctorial properties and fragmented elastic fibers were revealed near the rupture of the aortic wall. Adventitia showed severe hemorrhagic infiltration, dilated vasa vasorum.

Conclusions In aortic medianecrosis pathologic process was revealed in the whole vessel, not only in the area of aneurysm and dissection. Manifestations of variable severity of the same process were seen in different parts of aortic wall.

Aortic medianecrosis – Gsell-Erdheim syndrome – is a disease of aorta, associated with the lesions of elastic fibers of the media and with some other typical morphologic changes. Clinically dissecting aortic aneurysms manifests it.

O-73

Clinical morphology of acute coronary syndrome

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Background. Acute coronary syndrome (ACS) is "a useful operational term to refer to any constellation of clinical symptoms that are compatible with acute myocardial ischemia" (Braunwald E. et al., 2002). ACS's symtoms include unstable stenocardia without or with increased troponins and also early acute myocardial infarction without or with Q wave.

Methods. Autopsy material from 43 cases of ACS has been examined (33 males, 10 females, 39–60 years). Macro- and microscopic tests on myocardial ischemia were used including NBT-macrotest, polarizing microscopy, PAS-, Rego-, Lie, Sudan III-, Heidenhein-stain. In 7 cases morphofunctional comparisons with vital ECG data have been carried out.

Results. Vulnerable atherosclerotic plaques with focal hemorrhages into coronary intima have been found in all cases. The fibrous caps of plaques are loosened, infiltrated by mononuclear and foamy cells, and also impregnated by plasma proteins. As a sign of myocardial ischemia sarcomere hyperrelaxation has been found by polarizing microscopy associated with multiple contracture. Wavy deformation of muscular fibers was permanently seen. Microcirculatory disorders as an additional sign of ischemia were visible. NBT-test revealed focuses of myocardial ischemia in the left vantricle wall and in interventricular septum. On preliminary data by comparing the ECG with NBT-test, it was apparent that in early ischemia without Q wave (but with microscopic signs of ischemia) NBT-test was positive only with addition of lactate as substrate. In later stages of ischemia (myocardial infarction with Q wave) NBT-test was constantly positive.

Conclusion. Morphological basis of ACS is a myocardial ischemia provoced by rupture of vulnerable atherosclerotic plaques. Ischemia is detected on the lots of morphological signs on macro- and microscopic levels. Macroscopic test with NBT allow to define and to distinguish both early ischemia (unstable stenocardia) without Q wave and early necrosis (acute myocardial infarction) with occurence of Q wave.

O-74

Perivascular deposits - The role of EM in diagnosis of CADASIL and Nephrogenic Fibrosing Dermopathy (NFD) associated to gadolinium containing contrast agents.

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Peculiar small-sized perivascular deposits, preferable studied by electron microscopy (EM), play an important role in two different diseases. The first disease, known since 1993 as CADASIL (**Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy**), is an adult-onset neurologic disorder with recurrent strokes and progressive dementia caused by mutations in Notch-3 gene. Degeneration of arterial vascular smooth muscle cells and concurrent accumulation of granular osmiophilic material (**GOM**), which major component is the ectodomain of Notch-3, typify CADASIL. The pathognomonic GOM deposits were originally described in small blood vessels of the central nervous system. Later on involvement of many systemic tissues - including skin - was reported. Today it is common practice to utilize deep skin biopsies for CADASIL diagnosis confirmation. We report GOM deposits of two genetically confirmed CADASIL patients, whose skin biopsies were processed with and without routine postosmication. The EM evaluation revealed a similar deposit appearance in both tissue samples. This finding substantiates suspicions, that GOM is not truly osmiophilic and the term "GOM" seems to be a misnomer. In consequence, we propose to rename it: **VSMD = Vessel Smooth Muscle Deposit.**

Nephrogenic Fibrosing Dermopathy (NFD), now termed **Nephrogenic Systemic Fibrosis (NSF)**, is a recently emerged acquired disorder of the skin and systemic tissues. It is observed exclusively in patients with renal insufficiency exposed to gadolinium (Gd) used in contrast agents for MRI. Our EM study of skin samples of an NSF patient, utilizing electron spectroscopic imaging (ESI) and electron energy loss spectroscopic (EELS) microanalysis, revealed **Gd deposits** in a wide perivascular zone of the skin vessels. **Simultaneously iron was detected** in some Gd-deposits as well as in the adjacent tissue. This finding supports the Gd transmetallation hypothesis and its trigger function for the circulating fibroblasts. We provide ultrastructural evidence for the ability of lesional fibroblasts to differentiate into myofibroblastic cells.

O-75

Comparative ultrastructure of interstitial Cajal-like cells from different normal tissues

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Background Interstitial Cajal-like cells (ICLC) were identified in the last few years in a series of digestive and non-digestive organs. However, there is no available data describing similarities and differences between such cells, in terms of ultrastructure.

Method In this study we comparatively examined the ultrastructural features of ICLC identified in pancreas, gallbladder, mesentery, mammary gland, myometrium, and atrial or ventricular myocardium. Tissue samples were processed for ultrastructural investigation according with the usual protocol. Electron microscopy examination was performed with a Philips CM 12 transmission electron microscope at 60 kV. The images were recorded with Morada 11 megapixel CCD camera and analyzed with iTEM SIS software (Olympus).

Results Cells were identified using accepted transmission electron microscopy (TEM) criteria for ICLC: location in interstitium, vicinity to capillaries, nerve bundles, or other interstitial cells, very characteristic long cell processes, as well as quantitative data about organelles, plasmalemmal or cytoskeletal structural components. The processes of different ICLC were particularly long (e.g. from tens of μm in myocardium or pancreas to a mean length of 24.91 μm, in mesentery). A convolution index was calculated in order to measure the potential length of the very typical cell processes (e.g. 2.32 for mesentery, 2.47 for gallbladder). Appearance and spatial orientation in different tissues were revealed by measuring mean distances vs. main target cells of ICLC - nerve bundles, vessels, other connective cells and macrophages (e.g. 110.69, 115.80, 205.07 and 34.65 nm respectively in mesentery) or measuring distances at the level of close contacts with other cell types (e.g. 26.17 nm in a ICLC-smooth muscle contact in or 74 nm in an ICLC-eosinophil cell contact in myometrium).

Conclusion ICLC recognized in different tissues share significant ultrastructural features allowing ICLC to be viewed as a distinct interstitial cell type.

O-76

Exogenous surfactant causes direct lung injury and acivates lymphocyte-macrophage axis - electron microscope and flow cytometry studies

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Background: the cause of unsatisfactory results of surfactant treatment of acute respiratory insuficiency in adults remain unclear.

Methods: pathogen-free rats were given a single dose (150 mg of lipids/kg of b.w.) of semi-natural surfactant (curosurf). After 1, 6, 24 hours, 5, 10, 21, 42 days lungs from at least 5 animals were taken. Tissue secimens were prepared and examined by by light and transimission elecron microscope as well as morphometric studies were performed. We used cell cultures (macrophages and lymphocytes separated or mixed) for the studies of increasing surfactant concentration effect on cell proliferation. All results were statistically evaluated.

Results: in experimantal lungs average air-less areas (AALA) were fluctuating during observations, but in all time-groups they were greater than in controls ($p<0.0001$). In lungstaken short after treatment we found a raise of AALA from 28.15% (at 1 h) to 35.31% (at 24 h; $p<0.001$) and then a decrease. At this time experimental lungs were collapsed with parenchymal/intraalveolar edema. At 1 h observations in some samples only about 10% of area was aerated (vs. controls 82%; $p<0.0001$). Since 24 h enomous inflow of pulmonary macrophages into areas with abundance of exogenous surfactant was found and followed by proliferation of type II pneumocytes. In experimental lungs at 42nd day of observation AALA was 30.57% vs. 21.73% in controls ($p<0.001$). Alveolar septa were widened by the proliferation of fibroblasts as well as deposits of estracellular components (mainly collagen). Cell cultures revealed activation of macrophages with increasing doses of surfactant. The macrophage proliferation was augmented by the presence of pre-stimulated lymphocytes.

Conclusions: our results suggest that surfactant treatment possibly destroys normal lung tissue. Rapidly developing changes are partially reversible. Unfortunately they might lead to lung fibrosis. The lymphocyte-macrophage axis is activated depending on surfactant concentration.

O-77

Pathological evaluation of lentigo maligna

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Background: The pathological features of lentigo maligna (LM) can be subtle and difficult to evaluate in sun-damaged skin.

The aim of this project was to compare cases of LM with controls. We assessed 28 cases of LM. Eleven cases were formalin-fixed Mohs resections from difficult-to-treat recurrent or ill-defined LM with a control biopsy taken from the opposite side to the tumour. One case had another lesion removed at the time of surgery which was used as a control. The remaining 16 cases were matched with biopsies of basal cell carcinomas matched for sun damage and age.

Methods: Numbers of melanocytes per high power field were assessed by H&E and using a Melan A immunostain. HMB45 immunostaining was also used. Cases were graded as high grade if they possessed two or more of the following: cells above the dermo-epidermal junction; nests of melanocytes; confluence of melanocytes at the dermo-epidermal junction; a more uniform population of cells.

Results: Twenty cases were judged to be high grade and eight low grade LM. Four high grade lesions recurred in contrast to no low grade lesions.

Melanocyte counts in each case were compared with its control and a ratio was calculated. Cases where this ratio was greater than 2:1 were judged as highly informative. 21/27 (78%) cases were highly informative using H&E staining only and 17/24 (71%) cases were highly informative using a Melan A stain.

HMB45 staining was less well defined but was positive in all LMs and negative in 24/28 controls.

Conclusions:

1. Grading in LM is useful as all recurrences in this study occurred in high grade cases.
2. The use of a control biopsy alongside LM is helpful as the control: case ratio is usually greater than 2.
3. Positive HMB45 staining distinguished between cases and controls.

O-78

Consistent expression of the stem cell renewal factor BMI-1 in primary and metastatic melanoma

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Background: Stem cell-like cells have recently been identified in melanoma cell lines, but their relevance for melanoma pathogenesis is controversial. To characterize the stem cell signature of melanoma, expression of stem cell markers BMI-1 and nestin was studied in primary and metastatic melanomas. Because BMI-1 functions as a transcriptional repressor of the Ink4a/Arf locus, $p16^{ink4a}$ and $p14^{Arf}$ expression was also analyzed.

Method: 64 cutaneous melanomas, 165 melanoma metastases as well as 53 melanoma cell lines were investigated for BMI-1, Nestin, $p16^{ink4a}$ and $p14^{Arf}$ expression using immunohistochemistry.

Results: Increased nuclear BMI-1 expression was detectable in 41 of 64 (64%) primary melanomas, 117 of 165 melanoma metastases (71%) and 15 of 53 (28%) melanoma cell lines. High nestin expression was observed in 14 of 56 primary melanomas (25%), 84 of 165 melanoma metastases (50%) and 21 of 53 melanoma cell lines (40%). There was a significant correlation between BMI-1 and nestin expression in cell lines ($p=0.001$) and metastases ($p=0.02$). Cell lines obtained from melanoma metastases showed a significant higher BMI-1 expression compared to cell lines from primary melanoma ($p=0{,}001$). Further, primary melanoma lacking lymphatic metastases at presentation (pN0, $n=40$) were less frequently BMI-1 positive than melanomas presenting with lymphatic metastases (pN1; $n=24$) (52% versus 83%; $p=0.01$). In addition a high BMI-1/low $p16^{ink4a}$ expression pattern in primary melanoma was a significant predictor of metastasis by means of logistic regression analysis ($p=0.005$).

Conclusions: These data indicate that cells in primary melanomas and their metastases have stem cell properties. BMI-1 expression in primary melanoma appears to induce a metastatic tendency.

Furthermore BMI-1 mediated repression of $p16^{ink4a}$ seems contributing to an increased aggressive behavior of stem cell-like melanoma cells. Besides being of prognostic relevance, these data may provide new insights in melanoma tumor genesis.

O-79

TYROSINASE AND TRP2 EXPRESSION IN HIPOMELANOTIC MALIGNANT MELANOMA

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Background: Amelanotic malignant melanoma (MM) can be sometimes difficult to diagnose on hematoxilin-eozin (H&E) slides. Since the expression of consecrated melanoma markers may widely variated from case to case, new immunohistochemical markers should be used in order to establish the melanomatous characteristics of the tumor.
Method: We studied 24 cases of nodular MM, 4 of them with minimal focal production of melanin and 2 cases of malignant peripheral nerve sheath tumor (MPNST). We analyzed the expression of several immunohistochemical markers as S100 protein, HMB45, Melan A, tyrosinase and TRP2.
Results: All the MM cases presented S100 protein, HMB45 and melan A positivity, very variable in intensity or distribution. For S100 protein, 3 of the hipomelanotic cases had intense diffuse positivity, the other having faint diffuse positivity. HMB45 and melan A were unevenly distributed within the tumoral mass both in pigmented and hipomelanotic tumors with one case of hipomelanotic MM with very few positive cells. Tyrosinase and TRP2 were positive in some of the tumoral cells in pigmented nodular MM while the hipomelanotic MMs had intense diffuse pattern of positivity. Tyrosinase has fine granular cytoplasmic positivity while TRP2 has dot-like paranuclear and faint cytoplasmic pattern of positivity.
Both of the MPNST cases showed diffuse positivity for S100 protein while HMB45 and melan A stained small groups of tumoral cells. None of the MPNSTs had positivity for either tyrosinase or TRP2.
Conclusion: Tyrosinase and TRP2 are useful markers to be added to the immunohistochemistry panel of antibody when a diagnosis of hipo/amelanotic MM is suspected. Also, they may be used to exclude a MPNST.
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O-80

Difficulties in the diagnosis of vulval inflammatory disease

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Background: Diagnosis of vulval inflammatory disease can be difficult both clinically and histologically. The major problem is the distinction of lichen sclerosus (LS) from lichen planus (LP), when the features are not well established, is particularly challenging as both present with lichenoid inflammation. Some authors have suggested specific histopathological features to aid differentiation of these diseases and this study evaluated them.
Methods: 38 biopsies from 26 patients seen in Dermatology clinics were reviewed along with their clinical histories. Nine patients required more than one biopsy: 6 patients had 2 biopsies and 3 patients had 3 biopsies. An initial H&E diagnosis was made. Two different sets of diagnostic criteria as described by Fung et and al and by Regauer et al were used and the diagnoses reached by using these criteria were compared with the original biopsy diagnosis and the final clinical impression.
Results: Nine biopsies showed features of other inflammatory diseases: bullous pemphigoid; eczema; psoriasis; Hailey Hailey disease. Two biopsies showed unequivocal features of LP and 9 biopsies showed unequivocal features of LS. Sixteen biopsies were initially judged as non-specific. Ten of these scored for LS using one or both of Regauer's and Fung's criteria. Some so-called specific features such as loss of elastin from the papillary dermis and basement membrane thickening were found in LS and LP.
Conclusions:

1. 16 of 38 biopsies were initially called non-specific. 10 of these had features of LS according to criteria described by Fung et al and Regauer et al. This could mean either that LS is being significantly under-reported by pathologists using established criteria or that the reported criteria for early disease are too broad and include a range of non-specific entities.
2. Some so-called specific features were seen in both LS and LP making them unhelpful in making such a distinction.

O-81
Evaluation of Bcl-2 Expression in Aggressive and Non-aggressive Basal Cell Carcinomas

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Bcl-2, a well-known anti-apoptotic gene, promotes cell viability without cell proliferation. Expression of the bcl-2 oncogene is reported in certain low grade neoplasms including Basal Cell Carcinomas(BCCs). Bcl-2 expression in BCCs is contradictory, with 67–100% immunopositivity being reported. The purpose of this study was to evaluate bcl-2 expression in the indolent variants of BCC, namely superficial and circumscribed subtypes and their aggressive counterparts; infiltrative and morphea-like subtypes. Anti-human bcl-2 monoclonal antibody was used to identify its protein product in formalin-fixed tissue from 33 BCCs. 22 histopathologically non-aggressive and 11 aggressive subtypes were investigated. Quantity of decoration of tumor cells for bcl-2was graded in the following fashion: 0 to 25%, 26% to 50%, 51% to 75%, 76% to 100%. Intensity of decoration was evaluated as slight, moderate and intense. Bcl-2 expression was observed in all of the BCCs, but high bcl-2 expression was statistically a significant feature of non-aggressive BCCs($P=0.001$). Different bcl-2 expression in various non-aggressive and aggressive histopathological subtypes of BCCs suggests that despite the common derivation of these tumors from a primitive basaloid stem cell and a limited potential for metastasis, they form a heterogenous group of tumors. While the superficial and circumscribed BCCs are indolent slow-growing tumors with high bcl-2 labeling, the aggressive BCCs are infiltrative and morphea-like tumors with low bcl-2 labeling. High expression of bcl-2 may indicate a more favorable prognosis in BCCs.

O-82
PILAR LEIOMYOMA: A REVIEW OF 12 CASES WITH PARTICULAR EMPHASIS ON THE SYMPLASTIC VARIANT

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Background: Benign smooth muscle neoplasms of the skin have been classified into three categories: pilar leiomyomas, angioleiomyomas and genital leiomyomas. Previous studies have defined their clinico-pathological features. However, only few cases on the symplastic variant have been reported. Further studies on the clinico-pathological and immunohistochemical features of this variant, and its differential diagnosis with cutaneous leiomyosarcoma, are needed.

Method: We have studied the clinical and histological features of 12 consecutive cases of pilar leiomyoma with special emphasis on the symplastic variant, the potential diagnostic value of the immunohistochemical expression of smooth muscle and proliferative markers such as desmin, caldesmon and Ki67, and a correlation with clinical outcome.
Results: Piloleiomyomas are presented as dermal spindle cell proliferations usually organized in interlacing and short fascicles, with eosinophilic cytoplasm, elongated blunt-ended nuclei and loose chromatin. Three out of the twelve cases showed multifocal significant nuclear pleomorphism and prominent nucleoli resembling the symplastic leiomyomas of the uterus. 0 to 2 typical mitotic figures were found in these three cases, with a Ki67 proliferative index lower than 2%. Interestingly, one of the symplastic piloleiomyomas showed a prominent epithelioid component and 5% of Ki67 expression. All of them showed a smooth muscle immunophenotype.
Conclusion: Symplastic piloleiomyomas are not exceptional. In our series they represent the 25% of the cases, showing multifocal hyperchromatic and pleomorphic nuclei and a low proliferative rate, from less than 2%, in most cases, both conventional and symplastic type, and 5% in the epithelioid symplasic case. To the best of our knowledge, epithelioid symplastic pilar leiomyomas have not been previously described. Recognition of the spindle and epithelioid symplastic variants of piloleiomyoma, complemented with the proliferative index, may allow its distinction from cutaneous leiomyosarcoma.

O-83
Cutaneous Leishmaniasis

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Leishmaniasis is a parasitic disease caused by a haemoflagellate protozoan organism of the genus Leishmania. Cutaneous Leishmaniasis (Oriental Sore and Espundia) and the other main type Visceral Leishmaniasis (Kala-azar); are broadly classified as old world and new world depending on the endemic geographical area.
This disease is not endemic in Australia and all reported cases are imported, with the incubation period spanning from 2 to 30 years.
The case presented is a 32-year-old male migrant of Indian origin who was referred to a dermatologist for a painless, erythematous and scaly papule on his right cheek. A punch biopsy of the lesion was received which demonstrated plentiful Leishmania organisms in the histiocytes.

The life cycle, clinical features, Dermatopathologic Differential Diagnosis, of similar organisms in lesions will be discussed.

O-93
CHARACTERIZATION OF DIABETIC MASTOPATHY BY FINE NEEDLE ASPIRATION

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Background: Diabetic mastopathy occurs in patients with a long-standing insulin-dependent disease, and it courses with nodules that clinically and radiographically mimic breast cancer. The lesions may be multiple and bilateral. There are few cytological reports of this entity, most of them with non-conclusive findings or with insufficient samples.
Methods: We report the cytological findings of two different breast nodules arising in the right breast of a 35 year-old diabetic woman. Ultrasound-guided FNAB was performed with a 22-gauge needle, and the material smeared onto glass slides. After air-drying, the samples were stained with Diff-Quick, and immediately interpreted by a pathologist and a cytotechnologist.
Results: Both nodules yielded numerous blood vessels of the capillary type surrounded by a large number of lymphocytes. These cells had different sizes, predominating the small lymphocytes, and lacked atypical features. There were no epithelial cells in our samples. The diagnosis of diabetic (lymphocytic) mastopathy was rendered, avoiding unnecessary biopsy or excision of the tumors.
Conclusions: The presence of a capillary mesh admixed with a polymorphus lymphocytic cell population in an FNAB of a clinically malignant breast mass from a diabetic patient, provides sufficient information as to avoid unnecessary surgical procedures. Therefore, FNAB is a very useful tool in the management and follow-up of breast lesions in young diabetic women.

O-94
EVALUATING THE HEALTH OF THE VAGINAL FLORA USING CULTIVATION-INDEPENDENT METHODS

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Background: A healthy vaginal flora is of paramount importance. Our objective was to determine the morphotype of the adherent bacteria in liquid-based cytology (LBC) in smears with healthy and disturbed vaginal flora and to use PCR technology on the same fixed cell sample to establish DNA patterns of the 16S RNA genes of the bacteria in the sample.
Method: Thirty samples were randomly selected from a large group of cervical cell samples suspended in a commercial coagulant fixative "(BoonFix)". PCR was used to amplify DNA of five bacterial species: *Lactobacillus acidophilus*, *Lactobacillus crispatus, Lactobacillus jensenii, Gardnerella vaginalis* and *Mycoplasma hominis*. The LBC slides were then analyzed by light microscopy to estimate bacterial adhesion.
Result: DNA of lactobacilli was detected in all cell samples. Seventeen smears showed colonization with *Gardnerella vaginalis* (range 2.6×102 - 3.0×105 bacteria/μl BoonFix sample). Two cases were identified as dysbacteriotic with high DNA values for *Gardnerella vaginalis* and low values for *Lactobacillus crispatus*. The sample with the highest concentration for the (unhealthy) bacteria *Gardnerella vaginalis* showed an unequivocal *Gardnerella* infection. This cultivation independent method reveals a strong inverse relationship between *Gardnerella vaginalis* (unhealthy flora) and *Lactobacillus crispatus* (healthy flora).
Conclusion: This study indicates that the vaginal flora in liquid-based cervical samples can be evaluated because these samples are suitable for quantitative PCR analysis allowing evaluation of the health of the vaginal flora.

O-95
Regional differences in proliferation, DNA repair enzymes and p53 mutations in gliomas. Where to biopsy?

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Background: Gliomas comprise a spectrum of different tumor subtypes that share a diffuse infiltration of brain tissue and intratumor heterogeneity, which can be an issue when assessing tumor grade in small tissue samples. Invasion is regarded as one of the main reasons for poor therapeutic success. Mutations in the p53 gene are common in gliomas and are thought to arise early in the development of a malignant tumor. Methylguanine-methyltransferase (MGMT) is a DNA repair enzyme with an important role in cancer cell resistance to O6-alkylating drugs.
Design: The aim of this project was to study intra-tumor heterogeneity in gliomas, by investigating the differences between central and peripheral regions of 21 brain tumors (16 gliomas and 5 metastatic tumors) from gross total resection specimens, when these were readily available for

analysis. We studied the status of p53, Ki-67 and MGMT. We compared these results with the clinical outcome and response to therapy in these patients. Statistical analysis were done using SPSS computer program. Data were tested for significance by T-student test.

Results: Of the 16 gliomas, 15 were positive in at least one region for p53 and 11 showed MGMT immunoreactivity with significant differences between central and peripheral regions. Ki-67 expression was variable, with values ranging from 5 to 90% and a significantly higher central proliferation.

Patients with the diagnosis of gliomas received the established therapeutic protocol according to STUP which included quimio- (Temozolomide) and radiotherapy in high grade gliomas.

Of the 16 patients, 6 were alive and free of disease, 7 were alive with residual/recurrence tumor and 3 were dead of disease.

Conclusions: The results obtained in this study, reveal that clear differences exist between central and peripheral regions in brain tumors, particularly in regards to proliferation, and reinforce the importance of the selection of the sampled tumoral region in these cases. Although a larger study is ongoing, we believe that this work will contribute to advance the knowledge of the physiopathology of gliomas and may have an impact in treatment protocol designs.

O-84

Role of chromatin remodeling mediated by the HIV-1 Tat protein in the genesis of the HIV-1-associated malignancies

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BACKGROUND:The incidence of non-Hodkin's lymphoma (NHL) is greatly increased in HIV-infected individuals. Malignant lymphoma occurs in association with acquired immunodeficiency syndrome (AIDS). The vast majority of neoplasms are clinically aggressive, monoclonal B-cell neoplasms that exhibit Burkitt's, immunoblastic, or large cell lymphoma histopathology. The Tat protein of HIV is a likely candidate to contribute to tumour pathogenesis in HIV-infected patients. Extensive evidence indicates that Tat is a cofactor in the development of AIDS-related neoplasms and the protein has also been found to have an oncogenic role in vitro and in vivo. Deregulation of cellular genes and functions by Tat can also cause abnormalities that may contribute to the development of AIDS-associated disorders. The molecular mechanism underlying Tat's pleotropic activity may include the generation of heterodimers of Tat with cell cycle proteins.

RESULTS AND CONCLUSIONS: We have previously shown that Tat interacts with the RB2/p130 tumour suppressor gene product, resulting in uncontrolled cell proliferation. The interaction of Tat with cell cycle regulatory proteins alone may not be sufficient for neoplastic transformation in vivo and other cofactors may be required. Another mechanism, through which Tat may influence HIV-mediated transformation, is by hyper-activation of transcription by interacting with chromatin remodelling complexes. Recent findings indicate a complex interplay between viral proteins and host transcription regulatory machineries, including histone deacetylases (HLACs), histone acetyltransferases (HATs), cyclin-dependent kinases (CDKs), and histone metyltransferases (HMTs). The chromatin structure presents a significant barrier to transcription. These modifications and alterations of chromatin structure increase DNA accessibility to transcription factors and activators, thus promoting transcription initiation and efficient elongation. Many reports in the last several years have linked Tat transactivation to chromatin remodelling in vitro and in vivo. The aim of our study is to investigate whether Tat-mediated chromatin remodelling may have a role in HIV-associated transformation.

O-85

Proteomics characterization of formalin fixed breast cancer tissues

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Background: Breast cancer is a complex disease only incompletely characterized by clinicopathological parameters. Even though tumoral proteins are good markers and allow the identification of targets for specific treatment their limited number hampers the selection of the most appropriate diagnostic or therapeutic strategy. Proteomic techniques complement and further enlarge the wealth of information generated by genomics. In particular MSE data acquisition is a novel parallel peptide fragmentation protocol that allows high peptide detection efficiency, ideal for protein identification from very complex samples. Archival formalin-fixed paraffin-embedded (FFPE) tissues could represent an incredible source for examining the clinical course of diseases.

Methods:In the present study we compared the efficiency of protein identification from FFPE human breast cancers

samples with the unfixed counterpart. In order to explore different protein unlocking procedures which might enable a suitable recovery of polypeptides we have developed an in vitro approach using "tissue surrogate" samples for MSE analysis.
Results: Our results outline the possibility to obtain comparable peptide mass spectra both from unfixed and FFPE samples. The number of the identified proteins from unfixed cancer tissue was 160 (sequence coverage 48%); from Trypsin/EDTA treated FFPE tissues was 150 (sequence coverage 39%); whereas from FFPE untreated tissues the number of identified proteins was 35 (sequence coverage of 32%). Among the identified proteins the most relevant were annexin, ubiquitin, calmodulin, histonic proteins, MIF, tenascin.
Conclusions: Appropriate pretreatment to unlock the fixed proteins allowed us to obtain suitable protein expression profiles from FFPE tissues. Improvements of sequences analysis software could further increase the number of protein detected. The study of large numbers of archival FFPE tumors may represent an useful approach to assess breast cancer heterogeneity defining more reliable diagnostic/prognostic factors to selectively targeting the tumor cells.

O-86

Linear Discriminant Analysis of transcriptomic data reveals a 7-gene signature diagnostic of prostate cancer

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Background: Transcriptional profiling studies have unveiled new molecular markers of cancer, that are being applied to diagnosis in actual clinical environments. Quantitative PCR determinations for selected transcripts should be useful adjuncts for diagnosis, prognosis and monitoring of neoplasia. We have identified a small gene set that is diagnostic of prostate cancer, and have explored its applicability as a diagnostic aid.
Methods: We have used prostate carcinoma samples with homogeneous epithelial representations, normal prostate and pure stromal tissue and analysed them with Affymetrix Human Focus microarrays. Results were validated by low-throughput QPCR in whole tissue samples and micro-dissected specimens as well as by immunohistochemistry for selected genes.
Results: By microarray analysis, we have identified gene signatures that are highly discriminant of prostate cancer. Subsequent Linear Discriminant analysis (LDA) of micro-array and QPCR data supported a 7-gene signature which allowed robust discrimination between normal and tumoral prostate samples in 100% of cases, including samples distinct from those used in the original microarray analysis. In support of their tumor vs normal discriminant capacity, individual genes in this signature showed similar expression profiles when external datasets were interrogated.
Conclusions: Our transcriptomic studies of highly selected prostate cancer samples coupled to extensive validations and linear discriminant analyses have yielded a highly discriminant 7-gene transcriptional signature with diagnostic potential in clinical practice.
Supported by grants from the Ministerio de Educación (GEN2001–4856-C13, GEN2001–4865-C13–10 and SAF2005–05109), Ministerio de Sanidad (PI020231), Fundació Marató TV3, RTC of the Instituto Carlos III (ISCIII-RETIC RD06/0020) and the Fundación Ramón Areces.

O-87

Genomic alterations associated with the progression to invasive breast cancer revealed by array comparatlive genomic hybridization

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Background: Our knowledge of molecular events during breast cancer progression is limited. Preinvasive breast cancer (also known as duct carcinoma in situ, DCIS) is a heterogeneous group of lesions of difficult to predict prognosis. Genomes of DCIS and infiltrating duct carcinoma (IDC) have been compared previously and show a similarity of genomic alterations. We hypothesized that significant events can be detected by comparing lesions with a broader range of behavior: from pure DCIS (without IDC) to IDC associated with lymph node metastasis.
Method: Array comparative genomic hybridization was calibrated by self-self hybridization of normal DNA and used to analyze 6 cases of pure DCIS, 17 cases of paired

DCIS and IDC where 8 tumors had metastases in the axillary lymph nodes.

Results: We found a higher number of genomic alterations in pure DCIS than DCIS and IDC components of invasive tumors. In-situ and invasive components of the same tumor had similar genomic profiles. Pure DCIS differed from DCIS associated with IDC while the latter showed similarity within the group. IDCs positive for metastatic spread also showed similarity between tumors. Several alterations were detected preferentially in IDC and associated DCIS compared to pure DCIS. Gain on 17q22–24.2 was associated with higher histological grade, large IDC size, lymphatic/vascular invasion and lymph node metastasis ($p<0.05$).

Conclusion: These findings suggest that specific genomic events occur at different stages of breast cancer progression. DCIS associated with IDC may represent a clone with high invasive potential or an established invasive clone spreading inside the ducts. Gain on 17q22–24.2 is a candidate region for testing as a marker for high risk of invasion and nodal metastasis, which may prove useful for limited biopsy material.

O-88

Detection and genotyping of human papillomavirus DNA in 914 samples from a specialized Cervical Pathology Service

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Background: Cervical cancer represents the second most common malignancy in women worldwide. Almost all cases are caused by persistent infection with about 15 genotypes of human papilloma virus (HPV). A clear understanding of the molecular epidemiology of HPVs and the availability of powerful molecular diagnostic techniques has provided the background for prevention strategies of HPV-related carcinomas. The aim of this study was to investigate the frequency and genotype distribution of HPV infection in cervical brushings from women followed in a Cervical Pathology Service.

Methods: Cervical samples of 914 patients were collected for cytopathological and molecular test in the Pathology Cervical Service from May 2006 through October 2007. HPV infection was searched by PCR and viral typing was performed by dot blot hybridization.

Results: Out of the total number of tested women, 436 (47.7%) of them were positive to HPV Polymerase chain reaction (PCR). This high rate of HPV DNA positivity (adjusted prevalence of 6.8% in Southern Europe) is ascribed to our study population (women who attended to a specialized Gynaecological Service of cervical Pathology). The most frequent genotype detected in our series was HPV16, found in 18.1% of all positive samples, followed by HPV31 in 10.5%, HPV53 in 8.9%, HPV52 in 6.8%, HPV58 in 6.5%, HPV44 in 6.5%, HPV18 in 5.6%, HPV66 in 5.5% and HPV39 in 5%. Genotypes 56, 6, 33, 42, 59, 68, 35, 45 and 51 follow this group, with frequencies lower than 5%. A coinfection with two or more virus was found in 132 samples (30.3% of positive cases), 41 of these had HPV16 with other genotype. Correlation with cytological data will be done.

Conclusions: HPV16 alone or in coinfection is the most frequent type detected. According with other Spanish studies HPV18 is not a frequent type in our series. HPV31 and HPV53 are the second and third commonest types.

HPV types differ geographically and with the social phenomena of immigration HPV type-specific prevalence are varying; hence additional work should be done in providing information on frequency of different genotypes in Spain and to understand the mechanisms of HPV carcinogenesis.

O-89

FATTY ACID SYNTHASE AND KU80 AS NEW MARKERS FOR HER2 TRASTUZUMAB RESPONDER POPULATION SELECTION. MOLECULAR FOCUS ON A COMPLEX CROSS TALK

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Background Over expression of the EGFR family member Her2 is found in approximately 30% of breast cancers. Her2 currently represents one of the most appropriate targets for specific therapy. The determination of Her2 overexpression has become necessary for the selection of breast cancer patients responder to Herceptin therapy. Immunohistochemistry in combination with FISH is currently the most used method for this detection that still provide controversial results in term of selection of responder patients. In fact, a consistent number of Her2-positive tumors is not firstly responsive to HER2-driven therapy, indicating the need of a multiparametric approach on receptor functionality to improve the selection of real responder patients. We analysed the complex cross talk among the functional activated Her2 receptor and the

activation of the molecular target cascade closely linked to its functionality. Recent studies in breast cancer cells have revealed a bi-directional connection between Her-2/neu and fatty acid synthase (FASN), a major lipogenic enzyme catalyzing the synthesis of long-chain saturated fatty acid-sHer-2/neu overexpression stimulates the FASN promoter and concomitantly the presence of nuclear Ku80DNArepair protein, usually loss in advanced tumors, suggesting an indirect evidence of Her-2 transduction activity.

Methods In the present study we observed the pattern shift expression of Ku80 and FASN in 20 ductal infiltrating breast cancer biopsies matching their localization and expression to Her2 status determined by FISH and immunohistochemistry.

Results We observed that the overexpression of Her-2 due to polysomy, aneuploidy was correlated to the absence of Ku80 in the nuclei. Conversely, patients with Her2 ratio >>2 correlated to Ku80 and FASN overexpression only in 64%of cases.

Conclusions Data obtained could explain at least in part the existence of a cohort that lacks Her2 functionality and therefore do not respond to the therapy. These screening may further enhance the efficacy of trastuzumab therapy by selecting those patients most likely to respond.

O-90

Clusterin and IL-6: a new twin set of non-invasive molecular markers for colorectal cancer detection

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Background. Clusterin is an ubiquitous glycoprotein implicated in a large number of physiological processes and IL-6 is a pleiotropic cytokine with a broad range of functions on immune and non-immune cells. The expression of both Clusterin and IL-6 is regulated by TGF-β which plays an important role in the development of colorectal cancer and both proteins are involved in proliferation and apoptosis. These opposite effects are carried out by two different isoforms of Clusterin (cytoplasmic/secreted and nuclear, respectively) and by different signalling pathways that IL-6 is able to activate in cells.

We have already shown that in the adenoma-carcinoma sequence of colorectal cancer the pro-apoptotic nuclear Clusterin is lost, while there is an increase in the production of secreted Clusterin.

Method. Secreted Clusterin and IL-6 levels were assessed by ELISA in serum of healthy individuals and of patients with colorectal cancer. Clusterin was also assessed in stool specimens and immunohistochemistry was performed on surgical specimens.

Results. We evidence a significant increase of secreted Clusterin in serum and stool of patients with colorectal cancer compared to healthy individuals. The level of secreted Clusterin correlates with the grade and the metastatic potential of the tumour. The increase of secreted Clusterin was also observed by immunohistochemistry performed on surgical specimens of patients when tumour and normal mucosa were compared. Moreover an increase of IL-6 in serum and *in situ* was also shown to correlate with colon carcinoma stage.

Conclusion. The correlation among IL-6 and Clusterin levels and tumour progression suggests a role for these proteins as *in situ* and circulating prognostic markers for non-invasive colorectal cancer screening. Besides, the evaluation of Clusterin level in stool and in serum after surgical resection could be a good parameter to evaluate the relapse of the disease and for early detection of an ongoing metastatic process.

O-91

FGFR3 pathway identification by oligonucleotide microarrays

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There are two capital pathways in transitional cell carcinomas of the urinary bladder development, FGFR3 pathway for pTa and pT1 carcinomas and the p53 pathways for pT2 muscle infiltrating cases. The cellular human line RT-112 (from urinary bladder transitional cell carcinoma) has been showed to have FGFR3 overexpressed. Otherwise, PD173074 is a FGFR3 tyrosin-kinase activity inhibitor molecule developed by Pfizer. Our aims are to explore the different molecules implied in FGFR3 transduction pathway by oligonucleotide microarrays after inhibiting tyrosin-kinase activity in RT112 cells with PD173074

Methods: Four experiments were performed. RT112 cells were subjected to PD173074, FGFalpha plus heparin, FGFalpha plus heparin plus PD173074 and lastly RT112 control cells without treatment. Every experiment was performed by triplicate with and without DMSO (solvent of PD173074), in order to avoid the possible spurious effect of it. The analysis of gene expression has been realized using the technology GeneChip of Affymetrix (Human Genome U133 Plus 2.0). For the identification of changes in expression statistically significant between the groups of samples parametric tests were applied taking like significant those changes in $p<0.05$

Results: Hierarchical Clustering of the samples analyzed showed 30 sequences that were capable to differentiate gene expression between the four experiments. When we used the criteria of a fold change >2 to identificate significative sequences, we obtained a list of 50. 10 of them were included in MAPK signalling pathway, 6 in cytokine-cytokine receptor interaction and 4 in hemapoietic cell lineage signal transduction.
Conclusions: We have identified several molecules implied in FGFR3 signal transduction that are affected by tyrosin-kinase activity inhibition that could be used as diagnostic or therapeutical targets in urinary bladder carcinoma development.

O-92

WT1 EXPRESSION AS A MARKER OF ADVANCED STAGES OF PRIMARY MALIGNANT MELANOMA

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Background Cutaneous malignant melanoma remains the leading cause of skin cancer death. Clinical and histological variables predicting survival including Breslow's index or ulceration have been identified. Although the potential relevance of biological variables still awaits an in-depth study, recently, a group of biological markers ($p16^{INK4a}$, Ki-67 and Bcl-6) have been related to prognosis.
WT1 was first identified as tumor suppressor gene, involved in the development of Wilms' tumor of the kidney. Contradictory functions have been described to the protein WT1; it can develop a role as a tumor suppressor in some cancers, but as a potential oncogene in others. Since WT1 has been identified as a molecular target for cancer immunotherapy, immunodetection of WT1 in tumor cells has become an essential step in cancer studies. Two isolated studies report the expression of WT1 gene in human melanoma cells.
Methods In the present study we compare the expression of WT1 among different types of nevi and different stages in primary malignant melanoma progression. Tissue microarrays containing normal tissues and 271 primary melanocytic lesions samples (163 primary malignant melanomas and 108 benign nevi) were studied by immunohistochemistry.
Results The present study shows staining for WT1 in 39, 6% of MM. WT1 expression is increased in advanced stages of MM progression: a significant ($p<0.05$) increased of expression WT1 was detected in vertical cases 46.5% vs. radial cases 16.0%, in high levels of Clark (IV, V) 57.6% vs. low levels (I, II, III) 29,4% and in thick melanomas (>1 mm) 52,4% vs. thin ones (≤1 mm) 23,8%. No significant change in WT1 expression was detected when focusing on ulceration, vascular invasion, tumor size, "satellites" or clinical variables.
Conclusions Our study demonstrates: 1. WT1 is predominantly expressed in the cytoplasm of the tumor cells as in other adult cancers; 2. WT1 expression is increased in advanced stages of MM progression; 3. We establish an association of WT1 protein expression with shorter overall survival.

Posters

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P-1

DEMOGRAPHIC, ETIOLOGIC AND HISTOLOGIC PULMONARY ANALISYS IN PATIENTS WITH HIV/AIDS AND ACUTE RESPIRATORY FAILURE – AN AUTOPSY STUDY

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BACKGROUND: The different aspects of pulmonary pathology in HIV/AIDS are unknown in autopsies. In this study were described the demographic data, etiologic and histologic pulmonary findings in different associated pathologies of 353 autopsies of patients with HIV/AIDS and acute respiratory failure (ARF) as cause of death between 1990 and 2000.
METHOD: Were obtained following data: age, sex, and major associated diseases (found at autopsy and/or previously the death). Pulmonary histopathology was categorized as: diffuse alveolar damage (DAD); pulmonary edema (PE); alveolar hemorrhage (AH); and acute interstitial pneumonia (AIP). Odds ratio (OR) of the AIDS-associated diseases develop specific histopathologic pattern was determined by logistic regression.
RESULTS: Were observed 263 HIV/AIDS-infected men and 90 women. The mean age was 35 years. Bacterial bronchopneumonia was present in 29% (154 cases), *Pneumocystis jiroveci* pneumonia (PJP) in 15% (78 cases), tuberculosis in 11% (55), severe sepsis and/or shock septic in 8% (41), cytomegalovirus in 7% (37). Pulmonary histopathology showed AIP in 28% (99 patients), DAD in 25% (89), PE in 9% (33) and AH in 8% (29). Multivariate analysis demonstrated significantly positive association

between PJP and AIP (OR, 4.51; 95% CI, 2.46 – 8.24; $p<0.001$), severe sepsis and/or shock septic and DAD (OR, 3.60; 95% CI, 1.78 -7.27; $p<0.001$), cytomegalovirus and AIP (OR, 2.22; 95% CI, 1.01 – 4.93; $p=0.05$).

CONCLUSION: For the first time we showed in autopsies the demographic data, etiologic diagnosis and respective histopathologic findings in patients with HIV/AIDS and ARF as cause of death. More studies are necessary to elucidate the complete pulmonary fisiopathologic mechanism involved with each AIDS-associated disease.

P-2

ENDOTHELIAL APOPTOSIS AND VASCULAR COLLAGEN V INTERACTION IN SYSTEMIC SCLEROSIS: A PRELIMINARY STUDY

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Background: Systemic sclerosis (SSc) is a multisystem disorder characterized by inflammation, fibrosis and vascular damage. One of the pathogenetic mechanism proposed is related to direct binding of collagen V epitopes on endothelial cells, enhancing synthesis of collagen by fibroblasts or myofibroblasts. Prolonged attack can provoke basement membrane injury and exposition of hidden collagen V epitopes, originating neoantigens, which will trigger an auto-immune disease. The aim of this study was to examine in vascular and lung septal interstitium the relationship among collagen types I, III and V, endothelial and alveolar epithelial apoptosis and pulmonary function tests in patients with SSc.

Method: We examined the amount of collagen types I, III and V in vascular and septal interstitium using immunofluorescence and morphometric analysis. Endothelium and epithelium apoptosis expression was determined by TUNEL and quantified by point counting technique in 10 open lung biopsies of patients with SSc without pulmonary hypertension. The pulmonary function tests were analyzed in theses cases and correlated with collagen and apoptosis results.

Results: We observed, in decreased order, amount of collagen type III, I and V in septal interstitium. In vascular interstitium, the collagen type I was increased, followed by types V and III. The apoptosis index was higher in endothelial cells than alveolar cells. An inverse correlation was found between CVF, endothelial and alveolar cells apoptosis (p=0.01 and p=0.02, respectively).

Conclusions: We concluded that endothelial apoptosis and vascular collagen V interaction reinforce the vascular pathway in the SSc pathogenesis. Further studies are needed to determine whether this relationship is causal or consequential.

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P-3

DIFFERENCES IN AIRWAY REMODELING AMONG THE MAJOR HISTOLOGICAL PATTERNS OF IDIOPATHIC INTERSTITIAL PNEUMONIAS?

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Background: Structural alteration of the airways and lung parenchyma "remodeling" is a recognized feature of pulmonary fibrosis. In this study, we sought to validate the importance of relationships between bronchiolar collagen/elastic system, pulmonary functions tests and survival in the major histological patterns of idiopathic interstitial pneumonias (IIPs).

Method: We examined bronchiolar collagen/elastic system fibers in non-specific interstitial pneumonia (NSIP/NSIP=13), idiopathic pulmonary fibrosis (IPF/UIP=13), acute interstitial pneumonia (AIP/DAD=17) and bronchiolitis obliterans with organizing pneumonia (BOOP/OP=6) cases. We used the Picrosirius-polarization method, Weigert's resorcin-fuchsin histochemistry and morphometric analysis to evaluate the amount of bronchiolar collagen/elastic fibers, and their association with pulmonary function tests and survival.

Results: The bronchiolar measurement of collagen fibers was significantly higher in OP when compared with UIP; in the other groups (NSIP, DAD) there weren't significant differences. Analysing the amount of elastic fibers in the four groups we didn't observed statistically significant differences. The increase of collagen fibers in UIP was directly associated with dyspnea grade ($p=0.03$). Elastic fibers was directly associated with PaO_2 ($p=0.01$). In DAD we observed a direct significant correlation between amount of collagen fibers and PCO_2 ($p<0.001$) and an inverse correlation with VEF1 ($p=0,005$) and VEF1/ CVF (p=0,002). The most important predictor of survival in DAD was the amount of bronchiolar collagen deposited ($p=0.005$).

Conclusion: We concluded that a progressive bronchiolar fibroelastosis occurs in IIP histological patterns, probably indicating evolutionary adapted responses to airway injury. The more important spectrum of bronchiolar remodeling in OP and DAD suggests a more direct participation of the airways in their pathogenesis than UIP and NSIP. Further studies are needed to determine whether this relationship is causal or consequential.

Financial Support: FAPESP, CNPq

P-4

ARTERIAL AND INTERSTITIAL REMODELING PROCESSES IN NONSPECIFIC INTERSTITIAL PNEUMONIA: SYSTEMIC SCLEROSIS VERSUS IDIOPATHIC

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Background: Recently, the nonspecific interstitial pneumonia (NSIP) pattern was described as more prevalent in the collagen vascular disease (CVD) group, including systemic sclerosis (SSc). In this regard, some authors have studied pathogenetic mechanisms in idiopathic and CVD associated lung fibrosis to discover factors that might relate to better treatment and prognosis. As the pathogenesis of these two diseases seems to be different, we hypothesize that the repair mechanisms involving the collagen/elastic system also differ, leading to a diverse remodeling process.
Methods: Pulmonary biopsy specimens were examined from 40 patients, 22 with idiopathic-NSIP and 18 with nonspecific interstitial pneumonia associated with SSc. We compared the septal and vascular matrix remodeling (collagen/elastic fibers) by an image analysis system, vascular occlusion by semiquantitative analysis, pulmonary function tests and survival between the two groups.
Results: The content of septal collagen and elastic fibers, as well as the elastic fibers in the vascular interstitium, were higher in the SSc group ($p=0.01$, $p=0.001$ and $p<0.0001$, respectively). Among pulmonary function tests the DLCO/VA was affected to a greater extent in the SSc group (59% of the predicted value in SSc and 97% in the idiopathic group). There were no differences in the collagen content of the vascular interstitium, arterial occlusion, or survival between the two groups.
Conclusions: Although the fibrotic process is more intense in the SSc group, it does not affect the prognosis of these patients. Because the elastotic process is higher in the SSc group, this might suggest that autoimmune inflammatory mechanisms affecting the elastic fiber system could play a greater role in the pathogenesis and pulmonary remodeling process of SSc-NSIP than in idiopathic-NSIP.
Financial Support: FAPESP, CNPq.

P-5

Comparison of the dysadherin and E-cadherin expression in primary lung cancer and metastatic sites.

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Dysadherin, a cancer associated cell membrane glycoprotein, has been reported to downregulate E-cadherin. Aberrant expression of E-cadherin has been associated with the development of metastases in patients with cancer. Even though the expression of dysadherin and E-cadherin has been studied in primary non-small cell lung carcinoma, little is known about its expression at the distant metastases sites. We investigate by immunohistochemistry the ralationship between E-cadherin and dysadherin in 123 cases of primary lung carcinomas (53 squamous cell carcinomas, 21 adenocarcinomas, 13 large cell carcinomas, 24 small cell carcinoma and 12 epithelioid mesotheliomas), and their distant metastases. The intensity, the expression pattern and the percentage of neoplastic cell staining were recorded and the results were correlated with clinicopathological findings of the subjects. Dysadherin expression was expressed in 61 (49.59%) of the cases and increased dysadherin expression was significantly correlated with tumour size ($p=0.003$), distant metastases ($p=0.0034$), and metastasis size ($p=0.0008$). Reduced E-cadherin expression was noted in 58 (47.2%) of the cases, and was correlated with high grade tumour ($p=0.02$), infiltrative growth pattern ($p=0.042$), and advanced stage ($P=0.032$). Although the correlation between the expression of dysadherin and E-cadherin was not significant, a group of patients showed reduced E-cadherin expression with dysadherin overexpression. In lung carcinomas dysadherin expression seems to reflect tumour aggressiveness and may be considered a positive marker of poor prognosis when considered alone or/and in combination with downregulation of E-cadherin.

P-6

STRUCTURAL AND FUNCTIONAL CHANGES ASSOCIATED WITH PULMONARY OVERLOAD IN PIGS: PRELIMINARY STUDY

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Background: The precise mechanism of the lung microvascular barrier injury in patients with hemodynamic pulmonary overload is unclear. The aim of this study was to examine the structural and functional pulmonary changes after different levels of pulmonary overload.

Methods: This study was approved by the Institutional Ethical and Scientific Committees. Twenty Large White pigs were anesthetized and then placed on mechanical ventilation. After bilateral thoracotomy, the pulmonary arteries were exposed and the animals were divided into four groups: sham (n=5); clamp level I (n=5) with right lung overload by clamping of the left pulmonary artery; clamp level II (n=5) with right lung overload by clamping of the left pulmonary artery and right pulmonary artery branches to the inferior lobe and clamp level III (n=5) with right lung overload by clamping of the left pulmonary artery and right pulmonary artery branches to the inferior and mediastinal lobes. The arteries were clamped by 60 minutes using microvascular clamps and the pulmonary function was evaluated for dynamic gas changes (PaO_2/FiO_2). The pigs were sacrificed; the lungs were removed *in bloc*, weighed and processed using standard histological techniques to morphometric analysis.

Results: We observed that acute pulmonary overload in all levels of clamping produced a rapid and significant perivascular and interlobar edema when compared to the sham group and edema increased according to incremental levels of occlusion ($p<0.05$). Minimal alveolar hemorrhage and alveolar edema were occasionally present in the clamping groups. Mechanical pulmonary function changes were observed in the clamping groups characterized by decrease of PaO_2/FiO_2 correlated with different occlusion grade ($p>0.01$). Proportional increases of the overloaded lobes weigh in relation to the occlusion level were also observed in ($p>0.01$).

Conclusion: The acute pulmonary overload caused by increase of overload is an important factor for the damage of the alveolar/capillary barrier in lung mechanical stress injury.

Financial Support: FAPESP

P-7

Solitary fibrous tumours of pleura as a cuase of non-islet cell tumour hypoglycemia

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Background The solitary fibrous tumours of the pleura (SFTP) originate from submesothelial fibroblasts mostly arising from visceral pleura as localized, pedunculated and well circumscribed tumour protruded into pleural cavity. Majority of non-islet cell tumour hypoglycemia (NICTH) are SFTPs. The association of SFTP with hypoglycemia is referred as Doege-Potter syndrome. This syndrome is described in 2–4% of SFTPs.

Patients: Fourty-eight SFTPs were diagnosed during the last 15 years in our institution and two of them with Doege–Potter syndrome. We performed EnVision System immunostaining (DAKO) with 3,3′-diaminobenzidine as a chromogen without antigen retrieval. We proved positivity of IGF-II and pro-IGF-II in the tumour cells confirmed that SFTP was the cause of hypoglycemia. *Clinical data:* The both patients were women, age of 55- and 68-years. They both suffered of headache and consciousness. Endocrine tests in the both patients showed an extremely reduced glucose level, blood concentration of insulin and C-peptide and large pleural mass, occuping almost the whole hemithorax was diagnosed in chest x-ray and CT examination. *Pathology:* Small, uniform sized spindle- and round- shaped tumour cells with scanty cytoplasm and without nuclear pleomorphism and mitoses were alternated in hypercellular and hypocellular areas. Tumour cells exhibited diffuse cytoplasmatic positivity of vimentin, strong and uniform reactivity of CD34 and positivity for bcl-2 also. The tumours were diagnosed as benign SFTPs. Dot-like reaction for insulin-like growth factor II (IGF-II) and pro-insulin-like growth factor II (pro-IGF-II) products were deposited in Golgi area of spindle tumour cells, proving that SFTPs were the cause of hypoglycemia.

Conclusion: Pathologists can confirm the suspicion that SFTP is a source of hypoglycemia appling IGF-II and pro-IGF-II on paraffin embeeded tissue spacemans, obtained on percutaneous needle biopsy as well as on surgical resection.

P-8

Studies of prevalence of ventilator-associated pneumonia in intensive care unit of the University Braz Cubas – Mogi das Cruzes Hospital (Brazil) and factors and characterize patient evolution

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Introduction: Pneumonia is the second leading nosocomial infection and presents high mortality rates. Ventilator-associated pneumonia (VAP) is the leading infection in intensive care units (ICUs). The incidence ranges from 9% to 68%, depending on the diagnostic method used and on the population studied. Its lethality is high, ranging from 33% to 71%, and the case fatality rate can reach up to 55%. Of all cases of hospital-acquired pneumonia, 86% are associated with mechanical ventilation(MV). However, only 9% to 27% of mechanically ventilated patients develop pneumonia. The prevalence reported is 21.7 to 35.6 cases/1000 MV days, compared with 3.2 cases/1000 days for patients not on ventilation. The proportion of intubated patients who develop pneumonia varies from 10% to 50%, with an approximaterisk of 1% to 3% per day of endo tracheal intubation.
Objective: To determine the prevalence of ventilator-associated pneumonia in an intensive care unit, as well as to identify related factors and characterize patient evolution. Methods: This study evaluated 98 patients on mechanical ventilation for more than 24 hours in a university hospital. Results: Ventilator-associated pneumonia developed in 43.2% of the patients, translating to 39.6 cases/1000 ventilator-days: 55.8% were caused by gram-negative agents (Pseudomonas aeruginosa accounting for 27%); and multidrug resistant organisms were identified in 43.4%. In the ventilator-associated pneumonia group, time on mechanical ventilation, time to mechanical ventilation weaning, hospital stays and intensive care unit stays were all longer ($p<0.001$). In addition, atelectasis, acute respiratory distress syndrome, pneumothorax, sinusitis, tracheobronchitis and infection with multidrug resistant organisms were more common in the ventilator-associated pneumonia group ($p<0.05$). Mortality rates in the intensive care unit were comparable to those observed in the hospital infirmary. Associations between ventilator-associated pneumonia and various factors are expressed as odds ratios and 95% confidence intervals: acute sinusitis (41.1; 3.4–441); > 10 days on mechanical ventilation (7.9; 4.1–14.2); immunosuppression (4.3; 1.3–14.3); acute respiratory distress syndrome (3.5; 1.4–9.0); atelectasis (3.8; 1.2–7.3); cardiac arrest (0.19; 0.05–0.66); and upper gastrointestinal tract bleeding (0.05; 0.009–0.62). The variables found to be associated with in-hospital death were as follows: chronic renal failure (26.1; 1.9–350.7); previous intensive care unit admission (15.6; 1.6–152.0); simplified acute physiologic score II > 50 (11.9; 3.4–42.0); and age > 55 years (4.4; 1.6–12.3). Conclusion: Ventilator-associated pneumonia increased the time on mechanical ventilation and the number of complications, as well as the length of intensive care unit and hospital stays, but did not affect mortality rates.

P-9

Mucinous bronchioloalveolar carcinoma with rhabdoid features

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Background: A unique case of lung carcinoma with combined features of bronchioloalveolar carcinoma, invasive adenocarcinoma and rhabdoid poorly differentiated carcinoma is described.
Clinical history and Methods: The patient, a 63-year-old man, presented with cough and chest pain. CT scan revealed a giant mass located in the right lower lobe. The lobectomy specimen showed a mass of 10 cm in diameter that invaded the visceral pleura. Tissue were fixed in 10% buffered formalin, paraffin embedded and stained with haematoxylin-eosin. Immunohistochemistry was done with Ventana automated stainer. Results: Histologically, the lesion showed three patterns of growth: most of the lesion (40%) was composed of columnar mucus-secreting cells with a typical lepidic growth, consistent with mucinous bronchioloalveolar carcinoma (BAC). In addition, areas of invasive adenocarcinoma were present (30%- second pattern). Finally, the third pattern was constituted by a solid proliferation (30% of the lesion) composed of rhabdoid cells showing abundant deeply eosinophilic cytoplasm and large vesicular nuclei with prominent nucleoli. In these areas, the neoplastic cells were arranged in a diffuse pattern, without glandular formation and nuclei were two /three times bigger than in the other two components. Binucleated cells were also frequently seen. Immunohistochemically, the neoplastic cells had a similar phenotype in all the three components: they were positive with cytokeratin 7 and vimentin. Vimentin was strong and diffuse in the rhabdoid elements, while it was weak and focal in the other two components. Muscular markers (desmin, myogenin and striated actin) were consistently negative. The patient survived for 37 months after surgery.
Conclusions: The occurrence of a rhabdoid phenotype is most frequently seen in association with poorly differentiated carcinomas. An association with well differentiated carcinomas with BAC pattern is very rare. The present case

is the second case in which rhabdoid features are seen in association with a mucinous BAC

P-10

PULMONARY MALACOPLAKIA ASSOCIATED WITH RHODOCOCCUS EQUI INFECTION IN A PATIENT WITH AIDS

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We describe a 35-year-old man infected with the human immunodeficiency virus, who on initial examination was found to have cavitary tumoral lung lesion in right lower lobe. Histologic examination revealed sheets of foamy intraalveolar macrophages with the typical histologic features of Michaelis-Gutmann bodies that contained coccobacillary organisms and some "targetlike" cytoplasmic inclusions, so pulmonary malacoplakia probably associated to Rhodococcus equi was diagnosed. Cultures of blood yielded growth of Gram-positive coccobacilli subsequently identified as Rhodococcus equi.

Rhodococcus equi is a pathogen for some animal species, an Gram-positive coccobacillus that cause pulmonary infections in immunocompromised people. Pulmonary malacoplakia associated with this opportunistic coccobacillus is unusual, and pseudotumoral lesions are exceptional, and the recognition of this unique entity is important because of its responsiveness to therapy.

P-11

Epithelial to mesenchymal transition phenotype correlates with clinicopathological features and brain metastases in non-small cell lung cancer

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Background: In epithelial to mesenchymal transition (EMT) has been implicated in the progression and metastasis of epithelial tumors. In lung cancer, individual EMT markers' expression has been studied and correlated with prognosis.

Methods: To better define the EMT phenotype of lung cancer we studied the immunohistochemical (IHC) expression of 6 proteins, E-Cadherin (ECad), N-Cadherin (NCad), β-Catenin (β-Cat), MMP-9, Integrin-β6 (Int-β6) and Vimentin in 209 adenocarcinomas (ADC) and 116 squamous cell carcinomas (SCC). EMT expression was correlated with EGFR and p-EGFR IHC and *EGFR* mutational status. We also analyzed EMT markers expressions' in 55 paired lung cancers with brain metastases.

Results: Tumors showed high expression of ECad in 37.5%, NCad 68.2%, β-Cat 95.9%, MMP-9 93.1%, Int-β6 56.2% and Vimentin 45.8% of cases. Female gender associated with higher ECad intensity and lower NCad cytoplasmic score. ADC correlated with higher ECad class and intensity, and NCad membrane and cytoplasmic intensities. Smoking correlated with lower ECad intensity and higher NCad membrane class and cytoplasmic score. Never smokers showed higher ECad and Int-β6 intensities, and former smokers had significant higher NCad membrane class and cytoplasmic score. EGFR class correlated positively with β-Cat membrane class, but negatively with Ncad membrane and cytoplasmic intensities. PEGFR class correlated with NCad membrane and cytoplasmic intensities, but associated negatively with higher Vimentin staining. Higher Int-β6 showed worse overall survival (HR=4.4). No correlations were seen between markers' expression and *EGFR* mutations. ADC brain metastases associated with higher Vimentin score and intensity, and MMP9 cytoplasmic intensity but with lower NCad cytoplasmic intensity.

Conclusions: Our findings indicate that in lung cancer EMT is a frequent phenomenon and correlates with tumor histology, smoking history and survival. The process may have a role in brain metastases occurrence in ADC. Further EMT characterization in lung cancer will provide better understanding of tumor progression and metastasis. (UT Lung Cancer SPORE P50CA70907)

P-12

Basaloid Carcinoma of the lung: a histologic and immunohistochemical study of 8 cases.

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Background: Basaloid carcinoma (BC) and large-cell neuroendocrine carcinoma (LCNEC) are 2 recently recognized variants of large-cell lung carcinomas that may overlap in their morphology, and are discriminated by expression of neuroendocrine markers and TTF-1 in LCNEC, and CK34βE12 in BC.

Method: BC was classified according to the 2004 WHO classification as variant of non-small cell lung carcinoma. Immunohistochemistry was performed using antibodies against the following markers: TTF-1, CK34βE12, CK 5/6, synaptophysin, chromogranin, CD10, p63 and C-Kit (CD117).

Results: 8 men had a mean age of 70 (range 59–77 years). The histopathological features were similar in all cases with

a lobular growth pattern of small cells with moderately hyperchromatic nuclei, with no prominent nucleoli, and with scant cytoplasm, a high mitotic rate, and peripheral palisading. All cases were negative for TTF-1, chromogranin and synaptophysin. CK34βE12 was diffusely expressed in 3 cases and focal in 5. CK 5/6 was stained focally in 5 cases and negative in 3. CD10 was strongly expressed in 6 cases, focal in 1 and negative in 1. p63 nuclear staining was positive in 3 cases and negative in 5. C-Kit was stained in variable intensity and distribution in 7 cases and negative in 1. **Conclusions:** These data show that TTF-1 and CK34βE12, in association with specific neuroendocrine markers and CD10, represent a useful panel of antibodies in differentiating carcinomas presenting with a solid pattern, palisading, or pseudorosettes. The morphologic and immunohistochemical phenotype suggest that BC is derived from a basal bronchial epithelial stem cell.

P-13

HER-2/neu in Squamous Cell Lung Cancer

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BACKGROUND: The present study was delineated to know the status of *HER-2/neu* gene in 60 cases of squamous cell carcinoma of the lung. Either immunohistochemical protein expression and *HER-2/neu* gene and chromosome 17 copy number by fluorescent *in situ* hybridization were characterized. The cases were collected from surgical specimens in stages IIB/IIIA of squamous cell carcinoma.

METHOD: Tissue microarrays were constructed from paraffin-embedded tissue tumors after selection of representative areas from each specimen. Immunohistochemistry for HER-2/*neu* protein expression was verified with polyclonal rabbit anti-human c-erbB-2 oncoprotein. Fluorescence *in situ* hybridization was also performed in tissue microarrays to measure HER-2/neu gene copy number and its the amplification status by applying the probe LSI Her-2/neu/CEP17 (Kreatech, Biotechnology).

RESULTS: It was not verified positivity for protein expression of HER-2 in all cases. None of the 60 squamous cell carcinoma of the lung assessable by FISH exhibited HER-2/neu gene amplification as defined by HER-2/neu/chromosome 17 ratio = or >2. High polysomy for chromosome 17 with increase in HER-2/neu gene copy number was determined in 27 cases. No statistically significant association was observed between absolute chromosome 17 or HER-2/neu and protein expression levels.

CONCLUSION: Gene amplification and protein expression of HER-2 in squamous cell carcinoma in this series suggest to be an infrequent event. High HER-2 gene copy number present in this study reforces the value of HER-2 as a member of EGFR/HER family of receptors and an important partner of EGFR heterodimeration, influencing the response to tirosine kinase inhibitors. Our results are a complement to the published studies to understand the status of either HER-2 protein expression and its gene amplification in a series of squamous cell carcinoma in surgical stages, independently of the presence of lymph node metastasis. It may be understood as a basic study, to correlate with those where patients have been submitted to tyrosine kinase inhibitors in advanced stages after determination of squamous differentiation, as in general, the published data is enlarged to non-small-cell carcinoma, without clear specification of histological patterns.

P-14

Intrathoracic splenosis mimicking intrathoracic neoplasm: a case report.

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Background: Splenosis is a rare condition described as ectopic splenic tissue implantation generally after a splenic rupture. Most of the reported cases are intraabdominal; thoracic splenosis is even much rarer and is typically a result of thoraco-abdominal trauma. It is usually an incidental finding. Hematopoiesis associated with splenosis was rarely reported in the literature. To our knowledge, recurrence of the disease was not yet reported.

Method: We report a case of a thoracic splenosis in a 32 year-old woman and we discuss the clinicapathologic features of this entity.

Results: Our patient had a history of a mini-thoracotomy secondary to a basi-thoracic pain and a right pleural effusion. A multinodular tumor inside the visceral pleura was excised and the resected tumor was diagnosed histologically as an inflammatory pseudotumor. Nine years later, the patient presented with a recurrence of her pleural effusion. Thoracic computed tomography showed a solid and necrotic tumor at the level of the right lobe. During the intra operative observation, a dark colored, soft, multinodular lesion inside the visceral pleura over an area of 6× 3 cm in dimension was observed. The lesion was excised. The histopathological examination of the specimen showed

splenic tissu in the pleura with hematpoiesis, so the lesion was accepted as splenosis and the diagnosis was redressed. Conclusion: Our report is particular by the intrathoracic localization of the splenosis, its association with hematopoiesis and its recurrence after surgical resection.

P-15

Congenital cystic adenomatoid malformation presenting in adulthood in association with adenocarcinoma

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Background: Congenital cystic adenomatoid malformation (CCAM) is a rare pulmonary malformation and refers to a disorganized lung tissue with different degrees of cystic changes. Stocker described five distinct types according to the size of the cysts and the microscopic appearance. It mostly affects newborn infants, but some lesions may be delayed until school age. The adulthood presentation is reported in fewer than 40 cases. Malignoma arising in CCAM is a very rare event.
Methods: A 33-year-old male, non-smoker, presented with chronic productive cough, dyspnoea, left thoracic pain and haemoptysis. He was complaining about recurrent episodes of purulent sputum and fever, diagnosed three times as having bronchopneumonia in the last year. X-ray examination revealed opacity in the left lower lobe, while additional CT scan confirms a large area of consolidation with cystic lesions. The patient underwent left lower lobectomy after suspicion of CCAM.
Results: Macroscopically, solid and mucoid surface areas were demonstrated with multiple cysts, measuring from 0.5–3 cm. Histologically, cystic bronchiolar-like structures were lined by pseudostratified columnar epithelium intermixed with mucogenic cells that exhibited a lepidic growth pattern in adjacent alveoli, characterizing a bronchioloalveolar carcinoma (BAC). Focally, some areas showed greater cellular atypias with invasion of the underlying stroma. However, there was no invasion into pleura or lymph nodes. Based on light microscopy morphology, the diagnosis of CCAM type I in association with BAC and foci of adenocarcinoma was established. After 2-year-follow-up there is no progression of malignancy.
Conclusions: The occurence of CCAM with rhabdomyosarcoma or BAC is rarely documented. We presented the twenty-first case of late-onset CCAM associated with BAC. Because BAC is seen in type I CCAM, mucinous cells could play a neoplastic precursor role. Since CCAMs poses risk of malignant transformation, many advocate early surgical resection, even in asymptomatic patients.

P-16

Immunohistochemical detection of Simian Virus 40 (SV40) in 30 mesothelioma and 97 sarcoma

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Introduction: SV40 has been associated to human neoplasia, as mesothelioma, brain tumors and lymphoma. The viral protein *Tag* is thought to inactivate host p53. These data have been subject of controversies, as some authors did not find evidences to support relationship between this virus and human neoplasia. In our country, there have been no studies addressing this issue.
Objective: To contribute in the study of the oncogenic potential of SV40 by immunodetection of *Tag* in cases of mesotelioma and sarcoma, diagnosed at our laboratory.
Material and Methods: Thirty mesothelioma and 97 sarcoma had their formalin fixed, paraffin embedded tissue arranged in two tissue microarrays and submitted to immunohistochemical detection of SV40 (monoclonal antibody Ab-2, clone Pab416, Oncogene Research Products, San Diego, CA, USA). Positive cases were graded as 1 = up to 30% positive nuclei; 2=30–70%+; 3 = more than 70% +.
Results: Ten mesothelioma were non reactive for SV40 (33.3%), 4 were grade 1 (13.3%), 6 grade 2 (20%) and 6 grade 3 (20%). In 4 cases evaluation was not possible (13.3%). None of the 97 sarcoma showed any reactivity.
Discussion/Conclusion: Although some authors criticize immunohistochemistry as a method not sufficiently sensitive and specific for some purposes, it is noticeable that 53.4% of our mesothelioma showed immunoreactivity for SV40, or 61.5% of the valid cases (data from the literature using PCR=40–85%). Sarcomas were all negative. Our data furnish further support to a pathogenetic role of SV40 in mesothelioma. Divergence among studies might be due to geographic variation. (FAS and JV are supported by the National Council for Scientific Research, CNPq).

P-17

Mesothelial hyperplasias of the pleura

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Background. Specimens from patients presenting with pleural effusion are usually obtained to rule out malignancy. The separation of benign from malignant mesothelial proliferations remains one of the most difficult

problems in the pathology of the serosal membranes. Inconclusive cases may be termed either Reactive Mesothelial Hyperplasia (RMH) or Atypical Mesothelial Hyperplasia (AMH), since it is far better than overdiagnosing Malignant Mesothelioma (MM). True stromal invasion seems to be the most accurate indicator of malignancy. Clinical, radiological and macroscopic data are relevant. Cytologic atypia and immunohistochemistry are often not helpful.

Method. We looked for patints with noninfectious pleural effusion and a diagnosis of mesothelial hyperplasia, during a nine year period, and studied their characteristics and clinical outcome. Those who finally had pleural metastasis were excluded.

Results. 36 cases were included. Mean age was 62 years. 20 were men. 31 had unilateral pleural effusion. In 30 patients the initial daignostic procedure was a thoracocenthesis. The first cytology report was: benign 11, acute inflammation 7, chronic inflammation 6, RMH 6, AMH 1, MM 2 and other malignancy 3. Final diagnosis was done with open pleural biopsy in 17 cases, thoracoscopy in 10, thoracocenthesis in 3 and tru-cut biopsy in 3. It was: RMH in 13 cases, MM in 11, AMH in 7, nonspecific chronic pleuritis in 2 and inconclusive in 3. The mortality rate was of 61%; 82% in patients with MM.

Conclusion. In patients with recurrent pleural exudates and a pathologic diagnosis of RMH, invasive diagnostic procedures are recommended, along with clinical and radiological follow up, to rule out MM.

P-18
EPITHELIAL-MYOEPITHELIAL TUMOUR: AN INFREQUENT FORM OF SALIVARY GLAND-TYPE PULMONARY NEOPLASIA

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BACKGROUND Tracheobronchial submucosal glands can be considered as pulmonary counterpart of minor salivary glands and, therefore, they can develop most of the tumours originated by them. However, in spite of the fact that these glands are widely distributed along tracheobronchial tree, salivary gland-type pulmonary tumours are rare.

METHODS We present the case of a 76 year-old female, with no significant personal history, to whom a pulmonary nodule is detected while studying unknown origin neutropenia. Upper right lobectomy is performed.

RESULTS After gross and microscopic examination and immunohistochemical profile, a diagnose of epithelial-myoepithelial tumour is made.

COMMENTS Salivary gland-type pulmonary tumours are rare. Among them, mucoepidermoid carcinoma and adenoid cystic carcinoma are the most frequent, whereas mixed neoplasias with epithelial and myoepithelial components are extraordinarily infrequent. In the lung, they are considered as low malignant potential tumours with capacity for local recurrence and less frequently for metastasize. No case with vascular, lymphatic or perineural invasion, neither with distant mestastases.

It has been suggested that myoepithelial cells play an important role in this type of tumours, based on a different immunostain pattern for p27/kip-1 between both neoplastic components. An aberrant subcelular location of this protein into myoepithelial cells would provoke loose of its growth inhibition function and would contribute to tumorigenesis because of a lack of restriction of myoepithelial component proliferation.

P-19
THE VEGF-SIGNALLING PATHWAY IN VASCULAR AND PERIVASCULAR TUMORS OF THE LUNG

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Background: Vascular Tumors (such as Haemangiopericytomas, Epitheloid Haemangioendotheliomas and Angiosarcomas) of the lung are very rare lesions. Due to their rare incidence no standardized therapy regimen is established. So far surgery is the only option of treatment. Especially in Haemangioendotheliomas, which can present as a diffuse or multifocal lesion, surgical resection is sometimes incomplete. A blockade of vascular endothelial growth factor receptors (VEGFR2 and VEGFR3) either by antibodies for their ligands or by kinase inhibitors have been increasingly used for the therapy of solid tumors.

Method: We investigated several factors of the VEGF-signalling pathway in two Haemangiopericytomas, seven

Epitheloid Haemangioendotheliomas and eleven Angiosarcomas by means of immunohistochemistry using commercially available antibodies for VEGFA, VEGFB, VEGFC, VEGFD, VEGFR2, VEGFR3 and endothelium specific kinase Tie2.
Results: The tumor cells of the haemangiopericytomas, epitheloid haemangioendotheliomas, and angiosarcomas reacted positively for Tie2, either VEGFR2 and/or VEGFR3. They showed a diverse reaction pattern for their ligands, revealing VEGFA and VEGFB the most predominant. In general, the tumor cells of the angiosarcomas showed a stronger positivity for the ligands.
Conclusion: The observed positivity for the factors of the VEGF-signalling pathway points towards a possible new therapeutic option by inhibiting VEGFR2 and VEGFR3.

P-20

EGFR EXPRESSION AND GENE AMPLIFICATION IN NON-SMALL CELL LUNG CARCINOMAS: CORRELATION BETWEEN GENE COPY NUMBER AND PROTEIN EXPRESSION

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Progress in lung cancer biology led to the identification of the epidermal growth factor receptor (EGFR) signaling pathway as a therapeutic target leading to the development of tyrosine kinase inhibitors (TKIs). We determined EGFR protein overexpression by immunohistochemical analysis and EGFR gene amplification by fluorescence in situ hybridization (FISH) in 132 formalin-fixed, paraffin-embedded lung tumors including squamous cell carcinoma (SCC; 68 patients), adenocarcinoma (ADC; 50 patients) and other non-small cell carcinoma (14 patients). Protein expression was assessed by immunohistochemistry and gene copy number was evaluated by fluorescent in situ hybridization (FISH). EGFR protein overexpression was observed in 67% of the NSCLC, more frequently in SCC than ADC (75% vs 44% $p<.001$). There were no statistical diffrences in EGFR expression with respect to sex, smoking history, BAC feature, nodal status or stage. EGFR FISH-positivity as represented by high polysomy and gene amplification was observed in 33.2% of the NSCLC patients. The FISH patterns were disomy (29.6%) and low polysomy (37.2%), high polysomy (21.9%) and gene amplification (11.4%). Protein expression levels significantly correlated with the gene copy number per tumor cell ($p=0.000$). The proportions of EGFR-expressing tumors according to the gene copy status were: 90% of gene amplified cases, 75% of high polysomy, 40% of low polysomy, and 25% of disomy cases. EGFR overexpression or high gene copy numbers had no significant influence on tumor recurrence. EGFR overexpression is frequent in NSCLC, is most prominent in SCC, and correlates with increased gene copy number per cell. Additional studies are needed to determine whether EGFR gene amplification or protein expression bears any informative value in predicting response to EGFR inhibitor therapy.

P-21

FOLLICULAR BRONCHIOLITIS. A REVIEW OF 11 CASES.

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BACKGROUND: Follicular bronchiolitis (FB) is a histopathologic finding that occurs in diverse clinical contexts such as hypersensitivity reactions, immunodeficiency, toxic exposure and collagen diseases. Is characterized by bronchiolar lymphoid tissue with germinal follicles. The current study was conducted to characterize histopathological features, and clinical-radiologic correlation.
METHODS: From 352 lung biopsies between 1999 and 2007, 11 cases (3.1%) of FB were diagnosed. Medical synthoms, radiologic and morphological findings were recorded.
RESULTS: The patients included 5 men and 6 women; the median age at diagnosis was 47 years (range, 15–73). Main symptoms were repeated respiratory infections and variable degree of dyspnea. Some patients had underlying systemic deseases that included: In one patient FB was associated with advanced AIDS, other 3 patients had Sjögren's Syndrome (one of them also with Systemic Lupus Eritematous, SLE), other an undifferentiated connective tissue disease and another with prolonged exposure to polyethylene-flock. CT findings included reticular and ground-glass opacities and small nodules. FB was the major histopathology pattern in 8 patients, associated secondary with nonspecific interstitial pneumonia, organizing pneumonia, and isolated cases of bronchiolitis obliterans, non epitelioid granulomas and interstitial fibrosis with anthracosis and silice (using polarized light), all of them associated to the FB. The immunochemical stain for lyfocites B and T was positive in all cases using CD20 and CD3. Clinical course was characterized by relative stability with partial response to steroid or anti CD4 monoclonal antibody therapy, but in one case the progressive worsening caused the death.
CONCLUSIONS: FB is a non very unusual pathology found associated with other diseases. The major lesion of FB is the lymphoid follicles around distal airways. The clinical course and prognosis for most patients is relatively good, but progressive lung disease is possible.

P-22

Pseudoangiosarcomatous carcinoma of the lung: a clinicopathologic study of four cases.

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Background: Pulmonary pseudoangiosarcomatous carcinoma (PPAC) is a rare type of lung cancer, defined by the presence of infiltrating, anastomosing channels that resemble vascular spaces. The 1999 WHO classification places it into the pleomorphic carcinoma (PC) category. A putative different prognosis of PPAC within the PC group has not been studied yet.

Methods: Four cases of resected PPAC and four cases of PC were studied. Immunohistochemical stains against cytokeratin 7(CK7), cytokeratin 5/6 (CK5/6), and TTF-1 were performed. Stains against CD31, CD34 and factor VIII-related antigen (FVIII) were made in PPAC cases, in order to rule out a true pulmonary angiosarcoma. Clinicopathologic comparison was undertaken between both groups. Survival curves were evaluated by the Kaplan-Meier method, and satistical signification estimated with the log-rank test.

Results: Patients with PPAC (mean age=70 years)had a mean tumor diameter of 11.3 cm. All cases were positive for CK7, at least focally; TTF-1 was positive in one case; CD31, CD34 and FVIII were negative. Three patients were in pathologic stage IIIa (two of them died at 0.7 and 5.8 months, one is alive with disease progression at 3 months) and one in stage IIb (alive and well at 4 months). Patients with PC (mean age=58.7 years) had a mean tumor diameter of 4.05 cm. All cases showed a weak positivity for CK7; TTF-1 was positive in one case. Two patients were in stage IIb, one in stage IIIa, and one in stage IV (all alive with disease progression, with a mean follow up of 16.5 months). No significant differences were observed between both groups in terms of survival.

Conclusions: Clinical data suggest an aggressive behavior of PPAC. However, the limited number of cases analyzed does not permit to establish this histologic subtype as a predictor of survival within the PC group.

P-23

RECURRENT PRIMARY LUNG LIPOSARCOMA (A CASE REPORT)

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Background: Liposarcoma is one of the most common malignant soft tissue tumors in adults and the most frequent primary sites are the extremities and retroperitoneum. Intrathoracic origin is unusual, and most of those develop in the mediastinum. Primary pulmonary liposarcoma has been reported in only a limited number of cases worldwide. The clinical behavior and prognosis seem to correlate with the histologic classification of the tumor. The extend of surgical resection is one of the most important prognostic factor. Local recurrence may occur if the tumor is not completely excised. Metastasis to various structures including lung, pleura, liver, mediastinal lymph nodes and bone are described, specially in the poorly differentiated varieties.

Case Report: There was 10 cm in diamater mass, in the patient's chest x-ray whose applied to our hospital with the complaints of chest pain and dispnea. A mass which was thought to be recurrent, surgically resected and submitted to our pathology department. Surgical materials were couple of tissue pieces which 5×4×3 cm in diameters is the largest and have yellow, shiny and diffuse appearance in their cut surfaces. Microscopically, the tumour consisted of proliferating lipoblasts with considerable nuclear pleomorfism in a stroma containing a rich capillary network. The case was diagnosed as liposarcoma after histopathological and immunohistochemical analysis. Patients previous history revealed that she had been operated and diagnosed as liposarcoma of the right lung in another institution and put in to postoperative chemoteraphy programme. In order to exclude the possibility metastasis from primary tumor, whole body scan performed but there was no one, and this tumor is accepted as the primary.

Conclusion: Primary pulmonary liposarcoma has been reported in only limited number of cases worldwide. Although it is extremely rare, primary pulmonary liposarcoma should be included in the differential diagnosis of lung tumors.

P-24

Amiodarone induces pulmonary toxicity and fibrosis via phospholipidosis mediated apoptosis

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Background Amiodarone (AMD) is a potent anti-arrhythmic drug frequently used for treating ventricular and supraventricular arrhythmias. The use of AMD is limited by its toxic side effects, the most severe of which is the Amiodarone-Induced Pulmonary Toxicity (AIPT). The pathology of AIPT, which includes alveolitis, phospholipidosis, and irreversible fibrosis, is well described. However, the mechanism underlying the involvement of AMD to the pathogenic molecular machinery of the disease remains unclear.

Apoptosis of alveolar epithelial cells appear to play an important role in AIPT. It is known that an individual stimulus may initiate apoptosis; however, the pathways transducing these signals are often regulated by other cellular events such as phospholipidosis. In the present study we investigate the involvement of AMD induced phospholipidosis to the triggering and regulation of apoptosis.

Method The immunohistochemical expression of phospholipase C gamma-1 (PLC-γ1), activated-caspase-3, activated-caspase-8, Bcl-2, Survivin, and c-Flip, was studied in 33 samples of pulmonary tissue from Wistar rats after oral administration of AMD for 14 days at the minimum therapeutic dose (30 mg/kg/day). Apoptosis was detected by the TUNEL method and the apoptotic index (AI) was calculated.

Results Our results indicated that administration of AMD was correlated with increased apoptosis, increased expression of activated caspases-3 and -8, and decreased expression of PLC-γ1, Bcl-2, Survivin and c-Flip. The expression of pro-apoptotic proteins activated caspase-3 and -8 showed significant positive correlation with the AI, while the anti-apoptotic molecule Bcl-2 was positively correlated with the inhibitors of apoptosis Survivin and c-Flip. Activated caspase-8 exhibit significant negative correlation with c-Flip and Bcl-2. Finally, the expression of PLC-γ1 was positively correlated with Bcl-2 and Survivin.

Conclusion Our data indicate that administration of AMD enhances apoptosis of alveolar epithelial cells mediated by inhibition of PLC-γ1. Activated caspases-3 and -8 seem to act as the effector molecules of apoptosis in AIPT.

P-25

Diagnostic of mesothelioma in routine practice

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Pathological confirmation, either cytological and or histological diagnosis, is recommended in any patient in whom mesothelioma is suspected. The aim of study was to analyse our routine diagnostic practice in mesothelioma patients.

We retrospectively reviewed all patients with pathological diagnosis of mesothelioma in the last ten years. Pathological diagnosis was obtained from pleural effusion fluid cytology and or histhology of various pleural specimens (blind or guided needle biopsy, medical or surgical thoracoscopic biopsy, pleurectomy). Cytospins were done out of pleural effusion samples, further on stained by MGG and Papanicolaou. Immunocytochemistry was done on cytospins and on cell blocks. Tissue specimens were formalin fixed and paraffin embedded. Slides were HE stained and sometimes additional stainings were done, AB and PAS with diastase pretreatment. Immunohistochemistry was performed using different antibodies. Histopathological classification into three main groups was done according to WHO.

In ten years period 183 cases of mesothelioma were diagonosed in our lab. There were 145 cases diagnosed from pleural effusion fluid cytology and 123 cases had histological diagnosis from tissue specimens (91 needle biopsies, 64 medical thoracoscopic biopsies, 25 surgical biopsies and 4 pleural resected specimens). There were 99 cases with both cytological and histological diagnosis of mesothelioma. Epithelioid type was the most predominant one, biphasic was diagnosed in larger histological specimens and there was no case of cytologicaly diagnosed sarcomatoid type. Ten years ago we used pankeratin, vimentin, CEA along with histochemistry (AB and PAS with diastase). Then came period of testing various mesothelioma markers (thrombomodulin, WT-1, CD15, HBME-1, mesothelin). In the last years a panel of four antibodies was applied strictly (BerEP4, MOC31, CK5/6 and caretinin).

Our routine diagnostic practice for mesothelioma disclosed high percentage of cases being diagnosed from cytology, while almost one half of cases were confirmed cytologicaly and histologicaly. Immuncyto- and immunohistochemistry revealed three different panels of antibodies used through ten years period.

P-26

Bronchocentric granulomatosis associated with pulmonary hidatidosis

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Background Bronchocentric granulomatosis (BG) is a non-specific necrotizing granulomatousreaction surrounding the peripheral conducting airways. In asthmatic patients, BG is considered a hypersensitivity reaction to intrabronchial fungi. However, non-asthmatic patients may develop BG without signs for endobronchial fungal infections, but probably as a consequence of other pulmonary conditions.

Method & Results We report the case of a 31-year-old woman, previously healthy, referred to our hospital because of an intermittent cough and hemoptysis. Radiographs and CT scan of the chest disclosed 2 cystic masses in the right lobe with typical appearance of hydatic cysts. A surgical resection of the two cysts was done. Pathological examination of the resected specimens confirmed the diagnosis. Histology revealed anassociated bronchocentric granulomatosis with foreign-body giant cells containing fragments of hydatic membrane and scolex.

Conclusion BG associated with hydatidosis is exceptionally rare. To our knowledge, this is the fourth case reported. Although the aetiology of BG has not been fully elucidated, the current pathogenetic mechanism is considered to be an immunologic reaction against endobronchial antigens.

P-27

Steroid hormone receptors in non small cell lung carcinomas

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Background Steroid hormone receptors have been studied in non-small cell lung carcinomas (NSCLC) in the perspective of gender-associated differences in tumour types and prognosis. During recent years receptor subtypes for both oestrogen (ERα/ERβ) and progesterone (PRA/PRB) have been identified and conflicting results concerning their expression have been reported.

Methods A consecutive material of 270 NSCLC surgically treated at Örebro University hospital between 1990 and 1995 has been studied. The tumours consisted of 126 squamous cell carcinomas (SCC), 140 adenocarcinomas (ADCA), 3 adenosquamous carcinomas and one large cell carcinoma. 172 of the patients were males and 98 females. Immunohistochemistry was performed, including heat epitope antigen retrieval, with monoclonal antibodies to ERα, ERβ, PRA and PRB using Envision® visualization procedure. Nuclear positivity was scored positive when >10% of tumours cells were stained.

Results ERα expression was focally present in 4 adenocarcinomas.

ERβ was present in 63 tumours. ERβ expression was more frequent in SCC, 29%, then in adenocarcinomas, 19%, p= 0.04 (Pearson chi^2-test).

The differences between histological types could be seen in the male group (p 0.03) while it failed to reach statistical significance amongst women. Overall, female tumours were ERβ positive in 28% whilst male tumours in 21%, (ns, chi^2-test). There was no difference concerning mean age between ERβ groups in either gender.

None of the tumours expressed PRA or PRB.

Discussion Taking our and previously published data into consideration, discrepancies in ERα expressions seems to be antibody dependent. Our data supports that only a very small fraction of NSCLC expresses ERα. The absence of PR expression is in concordance with the absence of functional ERα signalling.

ERβ expression occurs frequently with a slight predominance in SCC compared to ADCA, without influence of gender. Whether ERβ mediated effects occurs in NSCLC remains to be studied.

P-28 WITHDRAWN

P-29

VEGF ROLE IN THE SPECIFIC PATTERN OF PULMONARY DAMAGE INDUCED BY MTOR INHIBITION WITH HIGH BLOOD LEVELS IN A RAT MODEL.

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Background: Pulmonary toxicity is an infrequent, but possibly severe side effect of mTOR inhibitor treatment. Its pathophysiology remains unknown so far.

Methods: 8 Wistar rats were treated with sirolimus with three weekly intraperitoneal injections of 1 mg/kg BW each

for three months (SRL group), and 8 rats were treated with vehicle (VEH group). After three months the animals were sacrificed for analysis. Pulmonary tissue was put in 3.5% formalin and frozen in OTC for further analysis. HE, PAS and oil red stains were performed. The histological features were quantified by a blinded lung pathologist according to severity on a scale of 0 to 3 (0=none, 1=low, 2=moderate, 3=severe). Immunohistochemical VEGF staining was quantified according to the same intensity scale.
Results: Whole blood trough concentrations after three months were 39 ng/mL. The lungs of SRL-treated rats showed a marked perivascular infiltrate of small-sized vessels (1.4±0.7 vs. 0.25±0.5; $p<0.01$). Furthermore, lungs of SRL-treated animals showed segments with marked accumulation of intra-alveolar macrophages and material (macrophages SRL vs. VEH 2.1±0.6 vs. 0±0, $p<0.01$; intra-alveolar material SRL vs. VEH 2.0±0.8 vs. 0±0, $p<0.01$). This material was intracellular, parts of it being PAS-positive and other parts oil red positive. The lungs of SRL-treated rats showed less intense staining for VEGF in vessels and interstitial.
Conclusion: In an experimental model exposure to high-dose SRL treatment leads to a histological pulmonary characterized by perivascular infiltrate and intra-alveolar macrophages and proteinaceous and lipidic material, which is associated with less intense VEGF staining.

Haematopathology

P-30
Primary Cutaneous Small-Medium CD4+ T-Cell Lymphomas. A Heterogeneous Group of Tumors with Different Clinicopathologic Features and Outcome

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Purpose: To define the clinical and pathologic characteristics of primary cutaneous small/medium CD4+ T-cell lymphoma (PCSM-TCL) and identify parameters of prognostic significance.
Patients and Methods: We have investigated 24 patients with primary cutaneous lymphomas composed of small/medium mature T-cells with a βF1, CD3, CD4+ and/or non cytotoxic, CD8- and CD30- phenotype. The proliferation index and CD8+ infiltrating cells were quantified with an automated image analysis system.
Results: Sixteen patients presenting with solitary or localized plaques or small nodules (<3 cm) had an indolent course. Only three patients experienced repeated cutaneous relapses and none of them died of the disease after 1–168 months (median 17 months) of follow-up. The tumors had a low proliferation (median Ki-67, 9% ±5) and an intense infiltrate of reactive CD8+ (median, 20% ± 11.7). Five patients presenting with rapidly evolving large tumors or nodules (≥5 cm) had an aggressive disease and died with extracutaneous dissemination 18–36 months after diagnosis (median 23 months). These tumors had a significantly higher proliferation (median Ki-67, 22% ±11.3) ($p<0.05$), and lower number of infiltrating CD8+ (median, 1% ±3) ($p<0.05$) than the previous group. A third group of three patients had a peculiar clinical presentation with multifocal relapsing lesions without extracutaneous dissemination after a long period of follow-up ranging from 41 to 92 months. Histologically these cases had an intense infiltrate of eosinophils.
Conclusion: PCSM-TCL is a heterogeneous group of tumors with differentiated clinical and pathological characteristics with impact in the outcome of the patients.

P-31 WITHDRAWN

P-32
Peripheral cutaneous T-cell lymphoma, unspecified, rich in giant cells: a case report

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Background: Peripheral unspecified T lymphoma are T lymphocyte neoplasias with innumerous morphological subtypes corresponding to distinct clinical aspects, renown for their difficulty for inclusion in specific entities. The report highlights a rare association of this entity with granulomatous reaction in multinucleated giant cells, which could obscure the main diagnosis. Case report: A 28 year-old Caucasian male presented infiltrated erythematous violaceous lesions, 20 cm in diameter on the right thigh and gluteus for 3 years. Examination revealed a right groin lymphadenomegaly measuring 2 cm in diameter, negative for B symptoms. The initial clinical hypothesis was non-Hodgkin lymphoma. Results: Microscopy revealed an infiltrated lymphoid neoplasia occupying surface and deep dermis with destruction of adjoining tissues, constituted by small lymphocytes. Immunohistochemical studies were

positive for CD3, CD4 in the lymphoid cells and CD68 and lisozime in the giant cells. T cell clonality proved positive by the genotyping method; BAAR and fungi tests were negative. The final diagnosis was peripheral T-cell lymphoma unspecified rich in giant cells. The CHOMP scheme was indicated. Conclusion: Peripheral T lymphoma is an entity that includes innumerous morphological aspects, though its association with granulomatous reaction rich in foreign-body type giant cells is rare. Presence of such cells can occur in other non-Hodgkin lymphomas like B lymphomas, angioimmunoblastic T lymphomas and classic Hodgkin lymphoma. The presence of giant cells could lead to an inconsistent diagnosis of infectious disease, requiring the exclusion of specific agents using special stains and confirmation of lymphoproliferative disease thorough auxiliary studies.

P-33

An unusual presacral mass in a thalssemic patient

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Background: Extramedullary hematopoiesis occurs as a compensatory mechanism for bone marrow dysfunction in chronic hemolytic anemias including thalassemia. It most often involves the liver, spleen and lymph nodes. The presacral area is an unusual site of extramedullary hematopoiesis as only 21 cases have been reported in literature.

Case report: in this paper, the authors describe a new case of a symptomatic presacral extramedullary hematopoiesis in a 53-year-old man with thalassemia intermedia. Diagnosis was suggested by the association of imaging findings and knowledge of the clinical context. Histopathological examination of a CT-guided core biopsy and fine needle aspiration of the mass established the diagnosis of extramedullary hematopoiesis.

Conclusion: We conclude that CT-guided biopsy and fine needle aspiration are efficient means to obtain final diagnosis of atypical extramedullary hematopoiesis localizations.

P-34

Immunohistochemical expression and methylation status of MGMT in diffuse large B-cells lymphomas

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BACKGROUND: Loss of O6-methylguanine-DNA-methyltransferase (MGMT) expression and presence of MGMT promoter methylation have been both proposed as favourable prognostic markers in diffuse large B-cell lymphomas (DLBCLs). However, there are very few studies which evaluate the relationship between the lack of immunoreactivity (IR) for MGMT and the methylation status of its promoter.

MATERIALS AND METHODS: We studied 74 patients with primary nodal DLBCL, all treated with cyclophosphamide-containing regimens and with available follow-up information. MGMT-IR was detected using a specific monoclonal antibody (Chemicon International Inc, Temecula, CA) and Ultravision Amplification System. The immunohistochemical results were evaluated in a semiquantitative way, using a four-titered score: 0 (no expression), L (Low, IR in 5–25% of neoplastic cells), I (Intermediate, IR in 20–50%), H (High, IR in more than 50%). In addition, the metilation status of MGMT promoter was analyzed in a subset of cases using a quantitative method based on Real Time methylation-specific PCR (Lo et al., 1999). For each sample we determined a methylation index (MI) and we considered cases with MI $\geq$20 as methylated.

RESULTS: At immunohistochemical analysis18 cases scored 0, 10 scored L, 16 scored I and 29 scored H. We considered all cases with score 0 or L as lacking MGMT expression (28 out of 74 cases, 38%), and all of them were studied for MGMT promoter metilation. We also analyzed the metilation status of 20 cases with score I (10 cases) or H (10 cases). The results of metilation analysis showed that all but 1 cases with immunohistochemical (IHC) score of I or H were unmetilated, while, only 52% of cases with IHC score of 0 or L (15 out of 28 cases), with no difference between the two groups, showed MGMT promoter metilation. In addition, the survival analysis showed significantly longer overall survival and lymphoma-free survival in DLBCLs with MGMT promoter metilation compared with unmetilated cases, while the difference was not statistically significant when the cases with IHC score of 0/L and I/H were compared.

CONCLUSIONS: These data suggest that the MGMT promoter metilation status, and not the loss of protein expression, is a favourable prognostic factor in primary

nodal DLBCLs treated with cyclophosphamide-containing regimens. On the other hand, immunohistochemistry seems to be an useful tool to identify unmethylated cases, while a methylation analysis should be performed when there is no or low expression of MGMT.

P-35

The evaluation of relation between staging of Hodgkin disease

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Hodgkin lymphoma is an uncommon cancer of the lymphatic system, mostly of the young. The staging system of the disease has been effectively used for treatment decision making. lt has recently been proposed that some serologic factors such as Lactate Dehydrogenase(LDH), Erythrocyte sedimentation rate (ESR)and C-reactive protein (CRP)are useful markers. In this study we aimed at making out the the evaluation of relation between staging of Hodgkin disease and serum levels of ESR, CRP and LDH of the known cases of Hodgkin.
Methods and Materials: Known cases were assessed for age, sex, LDH, ESR, CRP, tumor histologic type and stage, in hematological ward of Farshchian hospital, Hamedan provience, during a three year period. The data were analyzed by SPSS version.10
Results: The mean age was 32.84 years. 52% were male and 48% were female. Regarding the types we found 42% of Mixed cellularity, 40% of Nodular Sclerosis, 18% of Lymphocyte Prodominace. Stage of Them was 32%,34%, 7%, 16% respectively.
The erythrocyte sedimentation rate (ESR) was over 30 in 34% of patients. CRP was identified in 30% of all samples. LDH was over 32 .in 24% of patients
There was a significantly relationship between ESR over 30 and stage of diseases ($p=0.001$) and age over 45 years of patients ($p=O.OOO$). we also found a significant relationship between LDH over 220 and stage of diseases ($p< 0.05$). The relationship between ESR over 30 and sex and tumor type were not significant ($p>0.05$). The relationship between CRP and age, sex, tumor type and stage were not significant ($p>0.05$). Also we could not find any significant relationship between. LDH and age, sex and tumor type ($p>0.05$)
Conclusion: This study found a significant relationship between serum levels of ESR and stage of diseases and age over 45 years of patients. Also a significant relation between LDH over 220 and stage of Hodgkin disease.

P-36

Correlation between human herpesvirus-8 with multiple myeloma

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Introduction: Multiple myeloma is a plasma cell neoplasm characterized by involvement of the skeleton at multiple sites.some viruses such as HHV-8 implicated in pathogenesis of disease but this role is not clear.
The goal of our study was to show direct evidence of HHV-8 in multiple myeloma patients with use of immunohistochemical technique.
Materials and Methods: 30 cases of Multiple myeloma and equal number of normal bone marrow tissue were selected and Immunohistochemstry was performed with HHV-8 marker. slides were examined for immunoreactivity in cells nuclei and positive or negative results were reported.The data studied and analysed with pearson chi-square test,also 2×2 contingency tables and Fischer's exact test by specialist of statistics and Differences with p-value under 0.05 ($P\leq0.05$) assumed valuable.
Results: 73.3% of patients was male and 26.7% was females. The age ranged was 58.70 ± 13.52 for mean ± standard deviation. Fisher exact test showed no difference between two group of case and control ($P=0.11$) for HHV-8 Immunoreactivity and positive staining in MM was seen in 4 cases (13.3%) and zero (0%) in control group.
Conclusions: Our finding showed that HHV8 infection did not seem influence on pathogenesis of multiple myeloma.
Key words: herpesvirus - multiple myeloma-Immunohistochemistry.

P-37

Association between ABO and Rhesus blood group systems among confirmed Human T Lymphotropic Virus-1-infected patients in Northeast Iran

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Background:The distribution of ABO and Rhesus blood group types was investigated in 984 randomely selected Human T Lymphotropic Virus-1(HTLV-1) infected blood donor from April 2004 to March 2007.One thousand and eighty one healthy controls who admitted for blood donation in this period,were enrolled in this study.
Study design: Infected and control individuals were from the same region and their ABO/Rhesus blood group

systems were determined by the standard tube test technique.
All blood samples were screened for HTLV-1 using enzyme-linked immunosorbant assay (ELISA) and positive samples were confirmed by Western blot (WB).
Results: The unmached analyses showed significant differences in frequency of A+ blood group between healthy controls and HTLV-1 infected individuals(OR=0.8,95%CI=0.66–0.97) and also significant association was observed between these two groups(OR=1.42,95%CI=1.1–1.99,P=0.021).
No significant difference of blood group (A-, B+, B-, O+, O- and AB-) was observed between cases and controls.
Conclusion: Our results might suggest that A+ blood group decrease the risk of HTLV-1 infection in healthy controls, while the AB+ blood group is more frequent in HTLV-1 carriers and increase the risk of HTLV-1 infection.
Keyword: ABO Blood group, Rhesus blood group, Human T Lymphotropic virus-1.

P-38

Clonality study in malignant lymphomas - immunoglobulin gene rearrangement

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Introduction: Immunoglobuline gene (IgH) rearrangementstudy permits the analysis of the lymphoid cell line. This is possible due tothe fact that in a normal tissue (after somatic mutation of the immunoglobulingenes took place) there are numerous different cellular clones arising from thesame progenitor cell. Tumoural cells have the same IgH genes. This permits us,by the amplification of certain segments of the immunoglobulin gene, toevaluate the clonality of lymphoid tissue.
Materials and methods: We performed our study on 25 cases of malignant lymphoma,using paraffin embedded tissue samples. IgH gene rearrangement study was used toassess clonality, using PCR method (primers used: forward VH, reverse JH;framework region 3). For the analysis of the PCR products we used twodifferents methods: capillary electrophoresis and conventionalgel-electrophoresis. In the meantime we reviewed the available literature inorder to summarize the latest developments in the field.
Results: From the total of 25 cases 14 proved to be monoclonal, 2oligoclonal, 7 policlonal and 2 not interpretable. There are important differencesbetween the two methods and the costs are also different. The literature offersa great source of information highlighting the importance and utility ofclonality assessment in lymphomas.
Conclusions,discussions: The assessment of clonality by the analysis of IgH generearrangements with PCR offers additional information in the study of malignantlymphomas offering valuable information about the clonality of the cells. Therelatively high costs of the method does not permitted us the application of iton a large scale, however does not hinder its use in research and even in thediagnosis of difficult cases.

P-39

Atypical Hodgkin-like lymphadenopathy associated with CMV infection in a patient with hyper-IgM syndrome

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Introduction: CMV is a common DNA virus which causes up to 7% of cases of the mononucleosis syndrome. In contrast to EBV, CMV infection has never been formally reported in association with an “atypical” lymphadenopathy with Hodgkin-like features, although, in CMV lymphadenitides, infected cells may sometimes vaguely resemble variants of Reed-Sternberg (RS) cells.
Aims: We describe the histopathological and immunohistochemical features of an atypical lymphadenopathy with classical RS cells, associated with CMV infection, in a patient with hyper IgM syndrome.
Case report: A 16-year-old boy with a long history of recurrent infections and a previous diagnosis of hyper-IgM syndrome, presented with a persistent massive lymphadenopathy for the past six years. Lymph node biopsies showed follicular and paracortical nodular hyperplasia, rare foci of “dirty necrosis” and worrisome features, such as thickening of capsule and frequent atypical cells in interfollicular areas. Some of these were activated immunoblasts, but others were indistinguishable from classical RS cells. The diagnosis of CMV infection was established by serology, PCR and immunohistochemistry, with both types of atypical cells, a few neutrophils and sparse lymphocytes being consistently positive for the virus. Hodgkin’s lymphoma was ruled out by the lack of CD15 positivity and of characteristic clinical and evolutive features.
Conclusion: From the data presented, one cannot assume that the atypical changes found in the lymph node were due to a direct action of CMV or to the hyper-IgM syndrome itself. Most important, however, is to recognize that classical RS cells may occur in the context of CMV infection and that they should not prompt a misdiagnosis of

Hodgkin's lymphoma. (JV is supported by the National Council for Scientific Research, CNPq).

P-40

Aberrant CpG island methylation profiles of multiple genes in non-Hodgkin's lymphomas

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Background: Aberrant methylation of CpG islands in promoter regions is one of the major mechanisms for silencing of tumor suppressor genes in various types of human cancers including non-Hodgkin's lymphomas (NHL). In this study, we investigated the aberrant promoter methylation status of known or suspected tumor suppressor genes in NHLs and compared the methylation profiles between B-cell and T/NK-cell NHLs. Methods: 54 cases of B-cell NHLs and 16 cases of T/NK-cell NHLs were examined for the methylation status of eight tumor-related genes using methylation specific PCR.

Results: CpG islands methylation was variously found in eight genes as follows; *DAPK* (71%), *MT1G* (70%), *p16* (53%), *CDH1* (53%), *THBS1* (56%), *MGMT* (27.1%), *COX2* (13%), and *RUNX3* (11.4%), in order of frequency. In six cases (8%), methylation was not observed in any of theses genes. Overall methylation index of B-cell NHLs (0.48) was significantly higher than that of T/NK-cell NHLs (0.32). And *THBS1* gene methylation was much more prominent in B-cell NHLs (67%) than in T/NK-cell NHLs (20%).

Conclusion: This study suggests that aberrant CpG island methylation is a frequent event in NHLs, and T/NK-cell and B-cell NHLs show overlapping but distinct methylation profiles between each other.

P-41

Primary diffuse large b-cell lymphoma of bone: unusual presentation

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Introduction: Primary lymphoma of bone (PLB) is an extremely rare condition, habitually confused with others bone primary injuries. Any localization of skeleton can be involved, but a trend exists to reach bones with persistent bone marrow. Pelvic bones and vertebral column are usually reached. The aim of this report is to present a case of PLB. The patient had a rare lesion im vertebral column. This led us to review the literature for a survey of incidence of PLB.

Method and results: A 41-year-old woman was admitted with a six-month history of low back pain that moves to the inferior limb. In radiography, there is bone destruction with pathological fractures in L1 vertebral body. A lumbosacral spine CT showed increase on the soft paravertebrates between T12 to L3 and fractures at T12 to L1. Immunohistochemistry revealed positives CD20 and CD45. She was diagnosed with primary diffuse large B-cell lymphoma of vertebral column. She was treated with combination chemotherapy consisting of cyclophosphamide, adryamicin, vincristine, predonisolone and etoposide, followed by radiotherapy in the thoracolumbar region. There was no evidence of recurrence at the 12-month follow-up.

Conclusion: Primary non-hodgkin's lymphoma of bone constitutes 3% to 7% of all bone malignant tumors and approximately 3% of all extra nodal lymphomas. Although the radiographic appearance of disease is variable and non-specific, one typical pattern is a solitary lytic lesion near the end of the long bone. On the basis of present case and a comprehensive review of similar cases in the literature involving patients with PLB, it appears that younger age, advanced-stage disease, multiple-bone involvement, and non-large-cell histology are associated with decrease survival compared with older age, localized disease, single-bone involvement, and large-cell histology, respectively. The scarcity of cases in the literature with this localization and morphology confirm the rarity of case.

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Primary non-hodgkin's lymphoma involving the proximal phalanx of the right thumb and the femur: case report

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Introduction: Primary lymphoma of bone (PLB) represents an uncommon bone tumour with relatively homogeneous morphology and clinical behaviour. Distinguishing PLB from other bone tumors is important because the former has a better response to therapy and a better prognosis, specially when compared with patients with systemic disease. Long bones are usually compromised. Femur is the most common isolated site (25%). The authors

discussed a case of PLB involving the proximal phalanx of right thumb and right distal femur. Method and results: A 63-year-old man was admitted with a four-month history of difficulty in walking and pain in right knee joint. Physical examination revealed a mass in the right knee joint. He also complained for pain of the right thumb. Two months before, a plain radiograph showed pathologic fractures of right distal femur and proximal phalanx of right thumb with periosteal reaction. Biopsy of the tumour demonstrated proliferation of abnormal lymphoid cells. Immunohistochemistry revealed positive CD20. She was diagnosed with primary diffuse large B-cell lymphoma of femur. He was treated with combination chemotherapy consisting of cyclophosphamide, adriamycin, vincristine, predonisolone and etoposide, followed by radiotherapy on the right distal femur and proximal phalanx of right thumb. There was no evidence of recurrence at 14-month follow-up. Conclusion: PLB constitutes 3% to 7% of all bone malignant tumours and approximately 3% of all extranodal lymphomas. Although radiographic appearance of disease is variable and non-specific, one typical pattern of primary bone malignant lymphoma is a solitary lytic lesion near the end of long bone that has a permeative pattern. Appropriate treatment depends on histologic type and stage. Review of the literature has shown that combined modality therapy does confer a better prognosis for patients having PLB. The scarcity of cases in the literature with this localization and morphology confirm the rarity of the case.

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SYSTEMIC ANAPLASTIC LARGE CELL LYMPHOMA - A MULTICENTRIC CLINICO-PATHOLOGICAL STUDY

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First described by H Stein in 1985, Anaplastic Large Cell Lymphoma (ALCL) comprises only about 5% of Non-Hodgkin's Lymphoma (NHL), but represent the 4th NHL for children. The WHO Classification recognizes a primary systemic and a primary coutaneous ALCL. 50–80% of the systemic ALCL present the translocation t(2;5)(p23;q35) with a better prognosis. We present the results of a multicentric clinico-pathological study. We included in this study 71 cases of primitive systemic ALCL (with available clinical data) diagnosed in our institutes between July 1st, 1997 and May 31st, 2007. The diagnosis was made by histopathology and immunohisthochemistry methods; clinical data were from the patient clinical card; data were statistically processed using SPSS program, version 13 and the new version of program STATA. Histological, the common variant of ALCL was the most frequently (76.4%), followed by small-cell variant (8.8%) and lympho-histiocytic variant (5.8%). All the cases expressed CD30; 72.1% were T-cell type, and 27.9% null cell type. Most of the T-cell ALCL were CD4+CD8- (40%) or CD4-CD8- (40%). 81.8% of cases were EMA positive (all ALK+, frequently associated with T-cell type,). Half of the cases expressed ALK (83.6% nuclear and cytoplasmic, associated with translocation t(2;5), and 16.6% only cytoplasmic, indicating a X-ALK translocation). CD15 was positive in 28.5% of cases, and all the cases expressed NF-kB; the protein of RANK oncogene was positive in 82.4% cases, uncorrelated with bone determination; only 44% cases expressed RET, 82% of these ALK+. The patients were predominantly male (M/F=2.08) with a median age 41.0 years, with a bimodal age distribution. For 67.6% of the patients (74% for ALK+ cases) the diagnosis was made in a disseminated clinical stage (III/IV Ann Arbor); B symptoms were present in 81.7% cases. 50.7% of the cases presented extranode disease (skin 28.9%, pleuro-pulmonary 26.3%; bone 21%, bone marrow 18.4%; liver 18.4%), frequently associated with EMA+. Because of the younger age, lower LDH and good performance status, the International Prognosis Index (IPI) was low/intermediate-low for 66.7% of the patients. The treatment was very varied, uncorrelated with ALK expression (63.6% CHOP or CHOP-derived cures). The complete remission (CR) rate was 60%; the evolution was difficult, with multiple relapses (57% in the first year); at 3 years, the overall survival (OS) was 69%, and disease free survival (DFS) only 23%; 20.3% of the patients died, 3/4 on the first 3 months after diagnosis. The pediatric lot included 11 cases (3–18 years old); 77.7% of cases were ALK+, and all expressed EMA. Extranode disease was more frequently (82.0%). For our lot, the favorable prognosis factors included ALK+, EMA+, the absence of extranode disease, IPI 0–2, RET-; the unfavorable factors were ALK-, skin infiltration, mediastinal mass, IPI 3–5, EMA-, RET+.

The ALCL diagnosis remains a difficult one. The expression of ALK identify a better prognosis group; for ALK-cases, the group EMA+ (more disseminated clinical stage,

lower IPI, extranode disease) appear more related with ALK+ group comparing with EMA- group

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Immunohistochemical expression of DNA topoisomerase IIalpha in diffuse large B-cell lymphomas

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Background Topoisomerase IIalpha (Topo IIα) protein is involved in DNA replication and transcription and is the target for a number of chemotherapeutic drugs. Topo IIα immunohistochemical expression is a marker of cell proliferation in many human malignancies. Therefore, diffuse large B-cell lymphomas (DLBCL) were studied for the expression of Topo IIα protein in relation to 1) proliferation associated proteins and 2) the bcl6/CD10/MUM1 differentiation immunophenotypes.

Method Sixty four cases of de novo DLBCL were studied by immunohistochemistry for the expression of Topo IIα protein. The expression of the protein was analyzed in relation to the previously reported expression of major cell cycle proteins (p53, HDM2, p21, p14, Rb, p16, p27, Ki67/MIB1 and cyclins A, B1, D2, D3 and E) and the bcl6/CD10/MUM1 differentiation immunophenotypes. Spearman's correlation coefficient and Mann-Whitney tests were used for the statistical analysis.

Results Expression of the Topo IIα protein was observed in all DLBC cases (Mean: 74.53%, Std Deviation: 19.26). Significant positive correlations were found between Topo IIα and the expression of Ki67, Rb, and cyclin D2 proteins ($p=0.002$, $p=0.018$ and $p=0.028$, respectively). In addition, Topo IIα protein was positively correlated with CD10 ($p<0.001$) and bcl6 ($p=0.010$) proteins and was expressed more frequently in germinal center (GC: bcl6+/CD10±/MUM1- and bcl6-/CD10+/MUM1-) B-cell-like DLBCL than in non-germinal center (non-GC: bcl6±/CD10-/MUM1+) B-cell-like DLBCL ($p=0.048$).

Conclusion The above findings indicate that Topo IIα is highly expressed in DLBCL and is associated with increased proliferation status. Moreover, DLBCL with GC B-cell-like immunophenotypic profile are associated with higher expression of Topo IIα protein in comparison to DLBCL with non GC B-cell like profile. This finding might be related with the better prognosis of GC-DLBCL since Topo IIα isoenzyme is target for anthracycline-based chemotherapy.

P-45

Primary bone lymphoma: a descriptive study of 11 patients

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Background: Primary bone lymphoma is a rare entity that constitutes less than 5% of all extranodal lymphomas. A retrospective analysis was performed to describe clinical features, radiology, histological type and immunophenotype of primary bone lymphomas (PBL) in our institution.

Methods: We identified 11 patients with PBL between 1998–2007. Diagnosis was made based on x-ray, CT scans, MRI, bone biopsy, and in some cases, gallium scan, bone marrow biopsy and fine needle aspiration. We reviewed the clinical features, radiology findings and morphology. The cases were reclassified according to the WHO 2001 classification after examination of H&E slides and available immunohistochemical stains.

Results: Our series comprises 9 men and 2 women with a median age of 57 years (range 29–75). One third of them had disease in long bones, 18% in the spine and 27% had politopic disease. Radiological findings were lytic, sclerotic or mixed lesions often misdiagnosed as sarcoma. The clinical stage was IV in 8 cases and I in 3 cases. Ten patients had diffuse large B-cell lymphoma (DLBCL) and 1 patient had anaplastic large cell lymphoma. Six (60%) cases of DLBCL had an activated phenotype and four (40%) cases had a germinal center phenotype. All patients were treated with chemotherapy and 36% reached complete remission.

Conclusions: In our study, PBL was located in long bones or spine in 45% of cases. The main histological type was DLBCL with an activated phenotype in 60% of cases.

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INTRAVASCULAR LARGE B-CELL LYMPHOMA MIMICKING PULMONARY INTERSTITIAL DISEASE

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Introduction: We present a case of an intravascular lymphoma which manifested by fever, fatigue and weakness. The initial diagnosis was pulmonary interstitial disease.

Clinical Case: Fifty-four-year-old female patient with thalassemia minor ankylosing spondylitis taking methotrexate and corticosteroids until one month before the appearence of fever, night sweats, anorexia, weight loss, fatigue and weakness. Laboratory tests revealed persistently elevated inflammatory parameters, elevated LDH and partial respiratory insufficiency. The thoracic C.T. scan demonstrated a reticular pattern with interlobular septal thickening and bilateral ground glass appearence. A diagnosis of pulmonary fibrosis was made and the patient was medicated with azathioprine and corticosteroids. The surgical pulmonary biopsy performed demonstrated a diffuse intravascular proliferation of large lymphoid cells with starry sky pattern not only within the bronchiolar vessels but also within the alveolar capilares; these cells were immunoreactive for B-cell markers and were negative for cyclin D1 and TdT. The final diagnosis was intravascular large B-cell lymphoma and the patient started treatment with CHOP and Rituximab with remarkable clinical improvement, being discharged and maintainig treatment in an ambulatory regimen.
Comments: Intravascular large B-cell lymphoma is a rare entity and its presentation as pulmonary interstitial disease is exceptional. The starry sky pattern that we observed has never been previously described. This type of lymphoma usually has a bad prognosis; however, like in the few cases in which an early diagnosis was made, it had a good response to chemotherapy.

P-47
Differential expression of VEGF and its receptors in plasmacytoma and multiple myeloma

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Background & Objective: Vascular Endothelial Growth Factor (VEGF) has an established role as multifunctional cytokine that potently stimulate tumorigenesis and angiogenesis in many solid tumors. However, the significance of VEGF expression in hemopoietic, especially plasma cell, neoplasms need further analysis to clarify their tumorigenic role. Materials & Methods: Fifteen cases diagnosed as Solitary Plasmacytoma (SP) of bone in addition to another fifteen cases diagnosed as Multiple Myeloma (MM) were selected. All cases will be investigated for the expression of vascular markers namely vascular endothelial growth factor (VEGF), and type IV collagen, in addition to VEGF receptors Flt-1 and Flk-1, and Bcl-2 was also used as an anti-apoptotic marker. Numbers of blood vessels were counted by using a micrometer in 500 unit squares (1 mm2) in SP and MM tumor tissues. Results & Conclusions: Most of the immunohistochemically revealed vessels within the tumor tissues were non-muscular venules. Statistically micro vessel density was higher in MM compared to SP according to site dependant analysis. Meanwhile, VEGF and Flt-1 show significant increase in their cytoplasmic expression in MM tumor cells compared to SP, indicating tumorigenic role for VEGF when its signal is perceived either paracrinally or autocrinally by Flt-1 receptor on neoplastic clonal plasma cells.

P-48
Plasmablastic lymphoma: a review of 15 cases

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Plasmablastic lymphoma is considered a variant of Diffuse Large B Cell Lymphoma in the WHO classification. Initially these lymphomas were recognized as proliferations of large B cells with immunoblastic morphology and plasma cell immunophenotype resembling the terminally differentiated B cell, characterized by negative or weak expression of mature B-cell markers (CD20) and positivity for plasma-cell associated antigens (CD38, CD138). They are more frecuent in patients infected with HIV and they have presented commonly in the mucosa of the oral cavity, other locations include stomach, rectum and lungs. The clinical behaviour is aggressive with poor reponse to therapy and short survival. On the other hand several studies have now recognized other lymphomas with similar morphological and phenotypic plasmablastic features but with diverse clinical and molecular characteristics suggesting that they may correspond to different disease entities. We submit 15 cases from our hospital to provide their clinical and pathological characteristics and to illustrate the diversity of these tumors.

P-49
Intranodal kaposi sarcoma

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75 years old patient presenteal with dispne, and caughing to the Internal Medicine Department of Taksim's Education and Research Hospital. Multiple mediastinal, bilaterally axillary, inguinal, celiak, vertebral and paraaortic lymphadenomegaly had seen by MRI, CT and USG. Hemathologically there was trombocytopenia and normokrom normocyter anemia. Be-

cause his PSA was high, prostatic biopsy was planned. And the result of the biopsy was prostatic adenocarsinoma. Besides axillary lymph node was excized and the histopathological result was intranodal kaposi sarcoma. HIV markers were negative. Before he had the diagnosis of chronic renal failure. Because of prostatic adenocarcinoma and chronic renal failure was immunologically insufficient, this prepared the situation for intranodal kaposi sarcoma. We present the case because it is quiet rare in literature.

P-50

Angiocentric T/NK cell lymphoma-sinonasal type

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BACKGROUND: MGS-"midline granuloma syndrome" is a clinical description of a broad spectrum of diseases, which are characterized by aggressive and progressive destruction of mucosa and adjacent structures of midface and upper aerodigestive tract. The differential diagnosis includes: Wegener's granulomatosis-WG and malignant lymphoma. After exclusion of WG and by immunophenotyping, nearly all remaining cases presenting as MGS are peripheral sinonasal angiocentric T-and/or NK-cell lymphoma. The natural history of these lymphomas is characterized through a rapidly progressive course with a poor prognosis.

METHOD: A 63 year old Serbian woman, presented with the complaint of persistent chronic, recurrent sinusitis-ozena. After development of "midline" destructive disease which involved the maxillar and ethmoid sinus also the soft and hard palates, operation revealed a diffuse infiltration of partly necrotic tissue. Histologic features were identified and immunophenotyping performed.

RESULTS: The histological features showed coagulative "geographic"necrosis, heavy inflammatory infiltrates including plasma cells, histiocytes end eosinophils. The polymorphous cell population obscured the neoplastic cells that were pleomorphic, small and medium sized cells to large hyperchromatic cells with irregular and elongated nuclei, prominent nucleoli and clear cytoplasm. Angiocentric growth was common with infiltration and destruction of the vessel wall. Tumor cells showed immunopositivity towards: CD3, CD45, CD30, Ki67(80%) while negative towards: CK, EMA, NSE, Chromogranin, Synaptophysin, CD20, CD79a, CD8, bcl 2.

CONCLUSION: ATNKCL in european population is rare and clinically of aggressive behavior because of mutilant and destructive process and consequently multiorgan failure. Because of its poor prognosis it should be included early into the differential diagnosis.

P-51

PROGRESSION OF LYMPHOMATOID GRANULOMATOSIS TO DIFFUSE LARGE B CELL LYMPHOMA

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Background: Lymphomatoid granulomatosis (LYG) is comprised of Epstein Barr virus (EBV) - positive B cells admixed with various proportion of reactive T cells. We present two cases of cutaneous LYG progressed to diffuse large B cell lymphoma over a period of 5 years.

Methods: We used clinical histories of 56 years old female and 61 years old male, and paraffin sections from surgically excised skin lesions. Paraffin sections were stained for H. H., Giemsa, Reticulin-Gomori, Zeil-Nilsen, and immunohistochemically for T cell, NK cell associated and B cell antigens, as well as for LMP1.

Results: The lesions started as multiple erythematous dermal papules on the back of both patients. Histological analysis in the first case revealed perivascular polymorphic infiltrate of lymphocytes without cellular atypia and absent necroses, that was defined as chronic vasculitis. In the second case there were found lymphocytic infiltrates in papillary dermis, and diagnosis of mycosis fungoides followed. The patients were treated intermittently with corticosteroids and PUVA, respectively. After a period of 5 years the lesions progressed to large subcutaneous tumor nodules overlayed with an atrophic livid skin mostly disposed at the back. These lesion demonstrated marked slightly nodular to diffuse lymphohistiocytic infiltrate, involving the upper and deep dermis, and subcutaneous fat tissue. On special stains one could see angiocentric invasive lesions and granulomas with few giant cells. The infiltrate consisted from large lymphoid cells that were positive for CD20, CD79a, bcl6+, with proliferative rate more than 80%, as was determined with Ki67. They were diffusely admixed with CD68+ histiocytes and CD3+, CD5+ T cells, mostly at the periphery of the nodules and lesion. Some of the cells were positive for LMP1.

Conclusion: These cases confirm that LYG is a rare angioinvasive and destructive lymphoproliferative disease with B cell origin and probably pathogenetically connected with EBV infection

Endocrine Pathology

P-52

A case of Somatostatin producing Endocrine (D-cell) tumour of Duodenum with literature review.

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Somatostatinomas are rare tumours accounting for 1–2% of endocrine tumours of the gastroenteropancreatico-hepatic axis. Somatostatin is produced from the D-type cells of pancreatic islets, gastrointestinal tract and central nervous system. Ganda et.al (1977) reported the first case originating in the pancreas. The primary extrapancreatic stomatostatinomas originating in the descending part of the duodenum, ampulla of vater and peri-ampullary areas was first documented by Alumets et.al in 1978.

In contrast to its pancreatic counterpart, the duodenal somatostatinomas are asymptomatic rarely accompanied by the somatostatinoma syndrome.

We report a case of a non-functional Somatostatin producing duodenal endocrine tumour in a seventy four year old female, who presented with melaena and anaemia, with a literature review of duodenal endocrine tumours.

P-53

MUCINOUS VARIANT OF PAPILLARY THYROID CARCINOMA

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Background: Mucin production in the human thyroid gland is an unusual event that can occur in follicular adenoma, and papillary (PTC), follicular, medullary, and mucoepidermoid carcinomas. Mucinous carcinoma of the thyroid is an extremely rare tumor characterized by clusters of neoplastic cells surrounded by extensive extracellular mucin deposition. Follicular and papillary carcinomas displaying focal mucin production should not be regarded as mucinous carcinoma. Metastatic carcinoma, on the other hand, must always be considered as a differential diagnosis when dealing with a thyroid tumor with a mucinous component.

Method: We describe the histopathological, immunohistochemical (Dako EnVision Peroxidase/DAB), and molecular features of a tumor presented in the thyroid gland in a 69-year-old-woman with multinodular goiter.

Results: The 1.5-cm tumor located in the isthmus was encapsulated and microscopically composed of cells with ribbon-like, solid, or follicular growth patterns with extensive extracellular mucin positive for mucicarmine and Alcian blue (pH 2.5). Some cytoplasmic vacuoles were found and nuclei were oval, enlarged, with irregular contours, nuclear grooves and occasional intranuclear inclusions. The nuclei exhibited dusty chromatin but no mitotic figures were found. Tumor cells were positive for TTF-1, CK7, CK19, galectin-3, HBME-1, p63, and vimentin. Focal positivity for thyroglobulin and thyroperoxidase was also found, but no reaction was detected for calcitonin, CK20 or p53. The proliferative index (MIB-1) was<2%. No mutations were found in exon 11 nor 15 of the *BRAF* gene.

Conclusion: The mucinous variant represents an extremely uncommon metaplastic variant of PTC that we should be aware of to avoid potential pitfalls of misdiagnosis.

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P-54

Cell Monotony, Mitotic Activity and Necrosis are Associated with Malignant Pheochromocytoma/ Paraganglioma

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Background: Adrenal pheochromocytoma and extra-adrenal paraganglioma are uncommon neoplasms. So far no definite histologic features are able to predict aggressive behavior of pheochromocytoma or paraganglioma. This study is an attempt to identify morphologic features associated with malignant pheochromocytoma or paraganglioma.

Design: We reviewed 55 cases of pheochromocytoma and 43 cases of extra-adrenal paraganglioma from pathology archive at University of Wisconsin. After excluding cases without at least two year follow-up information or cases with tumor embolization prior to surgery, only 30 of the 98 cases are included in this study. 8 of the 30 cases are malignant (tumor metastasized to other organ or tissue). 22 cases of the 30 are clinical benign pheochromocytoma or paraganglioma, and are further classified as sporadic (n= 10) and hereditary (associated with MEN2A or von Hipple

Lindau syndrome) (n=6) pheochromocytoma, paraganglioma of head and neck region (n=6). Morphologic features of the tumors such as tumor architecture, sclerosing/hyalinizing changes, necrosis, capsular and vascular invasion, pleomorphism, nuclear hyperchromasia, cell size, nuclear and cytoplasmic (N/C) ratio and mitotic activity were examined by two pathologists.
Result: We found that relatively uniform tumor cells, small cell size, necrosis and mitotic activity are associated with malignant pheochromocytoma or paraganglioma (Table 1, below). Vascular invasion is an infrequent feature found in pheochromocytoma or paraganglioma. N/C ratio, nuclear hyperchromasia, sclerosing/hyalinizing changes and tumor architecture are indiscriminative features.
Conclusion: Tumor cell monotony, small cell size, necrosis and mitotic activity may be indicators of aggressive pheochromocytoma or paraganglioma of non-head/neck region.

P-55

A mixed medullary- follicular thyroid carcinoma discovered by fine needle aspiration.

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Mixed medullary and follicular cell carcinoma (MMFCC) of the thyroid is a rare neoplasm, constituting less than 5% of thyroid medullary carcinoma. A 57 year old female patient with tyroid enlargement was admitted in our institution. Thyroid ultrasonography revealed a 2 cm solid nodule within the right thyroid lobe. The fine needle aspiration was highly cellular and showed two seperate tumoral populations. the first one was made of several three dimensional cohesive clusters of follicular cells with round oval nuclei, and scant cytoplasm in a backgrounddevoid of significant colloid. The second one was made of rare non cohesive polygonal and pleomorphic shaped cells with eccentric nuclei, and an abundant granular cytoplasm.The aspiration biopsy was reported as malignant, the diagnosis of MMFCC was suspected. The patient underwent bilateral total thyroidectomy with bilateral mediastinorecurentiel lymphadenectomy.The diagnosis of MMFC of the thyroid was established by histological investigation using immunohistochemical staining.

P-56

Diagnostic usefulness of HMBE1 and CK19 AND EVALUATION OF THEIR EXPRESSION IN FOLLICULAR-PATTERNED LESIONS WITH QUESTIONABLE FEATURES OF PAPILLARY THYROID CARCINOMA.

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Objective: To evaluate the expression of HBME1 and cytokeratin 19 and their usefulness in diagnosis of follicular variant of papillary carcinoma.
Materiels and methods: We evaluated 30 follicular patterned thyroid carcinomas including 15 papillary carcinomas (PC) and follicular carcinomas (FC). All casers were examined by immunohistochemistry.
RESULTS:87% of papillary carcinomas expressed HBME1 as well as 13% of foliicular carcinomas. CK19 marked 73% of papillary carcinomas (the one negative case for CK19 was of oncocytic variant), and 13% of follicular carcinomas.
CONCLUSION: These results suggest that CK19 is a specific and sensitive marker for papillary carcinoma which is very helpful in the diagnosis of its follicular variant; the combination of HMBE1 increases its specificity.

P-57

CD10 expression in follicular patterend thyroid nodules

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Background: Follicular patterned thyroid nodules are a common problem in thyroid pathology and sometimes distinguishing benign from malignant nodules can be challenge. CD10 was studied and founded to be expressed in 79% of follicular patterned malignant thyroid nodules (i.e. follicular carcinoma and follicular variant of papillary carcinoma) and negative in benign counterparts. However, no further evaluation has been done in literature on this stain. Therefore, we conducted this study to evaluate CD10 expression in follicular patterned thyroid nodules.
Material & Methods: Fifty four cases of follicular patterned thyroid nodule were selected from Farwaniya hospital files as follow: Nodular hyperplasia 18 cases, follicular adenoma 16, follicular variant of papillary carcinoma 17 and follicular carcinoma 3 cases. CD10 was tested on one representative block from each case. The stain was reviewed by the two authors to reach an agreement and 10% was considered a cutoff point for positive result.
Results: The CD10 was expressed in different follicular patterned thyroid nodules as follow: 6/18 nodular hyper-

plasia, 2/16 follicular adenoma, 4/17 follicular variant of papillary carcinoma and 0/3 of follicular carcinoma. CD10 was expressed in 23.5% of benign nodules and 20% of malignant nodules.
Conclusion: CD10 is neither sensitive nor specific in distinguishing follicular patterned thyroid nodules.

P-58

Relationship between thrombospondin-1 and syndecan-1 in thyroid pathologies

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Thrombospondin-1 (TSP-1) is a multifunctional matrix protein implicated in cancer cell adhesion, migration, invasion, inhibition of angiogenesis, and activation of latent transforming growth factor-β. Syndecan-1 (SYN-1), is a transmembrane heparin sulphate proteoglycan that is evolved in cell-cell adhesion, organization of cell matrix adhesion and regulation of growth factor signalling. The purpose of this study was to examine the relationship between TSP-1 and SYN-1 in pathological human thyroid glands, using immunohistochemical methods in paraffin-embedded tissue. We studied 42 carcinomas, 14 adenomas, 30 cases of hyperplastic disorders, and 16 normal thyroid glands. Statistical analysis was performing using the SPSS for windows (versio 6.0) statistical package. Normal thyroid tissues showed immunostaining for TSP-1 confined to the interfollicular stroma, and to the vasculature endothelium. A similar degree of reactivity was found in hyperplastic lesions and adenomas. TSP-1 was strongly expressed in papillary carcinomas with desmoplastic stroma, but weak to moderate in cancer epithelium. SYN-1 was expressed in normal, hyperplastic and neoplasic thyroid cells. Reduction of SYN-1 was found in carcinomas compared with normal thyroid tissue ($p=0.04$). No relationship was found between TSP-1 and SYN-1. TSP-1 expression was restricted to stroma and might be act as protective effect against tumor progression; on the other hand, SYN-1 expression in epithelial tumor cells seems to show a close association with preserved epithelial morphology and differentiation.

P-59

Neoplastic transformation of thyroid gland is accompanied with changes of sialyltransferases expression in the affected tissue

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Background: Thyroid cancer belongs to the most frequent endocrine diseases. The factor often complicating its histopathological evaluation is the difficult differential diagnosis between benign and malignant lesions. Abnormal sialylation of cell structures is an important factor of the neoplastic transformation. This work evaluated the association between the neoplastic process and the changes of sialyltransferases expression in the thyroid gland.
Methods: The cases of thyroid gland tumors (papillary, follicular, oncocytic, medullary and anaplastic carcinomas, follicular adenomas) and parenchymatous goiter were analyzed. The total RNA was isolated from formalin-fixed in paraffin embedded material and analyzed by real-time RT-PCR using the specific primers for sialyltransferase-1 (alpha-2,6 linkage of sialic acid) and 4A, 4B and 4C (alpha-2,3 linkage). The results were correlated with the quantitative evaluation of protein sialylation by lectin histochemistry.
Results: The significant increase of sialyltransferase-4C level and sialyltransferase-4B to 4A index was reported in well differentiated follicular and papillary carcinoma when compared to the parenchymatous goiter and benign adenomas. The sialyltransferase-1 has not shown any changes in expression. These results correlate with the finding of strong luminal positivity found in well differentiated carcinomas especially in the alpha-2,3 linkage. Follicular cells in normal thyroid gland, adenomas and benign goiter showed weak expression of sialic acid.
Conclusion: The data showed only weak membrane-bound sialic acid positivity in benign adenomas and goiters in comparison with malignant papillary and follicular carcinomas, with alpha 2,3-linked sialic acid predomination. Increased membrane sialic acid expression may be the result of the increase of sialyltransferase-4C expression in transformed tissue. The presented molecular changes in thyroid gland may be an important diagnostic finding in the future, especially in respect to aspiration cytology evaluation and the differential diagnostics of thyroid gland follicular lesions.
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P-60

Changes of inducible nitric oxide synthase expression in thyroid gland diseases

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Background: Nitric oxide is a pleiotropic molecule with a variety of effects participating in the regulation of cellular functions under physiological and pathological conditions. Lately, it became an important topic in molecular biology research. The presented study evaluated the changes of NO synthase expression in thyroid gland diseases.
Methods: Inducible NO synthase expression was analyzed immunohistochemically in cases of papillary and follicular carcinomas, follicular adenomas, autoimmune Graves-Basedow and Hashimoto diseases and parenchymatous goiters of the thyroid gland. Slides were evaluated in optical microscope semiquantitatively and by morphometry.
Results: Weak and inconsistent membrane expression of i-NOS was found in parenchymatous goiter or benign follicular adenoma at the luminal site of follicular cells. Cytoplasmatic membrane, cytoplasm and colloid were negative. The increase of cytoplasmatic i-NOS expression was found in Hashimoto disease. Hyperplastic epithelium in Graves-Basedow disease showed strong nuclear expression of i-NOS with just inconsistent luminal membranes and apical cytoplasmatic positivity. The infiltrating lymphocytes were strongly positive in Hashimoto, but showed no i-NOS expression in Graves-Basedow disease. The moderate increase of strictly cytoplasmatic i-NOS expression was found in the cases of well differenciated follicular and papillary carcinoma.
Conclusion: Inducible nitric oxide synthase has been described to play a role in carcinogenesis as a mediator of DNA damage, neovascularization, vasodilatation, and metalloproteinase activation. Presented study indicate the increase of i-NOS expression in autoimmune diseases and in well differentiated thyroid tumors, but show only weak expression in benign follicular adenoma and normal thyroid gland tissue. It can be concluded that changes in NO synthase activity can be an important factor in the development of autoimmune thyroiditis, can play a role in neoplastic transformation and be a potential target for anticancer therapy in the future.
This work was supported parcially by grant APVT N° 20–025204 and grant MZSR N° 2006/23-UK-02

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SOLID CELL NEST HYPERPLASIA AND PAPILLARY THYROID CARCINOMA

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Background: Solid cell nests (SCNs) of the thyroid gland, usually considered as the remnants of the ultimobranchial body, are single or multiple foci of clusters of 2 types of cells referred to as “main cells” and “C cells”. The main cells of SCNs harbor the minimal properties of a stem cell phenotype, and may thus represent a pool of stem cells of the adult thyroid. We report a peculiar case of hyperplasia of SCNs associated with papillary carcinoma. Method: We describe the histopathological, immunohistochemical (Dako EnVision Peroxidase/DAB), and molecular features observed in the study of the right lobe of the thyroid in a 48-year-old man. The entire lobe was included in 25 formalin-fixed, paraffin-embedded tissue blocks. Results: The surgical specimen weighed 209 g, measured 12×7×6.5 cm, and showed a well-circumscribed nodule of 9 cm in diameter. Microscopically, the nodule was a follicular adenoma (FA) with focal calcification and cystic changes. Extranodular thyroid tissue showed an average of 18 (range 1–88) clusters (nests) of main cells of SCNs per section in the 25 tissue blocks. In one block, a solid nest of main cells merged with a follicular variant of papillary microcarcinoma (1.5 mm). The SCNs were positive for p63 and galectin-3, and negative for thyroglobulin. The papillary microcarcinoma was positive for thyroglobulin and galectin-3, and negative for p63. Tissue samples were obtained by laser captured microdissection and the same BRAFV600E mutation was found both in a pool of 5 solid nests we examined, as well as in the papillary microcarcinoma. Nodular hyperplasia and lymphoid aggregates occasionally associated with SCNs were also found in the histological study. No BRAF mutations were detected outside the SCNs and the papillary microcarcinoma. Conclusion: Our findings support the assumption that the solid cell nest hyperplasia may serve as a precursor lesion of papillary carcinoma of the thyroid. Supported by grant PI060209 from Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Spain.

P-62

Immunohistochemical Expression of Galectin-3, Cytokeratin 19 and HBME-1 in Papillary Microcarcinoma of the Thyroid Gland

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With wide use of US and FNA of the thyroid gland, the incidence of papillary microcarcinoma of the thyroid (PMCT) is rapidly increasing. We analysed the immunohistochemical expression of galectin-3, CK 19 and HBME-1 in 37 cases of PMCT to evaluate the diagnostic value of these molecular markers. Immunohistochemically, galectin-3 and HBME-1 expression was found in all cases. CK 19 expresseion was found in 36 cases. The adjacent normal thyroid parenchyma and accompanying benign lesions are negative for these markers. There are scattered foci of incomplete positive staining in cases of Hashimoto's thyroiditis, however.

Our findings suggest that the immunohistochemical staining using antibodies for galectin-3, CK 19 and HBME-1 is an useful adjunctive method for the histopathological diagnosis of PMCT.

P-63

BRAF MUTATION IN A THYROID GLAND WITH CONCURRENT ONCOCYTIC FOLLICULAR CARCINOMA, ONCOCYTIC PAPILLARY CARCINOMA, CLASSIC PAPILLARY MICROCARCINOMA AND HASHIMOTO THYROIDITIS.

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Background: Oncocytic (oxyphilic, Hürthle cell) lesions are composed predominantly of cells with abundant eosinophilic granular cytoplasm due to accumulation of mitochondria. For many years considered a special category of follicular cell tumors, in the most recent WHO classification of thyroid tumors, oncocytic carcinomas have been considered as variants of papillary, follicular, medullary or poorly differentiated carcinomas.

Method: We describe the histopathological, and molecular features (*BRAF* gene) of a peculiar thyroid mass presented in a 65-year-old man.

Results: Histological examination revealed, within a background of Hashimoto thyroiditis, an oncocytic follicular carcinoma, 6 cm in diameter and angioinvasive in the right lobe, concurring with two papillary carcinomas, one a Warthin tumor-like variant, 3 cm in diameter, and the other, a classical-type papillary microcarcinoma in the left lobe. The mutational study of exons 11 and 15 of the *BRAF* gene showed V600E mutation of exon 15 only in the Warthin tumor-like variant of papillary carcinoma, but not in the other two neoplasms nor in the Hashimoto thyroiditis sample.

Conclusion: Our findings support the view considering the oncocytic tumors as variants of different tumor types rather than a special follicular tumor category.

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HMGA2 as single marker for thyroid carcinomas

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Background: HMGA2 expression was reported of many tumours but not of tumour-free tissue. In some tumours, for example pancreas-carcinomas HMGA2 expression can be used for discrimination of carcinomas from chronic inflammatory changes.

In diagnostics of thyroid tumours, it can be very difficult to distinguish adenomas from micro invasive carcinomas by histology and quite impossible by cytology.

The aim of this study was to investigate whether the *HMGA2* expression can be used to detect malignant thyroid tumours.

Methods: 64 formalin-fixed paraffin-embedded thyroid tissues including normal tissue (n=3), thyroiditis (n=2) and follicular adenomas (n=19) as well as follicular (n=9), papillary (n=28), and anaplastic (n=3) carcinomas were tested for *HMGA2* expression using qRT-PCR. Immunohistochemistry was performed in a subgroup of five adenomas, two samples from healthy thyroid tissue, six FTCs, one poorly-differentiated FTC and five PTCs with an antibody raised against the HMGA2 protein.

Results: qRT-PCR showed expression differences of up to 400-fold between benign and malignant thyroid tumours. Based on *HMGA2* expression alone, it was possible to distinguish between benign and malignant thyroid tissues with a sensitivity of 95.0% and a specificity of 95.0%.

There was a highly significant ($p<0.001$) concordance with histology of the tumours which is the current gold standard of thyroid tumour diagnostics. Immunohistochemistry was clearly positive in all proven malignant tumours and negative in normal thyroid tissues and all the adenomas.
Conclusion: HMGA2 expression is a promising marker of malignancy in thyroid tumours. Further investigations will be undertaken for conformation of the retrospective results in advance and practice.

P-65

CONCURRENT THYROID CARCINOMAS IN AN ADOLESCENT GIRL: AN UNUSUAL PRESENTATION WITH AGGRESSIVE CLINICAL COURSE.

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Background Follicular carcinoma is usually seen in the fifth decade and commonly presents as an asymptomatic intra-thyroidal mass. In contrast, papillary carcinomas commonly occur at a younger age, although they are rare before the age of 20. We describe a case of a young girl with concurrent follicular and papillary carcinomas. To the best of our knowledge, this is the first report of such a case.
Method A 17 years-old girl presented with a history of multiple enlarging thyroid nodules slowly increasing in size. She had no history of radiation exposure. No family history of thyroid carcinoma was recorded. Three core biopsies, followed by two fine needle aspirations (FNAs) at three months interval were taken in 2004 from different nodules. Three years later, total thyroidectomy was performed as rapid increase in size of some of the nodules.
Results In biopsies and FNAs, macrofollicular lesions with papillary hyperplasia were detected. Total thyroidectomy specimen showed a multinodular thyroid weighting 111 gms, with multiple distinct nodules in both lobes and an unremarkable isthmus.
Microscopically, the left lobe demonstrated two angioinvasive follicular carcinomas (3.2 cm and 1.8 cm) with >50% poorly differentiated insular component, focal necrosis and a high mitotic count (14 mitosis/10 hpf). In the right lobe, one angioinvasive follicular carcinoma and one papillary carcinoma, follicular variant, were discovered. Both were 3 cms in greatest dimension. Postoperatively, the patient received radiotherapy (160 mCi) in a single dose two months after the surgery and she is well at 4 months follow up.
Conclusion Follicular thyroid carcinoma is a rare entity in children, but it is important to include them in the differential diagnosis of thyroid nodules in this age-group. In presence of rapidly increasing nodules (even with negative FNA), surgical excision may be warranted.

P-66

Genetics of radiation-induced thyroid tumors: an array-CGH (comparative genomic hybridization) study in a mouse model

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Background: Thyroid cancer is the most common endocrine malignancy in man. Although rearrangements of the ret/PTC-oncogenes are strongly correlated with the human irradiation-associated PTC, further genes are supposed to be essentially involved in tumorigenesis. The genetic mechanisms of human FTC indeed remain widely unknown up to now. Therefore, we performed a high resolution genome-wide array-CGH study in a mouse model of radiation-induced thyroid tumors to gain insights into carcinogenetic mechanisms.
Method: Iodine-deficient fed mother mice were injected with radioiodine to induce thyroid tumors in their litters. The DNA was isolated from the formalin-fixed paraffin-embedded thyroid tissue of 21 different (non-irradiated and radioiodine-treated) mice: two normal thyroids, 13 goiters, two FTA, and eight FTC. The analysis was done using a 1-Megabase BAC array-CGH.
Results: In 46% of the hyperplasias, a variety of small chromosomal gains and losses (0.5 to 119 Mb) was observed. Regional polyploidies on the chromosomes 4 and 5 (about 20 Mb) were demonstrated in an FTA. The FTCs exhibited frequent partial or complete chromosomal losses, predominantly of the chromosomes 4 (88%; p16), 9 (50%), and 14 (38%; Rb1).
Conclusion: The random gains and losses of hyperplasias represent genetic instability predisposing to malignant transformation. In similarity to the human counterpart, aneuploidies were demonstrated to be characteristics of the murine FTC, and seem to critically influence tumor development. In that respect, certain chromosomes have an increased risk for genomic damage. The commonly altered regions in FTCs and the polyploid amplification of the FTA give us a hint for the location of candidate tumor suppressor genes resp. oncogenes with effects on thyroid tumorigenesis. Losses of the tumor suppressor genes p16 and Rb1 are putative candidates playing a key role. Furthermore, in mice, histology and genomic alterations due to irradiation are similar to them in spontaneous tumors.

P-67

PHEOCHROMOCYTOMA WITH DIVERGENTE DIFFERENTIATION (COMPOSITE PHEOCHROMOCYTOMA) IN VON HIPPEL-LANDAU (VHL) DISEASE

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Background: Composite pheochromocytomas are rare tumors histologically characterized by features of pheochromocytoma/paraganglioma with those of ganglioneuroma, ganglioneuroblastoma, neuroblastoma or peripheral nerve sheath tumor. They have been described in association with NF1, MEN2A, and possibly neurocutaneous phakomatosis. The ocurrence of such tumors in the setting of VHL is unclear.
Methods: Thirty two cases of pheochromocytomas, 13 sporadic and 19 VHL associated, were retrieved from the NCI surgical pathology files. H&E sections and available IHC stains (chromogranin, synaptophysin, S100, and NFTP) were reviewed. Tumors with divergent differentiation were defined as neoplasms of a mixed phenotype exhibiting pheochromocytoma histology plus areas of an additional tumor type, supported by IHC stains.
Results: Among the VHL associated neoplasms, seven (7/19) pheochromocytomas with divergent differentiation were encountered. In all cases, the phenotype was that of focal to nearly equal admixtures of pheochromocytoma and ganglioneuroblastoma-like tumors. In one of the VHL associated cases, the ganglioneuroblastoma tumor showed increased mitotic activity and necrosis. Remainder VHL associated cases (12/19) and all sporadic tumors (13/13) were of classical histology.
Conclusions: Pheochromocytoma with divergent differentiation (composite pheochromocytoma) appears to frequently affect patients with VHL syndrome, at an younger age. Long term follow up may be necessary since metastasis from composite tumors are derived from the ganglioneuroblastoma component.

P-68

ENDOGENOUS AVIDIN BIOTIN ACTIVITY (EABA) IN THYROID PATHOLOGY – ARTIFACT OR MARKER

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BACKGROUND Immunohistochemicalmethods that use a high affinity of avidin and biotin (e.g. ABC, LSAB) arecharacterized by high sensitivity and wide known. However, non-specificreaction can be observed in frozen tissues and in paraffin embed material,increasing after heat induced epitope retrieval (HIER). The reaction caused byendogenous biotin or any another chemical compound that is an extremely highaffinity for avidin may cause diagnostic mistakes. The aim of ourinvestigation, is to study presence of endogenous avidin biotin activity (EABA)in thyreocytes originating from various thyroid pathological lesions(neoplastic and non-neoplastic).
Material and methods Theimmunohistochemical study was performed on paraffin-embedded specimenssurgically resected thyroid tissue from 75 patients with thyroid diseases: 11patients with nodular goiter, 9 with lymphocytic thyroiditis (LT), 8 withfollicular adenoma, 65 patients with papillary carcinoma (PTC) (typical,follicular, oxiphilic and cribriform-morular variants), 4 patients withfollicular carcinoma. In papillary carcinoma immunohistochemical study wasperformed both in primary tumors and metastases. After HIER incubation withstreptavidin from LSAB+ kit 25 min was done.
RESULTS Strong cytoplasmic EABA wasobserved in 56 of 65 (87.5%) PTC and in 8 of 9 cases of LT. Correlation betweenEABA in primary tumor and EABA in metastases was noted. Normal surroundingthyroid tissues show absence or weak EABA. Aberrant intranuclear localizationof biotin was noted in morules of cribriform-morular variant of PTC. Nostatistically significant correlation between patient's age, sex, metastasespresence and EABA was observed.
CONCLUSIONS Using of visualization systemsthat do not contain avidin or biotin (e.g. EnVision+ System-HRP) is preferablyfor immunohistochemistry of thyroid diseases. The nature of this reaction inPTC and LT needs further investigations.
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Expression of RegIV and Hath1 in neuroendocrine tumors

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Background: RegIV (RELP) is a c-type lectin with an unknown physiological function. It is expressed in selected neuroendocrine cells of the intestinal epithelium and it has also been found in certain gut-derived adenomas and carcinomas and in some neuroendocrine tumors. The basic helix-loop-helix (bHLH) transcription factor Hath1 is associated with neural development and with neuroendo-

crine differentiation of the gut epithelium. We investigated a panel of neuroendocrine tumors by immunohistochemistry and in situ hybridization for the expression and co-expression of these two proteins.
Methods: A total of 56 paraffin embedded endocrine tumor samples from different organs were immunohistochemically stained by monoclonal and polyclonal antibodies to RegIV and by a polyclonal antibody to Hath1. The specificity of the anti-Hath-1 antibody was validated by in situ hybridization.
Results: A robust expression of RegIV was seen in carcinoids of the gut and the stomach, in parathyroid adenomas, in Merkel cells carcinomas of the skin, and in pulmonary small-cell carcinoma. Mucocellular (signet ring cell) cancer of the stomach stained strongly for RegIV. In the gut carcinoids, only the marginal cells layers of the tumor in direct contact with the stroma expressed RegIV. Virtually no staining was seen in the pheochromocytomas, in pancreatic islets or in tumors of endocrine pancreas (insulomas). The staining pattern of Hath1 was similar that of RegIV in the intestinal carcinoids. Pancreatic islets, insulomas, and pheochromocytomas expressed Hath1, while the tumors of the stomach remained negative. The expression of Hath1 was consistently found in the cytoplasm of the neoplastic cells despite the function of the protein as a bHLH transcription factor.
Conclusions: The expression pattern of RegIV and Hath1 is partly overlapping but not identical, implying different regulatory mechanisms. The different staining patterns might add to the diagnostic and follow up tools for neuroendocrine tumors.

P-70
Immunohistochemical profile in metastatic neuroendocrine carcinomas (MNCs)

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Background: Neuroendocrine tumors (NETs) are heterogeneous, developing from a large variety of tissues. Histopathological aspects and neuroendocrine (NE) immunohistochemical (IHC) markers do not differentiate them, being frequently difficult to establish their primary origin. MNCs are relatively common encountered, representing 10–15% of unknown primary tumors (UPTs). UPTs have a particular biology with rapid progression and atypical metastases. The primary location of MNCs has impact on therapy and survival. We studied the expression of NE markers and cytokeratins (CK) in MNCs of UPTs, and looked for correlations with their histogenesis.
Method: 21 MNCs (M:F, 13:8; age range: 25–73 years, median: 52.7 years) from 53 consecutively archived in one year, formalin fixed paraffin embedded samples of NETs were reviewed. Three microns sections have been assessed using H&E stain and indirect tristadial ABC IHC method for a panel of 21 antibodies for NE differentiation, digestive - including pancreas, pulmonary, thyroid, and other origins.
Results: The sites of metastases in our study were: lymphnodes (8/21), liver (3/21), bone (2/21), brain (1/21), spleen (1/21), peritoneum (3/21), subcutis (1/21), and multiple site metastases (2/21). Beside the positivity for NE markers, in 9 tumors (42.85%) (moderate or well differentiated MNCs) the origin could be suggested by these results: digestive (2 cases): ChromograninA+/Synaptophisin+/-, NSE+/-, Gastrin+/-, Somatostatin+/-, CK7-/CEA-/CK20+/-; pancreatic (2 cases): NSE+/Synaptophisin+/CD56+/ChromograninA+/-, Gastrin+/Glucagone+/Insulin-/Somatostatin-/KL1+/MNF116+/CK7-/CK20-/VIM+/S100+/PGR+/-; pulmonary (2 cases): ChromograninA+/S100+/CD56+/-, NSE+/-, Synaptophisin+/-, TTF1+/Calcitonin-/KL1+/CEA+/CK7+/CK19+/CK20-; thyroid (3 cases): NSE+/ChromograninA+/Synaptophisin+/-, Calcitonin+/Thyreoglolbulin+/-, TTF1-/VIM+/S100+/KL1+/-, CK19+/-, CK7+/-. Some unusual particularities of immunophenotype were observed: digestive metastases were negative for CEA, and thyroidian metastases had no expression of TTF1. In our cases of poorly differentiated/undifferentiated metastatic carcinomas with NE differentiation, the primary origin could not be detected by IHC.
Conclusion: Histological diagnosis, associated with IHC algorithms, could suggest the primary origin in well or moderate differentiated MNCs. The origin of majoriry of poorly differentiated/undifferentiated NETs remains unclear after an IHC study.

P-71 WITHDRAWN

P-72
Anaplastic thyroid carcinoma with the remnants of a preexisting papillary thyroid carcinoma - case report

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Anaplastic thyroid carcinoma is an uncommon malignant tumor, more common in women than in men and it typically occurs in elder patients (7th decade or elder).

Clinically it is presented as a neck or thyroid mass that rapidly enlarges over a short period. In a high percentage of cases anaplastic carcinoma develops in patients with a preexisting thyroid lesion such as papillary carcinoma, but the probability of anaplastic transformation is considered low. CASE REPORT: A 70-year-old woman was admitted to hospital with a large bulky mass that distort the appearance of the neck and was firm to hard, and fixed to the adjacent structures, causing dysphagia and dyspnea. Ultrasonography showed hypofunctioning (cold) lesion. Bilateral total thyroidectomy was performed. Grossly, the tumor measured 11×9, 5×5 cm and occupied the whole thyroid gland, with extrathyroidal invasion. The cut surface was tan-white and firm to hard focally mottled and soft owing to necrosis and hemorrhage. Microscopically, the tumor had a solid nested growth pattern and was composed of epithelioid tumor cells, devoided of giant cells. The neoplastic cellular infiltrate was poorly differentiated, without colloid formation, markedly pleomorphic, with associated foci of confluent necrosis and individual cell necrosis. Tumor cells infiltrated extensively intrathyroidal and extrathyroidal tissue (muscle, nerves, and vessels) and vascular spaces. Remnants of preexisting papillary carcinoma of conventional and follicular type were present. Immunohistochemically tumor cells were positive for mixed high- and low-molecular weight keratins, EMA, vimentin and negative for thyroglobulin, chromogranin and calcitonin. As anaplastic thyroid carcinoma with the remnants of a preexisting papillary thyroid carcinoma is uncommon thyroid malignant tumor, it should be reported in order to increase the data in literature.

Gynaecological Pathology

P-73 WITHDRAWN

P-74
Morphologic and functional characteristic of smooth muscles' new growth of uterus un-der amplification of nucleolar organizers.

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It was recently discovered participation of argyrophilic proteins of nucleolar organizer areas in regulation of mitotic cycle and correlates with that of transcript activity of ribosome genes and also with indexes of proliferate cells activity.

Objective of the work is investigation of the activity of nucleolar organizers in leiomyomas and leiomyosarcoma corpus uteri myocytes.

Materials of investigation are 41 uterus, removed on account of myoma. 12 cases were cellular leiomyomas; 10- fibromyoma; 8 – bizzare leiomyomas; 6 - high-grade differentiated leiomyosarcomas and 5 are low-grade differentiated leiomyosarcomas. Histological sections of tumours were $AgNO_3$ imbued by two-stage method and calculated the average number of silver granules per 1 myocyte nucleus.

Results of the investigation made demonstrated that despite phase of the menstrual cycle in cellular leiomyoma, high-differentiated and low-differentiated leiomyosarcoma number of silver granules per 1 myocyte nucleus totaled 6,01±0,22, 12,11±0,46 and 18,8±1,06 respectively (ð<0,05). In bizzare leiomyoma number of silver granules totaled 6,6±0,33. The minimal number of silver granules was recorded in fibroma with marked sclerosis and hyalinosis i.e. 2,1±0,19.

Conclusion. Thus, calculation of the number of silver granules per 1 myocyte nucleus can be assume as a basis to differential diagnostics between cellular leiomyoma and high-differentiated leiomyosarcoma. Step growth of amplification of ribosome genes in chain from simple leiomyoma toward proliferating (mitotic activity) and further to leiomyosarcoma coupled with other molecular and biological criterion can be indexes of processes of growth of cellular anaplasia comparable with processes of dysplasia in epithelial structure. Presence of high level of silver granules in nucleus of atypical myocytes can be a sign of retrogression of their differentiation.

P-75
Intraoperative assessment of myometrial invasion in endometrioid adenocarcinoma

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Background: Intraoperative assessment of myometrial invasion in endometriod adenocarcinoma of the uterus can determine the surgical approach.

Methods: This is a retrospective study of 93 endometrioid adenocarcinomas surgically treated with hysterectomy, salpingooforectomy and pelvic lymphadenectomy with or without aortic lymphadenectomy during 11 years. The myiometrial invasion assessed by frozen sections during the surgical procedure is correlated with the one assessed in paraffin embedded tissue. Myometrial invasion is also correlated with other features such as age, histological grade according FIGO and lymph node involvement.

Results: Intraoperative study revealed absence of myometrial invasion in 19 cases, invasion of the inner half (IH) in 61 and of the outer half (OH) in 13. Definitive study showed absence of myometrial invasion in 9 cases, invasion of the IH in 68 and invasion of the OH in 16. The positive predictive value was 47% (95% CI: 25–70%) for absence of invasion, 93% (83–98%) for IH invasion, and 92% (82–100%) for OH invasion. In 10 cases intraoperative study was of absence of invasion and the definitive study revealed myometrial invasion (nine of them smaller than 2 mm). In four cases intraoperative study diagnosed invasion of the IH of the myometrium whereas the definitive diagnosis was of invasion of the outer myometrial half. In all of theses cases the uterus showed myomas and the tumor extensive necrosis. In one case the intraoperative diagnosis was of invasion of the OH and in definitive study only IH invasion could be demonstrated. In this case an endometrial ablation was done three weeks before. Myometrial invasion showed statistically significant relationship with age, histological grade and lymph node involvement.
Conclusions: Intraoperative assessment of myometrial invasion is a useful tool to establish the involvement of the inner or outer half. The absence of myometrial invasion by frozen section is less accurate.

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Coexistence of a mature cystic teratoma and a serous cystadenoma in the same ovary

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AIM: To describe a rare coexistence of two different benign cystic tumors in the same ovary, i.e. a mature cystic teratoma and a serous cystadenoma.
MATERIALS AND METHODS: A 32 year old female presented with a cystic tumor in the right adnexa. The tumor excision followed. We received a cystic specimen which measured 7.6×6×3 cm with two separated cystic lesions. Its surface was smooth. Ovarian tissue measuring 2.5×1×0.3 cm was also recognized. The first cyst was filled by serous fluid and had micropapillary architecture in limited regions of the inner surface. The mural thickness measured 0.2 cm. The second cyst was filled by sebum and hairs and its mual thickness measured between 0.2 to 0.4 cm. Multiple sections were taken, embedded in paraffin and stained with hematoxylin-eosin.
RESULTS: The microscopical examination showed the presence of two cystic tumors. The first was a serous cystadenoma and teh second was a mature teratoma. In the latter, skin, sebaseous and eccrine glands, hair follicles, intestinal and respiratory epithelium, cartilage, smooth muscle and adipose tissue were detected. No spsecific changes were identified in the excised ovarian tissue.
CONCLUSION: We believe that due to the rarity of this coexistence, after an exhaustive search of the literature, the pathologist should have it in mind.

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The implementation of prognostic index and risk grouping in surgically treated cervical carcinoma patients: A prospective validation study

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BACKGROUND: The objective of this prospective study was to validate the prognostic criteria defined by the results of our previous study in an independent population of surgically treated cervical carcinoma patients. METHOD: The study group consisted of 340 patients who underwent abdominal hysterectomy with pelvic lymphadenectomy as primary therapy between 2000 and 2005. Based on the scores of the variables (blood vessel invasion, lymph node metastases, tumor diameter, degree of inflammatory reaction at the invasive front, and minimum thickness of uninvolved cervical stroma/parametrial extension) and calculated prognostic index (PI) values, the patients were divided into three prognostic groups. RESULTS: During the follow-up period (range, 1.6–89.7, mean, 39.7± 22.2 months) recurrences were observed in 1% (1/97), 12.2% (16/131) and 23.2% (26/112) of the low-, intermediate-, or high-risk group patients, respectively. The 5-year disease-free survival (DFS) rates of the low, intermediate, and high-risk groups were 98.82%, 84.57%, and 74.01%, respectively. The differences in DFS rates were statistically significant ($P<0.0001$). In order to validate the model from our previous study, we have compared DFS rates between the groups. There was no difference in DFS rate between low-risk groups, in spite of the fact that majority of the patients in this study were not irradiated, while radiotherapy was administrated invariably to all the patients included in the original study. Similarly, DFS did not differ significantly between the intermediate-risk groups from both studies, which could be expected since radiotherapy was administrated to majority of the patients (125/131) in this study. In contrast, the high-risk group patients in this study had significantly higher DFS rate (74.01% vs. 44.24, $P=0.0010$), probably as the result of

the adjuvant chemotherapy administrated to 69% of them. CONCLUSION: PI could be a sound and reliable basis for an appropriate planning of the following therapeutical strategy of the surgically treated cervical cancer patients.

P-78

Extraovarian granulosa cell tumor: first case report from Colombia

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Objective: To describe the case of an extra ovarian GCT in a Colombian woman and to discuss about these malignancies.
Case Report: 69 years old female Patient, who came to the doctor with an abdominal pain, vomiting and no depositions, with 8 days of evolution.The woman presented pre existing conditions of hysterectomy with bilateral salpingoophorectomyfor placental acretism. At physical examination a mass was identified, locatedin the hypochondrium and left flank of 10×15 cm. This was confirmed inthe echogram and tomogram studies. Therefore the patient was taken to surgery,and a encapsulated retroperitoneal tumour was found.
PathologicalStudy: The pathology service received a 12×11.5×10 cm.and 950 gr. solid-cystic mass. The heterogenic surface court was well encapsulated, with a solid area (70%)and a cystic area with hemorrhagic contain (30%). The courts stained with hematoxylin andeosin were identified neoplastic lesion formed by round-nucleus cells withlongitudinal cleft and few eosinophil cytoplasms, arranged in nests, trabeculaeand solid sheets with plenty Call-Exner corps formation. Immunohistochemistry was positive forinhibin and negative for Melan A Calretinina. Making an extraovaric adult type GCTdiagnose.
Conclusions: The GCT can be originated from extraovarictissues in extremely rare cases, only 8 cases reported globally. These caseshave been informed in the broad ligament, adrenal glands, uterus and retro peritoneum. This case was originated in the retro peritoneum. Itis believed that the origin from ectopic gonadal tissue, or directly from themesonephron.

P-79

PBNIP-3-EXPRESSION AND ITS ASSOCIATION TO PATTERN OF INVASION AND PERITUMORAL STROMAL RESPONSE IN CARCINOMA OF THE CERVIX UTERI

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Background: Finger-like and spray-like pattern of invasion represent the most common types of tumoral growth patterns in carcinoma of the cervix uteri (CX) invading host tissue (Horn et al. 2006). Infiltrative growth usually induce new matrix formation by activating the peritumoral stromal cells; that is, desmoplastic stromal reaction (DSR) at the front of invasion (juxtatumoral stroma). Infiltrative growth and peritumoral stromal remodelling might be involved by apoptosis of tumor cells. BNIP3 is a mitochondrial protein and a proapoptotic member of the Bcl-2 family and expressed in different types of human cancer. Nothing is known regarding the association of BNIP-3 expression in CX and parameters of infiltrative growth.
Method: Biopsies of 50 cervical cancers (FIGO stage IB to IV) were stained with an anti-BNIP3 antibody. In accordance to own previous studies (Leo et al. 2006), cytoplasmic as well as nuclear staining results were counted as positive. Staining was counted performing an immunoreactive score (IRS = staining intensity (0–3) x calculated percentage of positive stained tumor cells (1–4). The IRS was correlated to pattern of invasion (finger-like and spray-like) and the grade of DSR. DSR was scored as none/weak and moderate/strong.
Results: Spray-like pattern of invasion was associated with high expression of BNIP-3 (mean-IRS 5.8 vs. 3.2; $p=0.17$). High juxtatumoral stromal remodelling, characterised by strong desmoplastic change was also associated with BNIP-3 overxpression (mean IRS 3.6 vs. 5.7; $p=0.082$).
Conclusions: The results suggest that alteration of the mitochondrial pro-apoptotic BNIP-3 is involved in the mode of pattern of invasion (representing the grade of tumor cell dissociation) and juxtatumoral stromal remodelling in carcinoma of the cervix uteri.

P-80

PATTERN OF INVASION IS OF PROGNOSTIC IMPACT IN CARCINOMA OF THE CERVIX UTERI

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Background: Different patterns of invasion (representing different grades of tumor cell dissociation) are associated with prognostic outcome in cancer. We evaluated the prognostic value of different patterns of invasion (PI) in cervical carcinomas (CX).

Methods: 611 surgically treated CX (FIGO IB to IIB) were re-evaluated histologically regarding the PI, using a three-level scoring system. Closed PI was defined as cohesive growth with well-delineated (pushing) borders. In finger-like PI the tumor grows in solid cords/trabecles. Highly dissociative growth in small groups or single cells was defined as spray-like PI. Types of PI were correlated to tumor stage, histomorphologic factors and prognostic outcome.

Results: 60% of the tumors showed a spray-like PI, 30% a finger-like PI, and only 7.4% were of the closed type. Spray-like PI showed a significant correlation with advanced stage disease, lymphovascular space involvement, poorly differentiated tumors and pelvic lymph node metastases. Spray-like PI was accompanied by a reduced 5-year overall survival when compared to the finger-like and closed PI (68.7% vs. 80.9% vs. 88.5%; p=0.0004). The prognostic impact of the PI disappeared in node-positive patients (p=0.06), but persisted in patients without pelvic lymph node disease (p=0.03). In multivariate analysis, using COX-regression model, the PI represented as independent prognostic factor.

Conclusions: Spray-like PI, (i.e. highest degree of tumor cell dissociation) is associated with advanced tumor stages, increased rate of recurrency and a reduced overall survival. In separate analysis of patients with and without lymph node metastases, the impact of PI persisted only in node-negative cases as an prognostic factor.

P-81

TUMOR SIZE IS AN INDEPENDENT PROGNOSTIC FACTOR IN FIGO STAGE II CERVICAL CARCINOMA

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Background: Tumor size is a well recognised prognostic factor in early stage cervical carcinoma (CX). But, limited knowledge exists about the value of timor size in surgically treated CX with extrauterine extension.

Methods: 245 cases of local advanced CX (FIGO stage IIA and IIB) who received upfront surgery, were evaluated regarding tumor size, regarding the prediction of pelvic lymph node involvement, recurrence free and overall survival during a median follow-up time of 54 months (95%-CI 45.4 – 62.6 months). Tumors larger than 4 cm were defined as bulky stage disease.

Results: Bulky disease was seen in 46.1% (113/245). 60.2% of these patients showed pelvic lymph node involvement, compared to 42.4% (56/132) in non-bulky tumors (p= 0.006; odds ratio: 2.2 [95% CI: 1.3 – 3.6]). Patients with bulky tumors showed an increase of recurrent disease (40.2% vs. 28.0%; p=0.045). The relative risk for recurrent disease was 1.97 (95%-CI: 1.3–3.0). The 5-year overall survival rate was significant lower (67.7% [95% CI: 58.2–74.8] vs. 49.5% [95% CI: 36.8–59.1]; p=0.0015).

In multivariate analysis, tumor stage, pelvic lymph node involvement and maximal tumor size, were independent prognostic factors.

Conclusions: The results suggest that tumor size, defining bulky disease as tumors larger than 4 cm, is of prognostic impact also in FIGO stage II cervical carcinomas. A revised FIGO/TNM classification system similar to the subgrouping of stage IB CX is recommended for stage II using a cut-off value of 4 cm as discriminator: stage IIA1 and stage IIB1 for tumors with ≤ 4 cm and IIA2 and IIB2 for tumors > 4 cm (i.e. bulky disease).

P-82

HEPATOID CARCINOMA OF THE OVARY

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Backround: Hepatoid carcinoma (HC) of the ovary is an ovarian carcinoma showing areas morphologically comparable to those of hepatocellular carcinoma. These areas are composed of cords of polygonal cells separated by sinusoids, occasionally featuring bile production and/or bile canaliculi formation. Hepatoid carcinoma is thought to be a different subtype from hepatoid-type yolk sac tumor based upon its pathologic features. In contrast to the much younger age of patients with ovarian hepatoid yolk sac tumor, the ages of the patients with hepatoid carcinoma ranged from 42 to 78 and none of them had gonadal dysgenesis or recognizable germ cell components within their tumors. The histiogenesis of HC is unknown but it has been described an association with tubular adenocarcinoma and serous carcinoma. This sug-

gests that it is a metaplastic tumor and represents a variant of the surface epithelial-stromal tumor.
Method and results: We describe a case of a 42 year-old woman who presented with abdominal pain. Physical examination and CT-scan revealed a large tumor in the left adnexal. She underwent a total hysterectomy and bilateral salpingo-oophorectomy with omentectomy. A left ovarian mass measuring 11×7×7 cm was found. The tumor was diagnosed as hepatoid carcinoma of the left ovary.
Conclusion: Based on the review of the literature we discuss the histological and immunohistochemical findings of these tumor and their differential diagnosis from other ovarian neoplasms, especially from hepatoid yolk sac tumors.

P-83

Immunolocalization of neurotrophin receptors tyrosine kinase receptors in Endometriosis Associated Ovarian Cancer (EAOC).

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Neurotrophins and their receptors have been localized in reproductive tract tissues including the ovary and endometrium. Of the neurotrophin receptors, tyrosine kinase receptor B (TrkB) is thought to be important in regulating malignant epithelial cancer cell resistance to attachment free mediated programmed cell death (Anoikis). Therefore, our objective was to immunolocalize TRK B in patients with a diagnosis of ovarian cancer and endometriosis. Endometriosis-associated ovarian cancer (EAOC) is a rare disease of unknown etiology that appears as either a low-grade tumor of endometrioid cell type or as a clear cell tumor in young women. Archived samples of formalin-fixed, paraffin embedded blocks from 15 women were obtained from the Department of Pathology at McMaster University Medical Center. Tissue sections of ovarian tumor, ovarian endometriosis, eutopic endometrium, and normal ovarian stroma were incubated with a polyclonal rabbit anti-human TrkB (1:250), Vectastain ABC Reagent, diaminobenzidine tetrahydrochloride as the chromagen, and counterstained with hematoxylin. TrkB positive epithelial cells were present in sections from 2/2 subjects with grade II ovarian endometriod tumors, 3/3 ovarian papillary serous tumors, and 2/2 with ovarian clear cell tumors. By comparison, ovarian endometrioid grade I tumors were negative (7/7), and immunostaining was generally absent in ovarian endometriosis (11 negative, 3 weak staining and 1 positive) and the eutopic endometrium (12 negative, 1 positive, and 1 weakly positive). TrkB is expressed in epithelial cells of more aggressive EAOC compared with less aggressive tumors or benign tissues.

P-84

Undifferentiated endometrial sarcoma with sex cord and smooth muscle immunophenotypical differentiation: a case report

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Backgound: Endometrial stromal sarcomas are rare tumours with two morphologically distinct entities; one is classified as low grade endometrial stromal sarcoma and the other as undifferentiated (high grade) sarcoma. Sometimes the classification of these tumours is a challenge because different histological cell types may be encounted in the same lesion. We present a case where immunohistochemistry was influential in the final histiotypic classification of uterine sarcoma.
Method: A retrospective review of a uterine tumour difficult to classify by histology alone. The lesion occurred in a 58 year-old lady and mesured 12×11×8 cm.
Result: The lesion involved the entire myometrium and replaced the endometrial lining. It was made of an admixture of two different sarcomatous growths. The main component consisted of a monotonous, small round cell proliferation with occasional sex cord like pattern and the alterations were compatible with a low-grade endometrial sarcoma. The second component contained a pleomorphic mesenchymal proliferation resembling a poorly differentiated epithelioid leiomyosarcoma. The low-grade cellular component reacted strongly and diffusely for actin, desmin, cladesmon, CD56 and ER but not for CD10. On the other hand, the high-grade carcinomatous areas stained strongly but focally for CD10 but not for smooth muscle immunomarkers. Neither component stained for PR, inhibin, calretinin, chromogranin, synaptophysin, CD99, epithelial markers and S-100 protein.
Based on these findings, the diagnosis of undifferentiated endometrial sarcoma with sex cord and smooth muscle differentiation was favoured.
Conclusion: In the field of gynaecological sarcomas, histological classification is sometimes difficult. Correlating morphological features with immunohistochemistry may help to better histiotype uterine sarcomas.

P-85

Ki-67 and P16ink4a immunoexpression strongly correlates with increasing grade of CIN and HPV infection

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Background: MIB-1 immunoquantitative features have been correlated with the grade of dysplasia in cervical epithelium, while P16 accumulation has been related to the presence of high grade intraepithelial lesions (HSIL) and detection of human papillomavirus (HPV). The aim of this study was to assess the value of these markers in assisting CIN grading.

Method: Our series included 7 normal cases, 8 HPV-suspicious, 42 CIN I, 31 CIN II, 35 CIN III and 2 invasive cervical squamous-cell carcinomas collected from 109 women. Immunohistochemical analysis of formalin-fixed, paraffin-embedded samples was performed by ABC immunoperoxidase staining, using mouse monoclonal antibodies Ki-67 and $P16^{INK4A}$. Results were reviewed by two pathologists. MIB-1 was classified in four categories according to the extension of nuclear staining within the epithelial thickness. Nuclear or nuclear and cytoplasmic diffuse strong staining was considered positive for P16. The presence of HPV was assessed by a commercial PCR-based assay and typified by hybridization to a low density microarray.

Results: A highly significant association ($p<0.0001$) was observed using Kendall's tau-c between increasing grade of CIN and MIB-1 expression. Further Chi-squared analysis of the 2 by 2 contingency table proved MIB-1 especially useful in discriminating the presence of CIN ($p=1.004e-^{09}$). Equally, a significant relation ($p=2.2\ e^{-16}$) was found between P16 immunoexpression and the presence of HSIL lesions, with 98% specificity, 98% positive predictive value and 78% negative predictive value. P16 accumulation was also indicative of the presence of HPV which was detected in 51 out of 53 P16 positive samples. Furthermore, 50 (98%) of these HPV positive cases contained High Risk HPV.

Conclusion: The combined use of these two markers provides a valuable tool in assessing the grade of CIN, the presence of HR-HPV infection and verifying the diagnosis of equivocal cases.

P-86

SYNCHRONOUS PRIMARY GYNOCOLOGICAL

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Background Synchronous tumor of endometrium and ovary may indicate either independently developing neoplasms or metastatic disease. Synchronous primary cancer occurring in both organs is relatively rare. Ovarian carcinosarcoma is very rare and aggressive tumor representing less than 2% of ovarian cancers. Papillary serous endometrial adenocarcinoma is a frequent endometrial tumor that is thought to occur under the influence of the estrogen receptor pathway. The etiology of synchronous malignancies is not clear, but the response of embryologically similar tissues to simultaneous carcinogens has been postulated

Method We report a rare case of simultaneous ovarian carcinosarcoma and papillary serous endometrial adenocarcinoma occurring in a 56-year-old-woman and we discuss clinicopathologic features and histogenesis of this association

Results The patient was admitted for post menopausal uterine bleeding, leucorrhoea and pelvic pain. Radiological explorations showed endometrial thichning with a left latero-uterine mass infiltrating the rectum. Histological examination of total abdominal hysterectomy with bilateral salpingo-oophorectomy specimen concluded to ovarian carcinosarcoma associated with papillary serous endometrial adenocarcinoma.

Conclusion To our knowledge, this is the first case in aspect of accompanying ovarian carcinosarcoma to papillary serous endometrial adenocarcinoma.

P-87

Gestational Choriocarcinoma. Study of an autosy case

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BACKGROUND: Gestational Choriocarcinoma may occur subsequent to a molar pregnancy, abortion, ectopic pregnancy or an uneventful gestation. Abnormal vaginal bleeding is one of the most frequents signs of presentation. However, occasionally, the discovery of the disease is associated with the presence of metastasis, being the lung the most frequent site, and haemoptisis the presenting symptom.

CASE REPORT: A thirty weeks pregnant, thirty-nine year old caucasian woman, presented at a local hospital with fever, dyspnoea, haemoptisis and toxic syndrome. Intersti-

tial alveolar infiltrates were seen in the thorax plain x-ray. Abdominal ecography demostrated a retropubic solid tumor suggestive of lymph node. She was admitted to the intensive care unit and underwent emergency caesarean surgery. During the intervention, a biopsy of the retro pubic lymph node was taken.
RESULTS: Pathology examination of the retro pubic lymph node was diagnosed as metastatatic choriocarcinoma. Thoracic CAT scan, obtained after the caesarean intervention, was rendered compatible with metastatic disease affecting both lungs. In spite of appropriate chemotherapy treatment, the patient took an unfavourable course and died. At autopsy, a necrotic vaginal nodule of 4 cm in diameter was identified. Aditionally multiple necrotic nodules were found in both lungs, affecting the pleura. Microscopic examination revealed diffuse alveolar damaged.
CONCLUSIONS: Although gestational choriocarcinoma may occur subsequent to a molar pregnancy, is not infrequent to see it in an otherwise normal gestation, and is a diagnosis to be considered when confronted with haemoptysis in a pregnant woman.
Patients at high risk have a survival rate of 85–95%. Death caused by choriocarcinoma is usually associated, as in our case, with distant haemorrhagic events and respiratory insufficiency.

P-88
P16INK4A IN CIN1. IS IT THE BIOMARKER OF PROGRESSION TO HIGH GRADE CIN ? A FOLLOW-UP STUDY of 154 PATIENTS CORRELATED TO HPV STATUS.

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Background: The management of CIN1 is difficult due to the lack of predictive criteria of progression. The objective of our retrospective study was to determine whether there is a correlation between the immunohistochemical staining pattern of $p16^{INK4A}$, a surrogate marker of HR-HPV activity in cervical epithelia, the HPV status and the outcome of CIN1.
Method: Biopsies of 154 patients with initial diagnosis of CIN1 and a mean follow-up of 76.8 months (12 – 240 months) without treatment were reviewed by two pathologists in a double-blind manner. Immunohistochemistry of $p16^{INK4A}$ (clone 16P07) and HPV detection by in situ hybridisation (Inform HPV III, Ventana) were performed on serial sections of paraffin embedded tissue from initial and control biopsies of the cervix. According to the evolution, three groups were defined: regression, persistence and progression.
Results: Reviewers confirmed 80 CIN1 (51.9%), whereas 18 biopsies were considered as normal, 21 as CIN2 and 35 as "squamous atypia NOS" (κ=0.64). Among 80 CIN1, 51 regressed (63.8%), 18 persisted (22.5%) and 11 progressed (13.8%).
$P16^{INK4A}$ staining was diffuse (>25% of cells), sporadic/focal (< 25%) or negative (< 1%) in 56 (70%), 18 (22.5%) and 6 (7.5%) cases respectively. All 56 HR-HPV positive CIN1 showed positive $p16^{INK4A}$ staining (45 diffuse, 11 focal). The 11 CIN1 with progression were all $p16^{INK4A}$ positive: 10 diffusely, 1 focally. Among the CIN1 without progression, 63 were $p16^{INK4A}$ positive (91.3%) and 6 negative (8.7%). Considering progression, $p16^{INK4A}$ positive staining has a sensibility, specificity, PPV and NPV of 100%, 8%, 14.9% and 100% respectively.
Conclusion: $P16^{INK4A}$ is an excellent diagnostic marker for HR-HPV induced CIN. Its low PPV for progression does not support the hypothesis of a prognostic marker. On the other hand, with a NPV of 100%, the absence of $p16^{INK4A}$ staining is highly correlated to the regression of CIN1.

P-89
IMMUNOHISTOCHEMICAL ASPECTS OF DIFFERENTIAL DIAGNOSIS IN MATURE AND IMMATURE TERATOMAS

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Background: Teratomas usually manifest as masses in descended or undescended testes, in extratesticular tissues where germ cells tumors occur and in ovary in females. Teratomas are germ cell tumors composed of an array of tissues derived from two or three embryonic layers in any combination. Immature teratomas occur predominantly in children and young people and represents 3% of ovarian teratomas and 5% of testicular teratomas.
Method: Our report presents two cases of immature teratomas. The first patient is a 34 year old man admitted in Surgical Department of County Hospital of Constanta for left testicular increased size in last six months and the second case is a 15 years old girl hospitalized in the same department for retroperitoneal tumoral mass. CT scan shows in both cases o testicular and ovarian mass with nonhomogeneous structure, surrounded by liquidian areas.
Results: Histological exam revealed in both situations immature teratoma with mesenchimal, epithelial adenocarcinoma and neuroepithelial features and mature zones with

condroid tissues. There are no specific immunohistochemical markers for teratoma, but individual antibodies can be used to identify particular tissues. In both cases, chromogranin, synaptophysin and AFP were positive. There was no correlation found for the rest of IHC markers used for these cases, markers that were positive or negative (PLAP, beta-HCG, CD 117, S 100 and other). Prognosis was favorable after surgery on young man, but the young woman was given chemotherapeutical support.
Conclusions: Immature teratomas are rare germ cell tumors. The incidence in children is 2% to 3% of ovarian and testicular tumors. Microscopic exam shows the certain diagnosis. In both cases the IHC confirms immature teratoma diagnosis. Imature intestinal-type of glands were positive for AFP, and neuroepithelial structures were positive for chromogranin and synaptophysin, that sustain immature teratoma.

P-90

Galectin-3 Expression in Human Endometrium and Decidua During The Menstrual Cycle and in Gestation

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Galectin-3, one of the member of beta-galactozide binding lectin family, binds to basal membrane glycoprotein and has a role in many biological events. Galectin-3 is important mediators of inflammation. Endometrial function and implantation involve many inflammatory mediators, galectin-3 might contribute to the modulation of the endometrial immune system. Galectin-3 have been shown to play an important role in cell adhesion, migration and chemotaxis. Galectin-3 may be involved in the establishment of endometrial receptivity by regulating the proliferation and adhesion of endometrial cells. In our study, the secrotory *endometrium* (n=30), proliferative endometrium (n=34) and *decidua* (n=41) were collected from patients undergoing *currettage* for benign reasons and from *induced abortions*. Expression of galectin-3 during the proliferative phase was considerably low ($p<0.001$). Expression of galectin -3 increased significantly in the late secretory phase *endometrium* and in the decidual tissue ($p<0.001$). This shows that galectin-3 plays an important role in implantation and beta-galactozide binding lectin family agents, like galectin-3, which take role in adhesion and inflammation can be used for the treatment of abortus in future.

P-91

Endometrial intraepithelial neoplasia and carcinoma- correlations between immunohistochemical markers expression

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Background: Identification of endometrial precancers has implemented EIN (endometrial intraepithelial neoplasia), defined on a combined molecular, histomorphometric, and clinical outcome diagnostic schema. Our research was designed to identify immunohistochemical markers, focused on several directions: hormonal receptors (ER, PR), proliferation (PCNA), apoptosis system (Fas, FasL), and matrix enzymes (MMP-2, MMP-9). Method: Immunohistochemistry was applied on 12 cases of non-neoplastic endometrium, 15 cases of EIN, and 6 cases of endometrial carcinomas. Results: ER and PR showed a concordant expression, with an increased expression in EIN compared to anovulatory endometrium, both in glandular and stromal components, and occasionally endothelial staining. ER and PR presented a focal overexpression in G1 and G2 endometrial endometrioid carcinomas and a weak expression or absence in undifferentiated (G3) endometrial carcinomas. PCNA index was variable but high in EIN and carcinomas, in glandular, stromal, and endothelial cells, correlated with ER and PR expression, with the maximum staining in the carcinomatous front of invasion. Fas/FasL system showed a deregulated expression in EIN and carcinomas, with a reduced Fas expression and a low FasL expression, in epithelial and immune cells. MMPs expression was increased in EIN, both in glandular and stromal components. Furthermore, MMP-9 expression was higher than that of MMP-2 and supplementary noticed in migrated cells and in myometrium. Conclusions: Overexpression of steroid receptors in EIN demonstrates the role of hormonal stimulation in carcinoma pathogenesis. PCNA, ER and PR coordinated increased expression suggests a correlation between hormonal status and cellular proliferation. Deregulated expression of Fas/FasL system probably enhances endometrial proliferation, immunohistochemical aspects suggesting that FasL positive epithelial endometrial cells, together with immune cells, may induce apoptosis in Fas positive cells. As markers of extracellular matrix degradation, MMP-2 and MMP-9 demonstrate the role of epitheliostromal interactions in the development and progression of endometrial neoplasia.

P-92

Prevalence of high risk types of human papillomavirus in previously unscreened, bosnian women in last eight years

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Background: Cervicalcancer is an important public health problem for adult women in developingcountries where it is the most or second most common cancer among women. Thevast majority of cervical cancer cases are caused by infection with certainsubtypes of human papilloma virus (HPV). Worldwide, cervical cancer claims thelives of 231 000 women annually, over 80% of whom live in developing countries.In developed countries, initiation and sustenance of cervical cytologyprogrammes involving the screening of sexually active women annually, or oncein every 2–5 years, have resulted in a large decline in cervical cancerincidence and mortality. Testing women ≥age 30 for high risk HPV is more sensitive as a primari screen than currently practiced cervical cytology.

Method: Identification of the presence of human papilomavirus was carried out by the Digene Hybride Capture II test for all patients.

Results: 4696 previously unscreenedwomen were tested for the presence of the HPV. 33.36% were positive for theHRHPV. We also analized the age of the women at the moment of the testing andassociation with some other type of infection (bacterial or fungal).

Conclusion: Data of the genitalHPV DVA prevalence in representative samples of populations in difierentcountries are lirnited, in spite of the largc number of rcports alreadypublished. When available the HPV DNA prevalence would be useful in makingprediction of expecting incidence and likely time trends of cervical cancer.Typical y the proportion of the HPV female carriers have been placed in the15–40% in the young, sexually active age group and between 3–10% in the 35 andabove age group.

P-93

LYMPHOEPITHELIOMA-LIKE CARCINOMA OF THE FEMALE GENITAL TRACT. AN IMMUNOHISTOCHEMICAL AND MOLECULAR STUDY OF 5 CASES.

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Introduction: Lymphoepitheliomas were originally described as a nasopharingeal neoplasm related to Epstein-Barr virus. Lymphoepithelioma like carcinoma (LELC) can also be found in the female genital tract (uterine cervix, endometrium and one case, not reported previously, in the fallopian tube). Importantly, LELC has better prognosis than squamous cell carcinoma variants, therefore its differential diagnosis has great importance. It's association with Epstein-Barr virus is still controversial in the uterine cervix. In addition, HPV is also a well known risk factor related to development of cervical cancer although the role that HPV plays in LELC development is not well studied. The aim of this study is to evaluate possible relationships between HPV, EBV and LELC in the female genital tract.

Material and methods: 4 cases of LELC of the uterine cervix and 1 case of the fallopian tube have been collected. Immunohistochemistry for p16, p53, Ki67, PCR based methods to detect EBV (EBNA2 and LMP1) and HPV serotyping by the HPV Clinical Arrays Kit, have been performed.

Results: The five cases were negative for EBV sequences. In two cases HPV was detected (serotypes 16 and 31) and, in three cases was negative. Immunohistochemistry showed overexpression of p16 in all cases and interestingly, p53 was positive in one of the cervical carcinomas (with HPV16 serotype) and in the tubaric carcinoma. In all cases, the Ki67 index was higher than 35%.

Conclusions: HPV was not found in every LELC and no EBV infection could be demonstrated in our series. On the contrary, p16 was overexpressed in all cases, including those without HPV infection. Our results together with some other reported series, suggest that other factors may be related with the development of gynecological LELC. Finally, our findings indicate that p16, p53 and HPV serotyping does not seem to help in the differential diagnosis with other squamous cell carcinoma variants.

P-94

Heterologous Mixed Mallignant Mullerian Tumor of the uterus. A case report and review of the literature.

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Background: Heterologous Mallignant Mixed Mullerian Tumor (HMMMT) of the female genital tract is a rare and highly malignant neoplasm with poor prognosis occurring mainly in postmenopausal women. Endometrium is the most frequent location of HMMMTs accounting for 1.5% of all uterine malignancies. Here we present such a case.

Method: A 70-year old patient admitted to our hospital with a stagonoid uterine bleeding and pain in the hypogastrium. Ultrasonographically the endometrium was thickened measuring 7 mm. The patient underwent a total hysterectomy measuring 11.5×11.5×6 cm and bilateral oophorectomy. We also received a specimen measuring 46×11×2 cm from the omentum and a tissue specimen from the urinary bladder wall measuring 2.5×2×1.5 cm.
Results: Grossly the tumor was soft and friable, tan to grey in color and accompanied by focal and extensive hemorrhage and necrosis. After thorough histological examination it was about a HMMMT involving most of the myometrial wall, the endometrium, the left salpinx and the mesosalpigium. Histologically the tumor consisted of high cellular spindle cells, compact small round cells and bizarre giant cells resembling leiomyosarcoma or fibrosarcoma. The stroma of the tumor was myxoid and highly vascular. Heterologous elements in the form of cartilage and bone were also present and the mitotic activity was marked. Immunoreactivity with keratin and EMA was found and excluded any carcinomatous component. SMA and myoglobulin was also performed to exclude rhabdomyosarcomatous component.
Conclusion: HMMMT is regarded by many authors as a subtype of uterine sarcoma with a significantly worse prognosis than FIGO grade 3 endometrial carcinomas. Breast Cancer survivors who were treated with aromatase inhibitors for many years are reported to have higher incidence of uterine sarcomas. Stage appears to be important prognostic factoring HMMMTs, although heterologous components are not certain whether are important in the prognosis. Only those HMMMTs with rhabdomyosarcoma differentiation were linked with worse prognosis.

P-95

How to differentiate Uterine Smooth Muscle Tumors with standard smooth muscle differentiation.

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Background: Many variants of leiomyoma mimicking malignancy exist. Here we present three rare cases of a Symplastic Leiomyoma (SL), Smooth Muscle Tumor of Low Mallignant Potential (SMTLMP) and High Cellular leiomyoma (HCL).
Method: The 1st case was about a 52-year old patient presented with hemorrhage, urgency and constipation. She underwent a total hysterectomy and a whitish-grey, circumscribed tumor measuring 25×22 cm was found in the uterus. The 2nd case was an incidental finding of leiomyoma-like lesion discovered ultrasonographically and involved a 32-year old patient treated for infertility. Myomectomy was performed and two tumors measuring 5×4.5 cm were obtained. The 3rd case was a 38 year old woman presented with menorrhagia. Total hysterectomy was performed and on dissection a voluminous cystic mass was observed measuring 9 cm.
Results: We examined in all three cases the degree of cellularity, atypia, mitosis and coagulative necrosis. Microscopically the 1st and the 2nd tumor were composed mainly of multinucleated, giant cells with hyperchromatic, prominent chromatin with moderate cellularity and pseudonuclear cytoplasmic inclusions. In the 3rd case the tumor was composed of monotonous appearing cells with round nuclei with evenly dispersed chromatin, small nucleoli and scant cytoplasm and high degree of cellularity. Atypia was observed only in the first case. The mitotic rate in all cases was low (<10/10HPF), and coagulative necrosis was found only in the 2nd case. According to our findings the first case was about a SL, the second was a SMTLMP and the third was HCL.
Conclusion: The diagnosis is based mainly on exclusion and the trivariate rule introduced by Kempson et al., that uses mitotic index, the degree of atypia and the coagulative tumor cell necrosis is helpful. Some authors use the term SMTLMP and some others instead use the term Smooth Muscle Tumor of Uncertain Unknown or Undetermined Malignant Potential.

P-96

Evaluation of angiogenesis in ovarian mucinous epithelial tumors: comparative analysis of new methods of semiautomated quantification

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Background: In some neoplasms, angiogenesis seems to favor not only growth, but also tumor spreading, therefore, bearing diagnostic/prognostic value. In ovarian mucinous tumors, due to shortage of studies and their methodological variability, the role of angiogenesis is not yet established.
Aims: to evaluate the diagnostic role of angiogenesis in ovarian epithelial mucinous neoplasms, comparing and validating different methods of microvessel quantification.
Methods: Twenty-one mucinous epithelial tumors (twelve benign, four borderline and five malignant), diagnosed at the Department of Pathology of our institution, from 01/1997 to 12/2003, were assessed through immunohisto-

chemical technique, using CD34 marker. Microvessel density (MVD) and parameters based upon endothelial area (total endothelial area [tENDA], mean endothelial area [mENDA] and percentual endothelial area [%ENDA]) were quantified using manual and semiautomated protocols with Imagelab (version 2.4). These different quantification methods were tested and compared for their relative ability to discriminate between the three diagnostic entities.

Results: In general, angiogenesis estimates were higher in the borderline (MVD=111.0±52; tENDA=15140.35±3000; mENDA=7.95±2) and malignant groups (MVD=95.6±60; tENDA=17712.13±17771; mENDA=31.83±68), in comparison to the benign group (MVD=94.5±46; tENDA=15292±9475; mENDA=7.94±3), except for % ENDA, whose mean estimates were higher within the benign and borderline groups. However, in none of these parameters the difference between groups reached statistical significance.

Conclusion: The present data, while preliminary, showed no significant association between parameters of vascular quantification and biological behavior of ovarian mucinous tumors, regardless of quantification method. The small number of cases and wide dispersion of values may have contributed for this finding. (JV and LA are supported by the National Council for Scientific Research, CNPq).

P-97

Selection of image format (TIFF vs. JPEG) in semi-automatic quantitative analysis of ovarian tumor angiogenesis

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Background: Although well-established in many neoplasms, the role of angiogenesis in ovarian tumors is still controversial. Computer Image Analysis Systems (CIAS) potentially improves accuracy and reproducibility of current vascular quantification. In CIAS, the use of high quality images (e.g., TIFF) is preferred over compressed images (JPEG) because, theoretically, loss of information observed in the latter could jeopardize quantitative analysis. However, this has not yet been formally demonstrated.

Aim: to compare distinct image formats with respect to different protocols of angiogenesis quantification.

Methods: Forty epithelial ovarian tumors (14 serous and 26 mucinous, including benign, borderline and malignant specimens) were included. Using a Nikon E200 microscope, adapted to a Nikon 995 Coolpix CCD camera, one TIFF and two distinct JPEG images ("basic and fine") were obtained from a single low-power field of a vascular 'hotspot', per case. Later, two more types of JPEG ("high and low") were obtained from the conversion of the TIFF images, using Adobe Photoshop. All five image types were assessed for microvessel density (MVD) and mean endothelial area (mENDA), using Imagelab 2.4 software.

Results: mean differences between TIFF and JPEGs were significantly different from zero ($p<0.001$) and varied from 89,5 to 1234,8, for MVD, e between -0,2 e -9,1, for mENDA. In both variables, the agreement between image formats (mean difference ±2X the standard deviation) was higher between TIFF and high quality JPEGs ("fine/high"). Furthermore, Spearman's correlation coefficient was high between TIFF and all (0.82–0.99) but JPEG "low" (0.61–69).

Conclusion: the significant differences found between TIFF and JPEGs precludes direct comparison between angiogenesis values obtained from different format images. Nevertheless, given their high degree of agreement/correlation with TIFF, high quality JPEGs can be used instead of TIFF images, whenever memory and computer processing speed are limiting factors. (JV and LA are supported by the National Council for Scientific Research, CNPq).

P-98

Malignant struma ovarii

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Malignant struma ovarii is a very rare form of germ cell tumor. Malignancy is represented by follicular carcinoma, papillary carcinoma or a mixed pattern, similar to the types of thyroid carcinoma. It is difficult to find out the real incidence of malignant struma ovarii, due to the different criteria for its diagnosis. Some authors described an incidence as high as 37% of struma ovarii cases and others estimated an incidence lower than 5%. Anyway, the malignant transformation is an uncommon event. Here we report one case of malignant struma ovarii. The 69-year-old woman complained of a three-month history of abdominal distension. A pelvic computed tomogram imaging study indicated a large irregularly-marginated complex mass in the right pelvic cavity and large amount of ascites. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. On operation, the right ovary was enlarged and partially ruptured. The right pelvic cavity showed large amount of a little bloody ascites about 2000 cc. On gross examination, the right ovary measured 10.5×2.5×5.7 cm and weighed 134.0 gm. The right ovary was multinodular and multicystic mass. It contained gelatinous material and sebum-like material with hairs.

Partial solid portion is pale tan to white and firm. No endophytic papillary growth is present in any of the loculi. The right ovary was predominantly composed of follicular structure of thyroid gland. Within the struma ovarii, a small focus of papillary thyroid carcinoma was found. Immunohistochemically, the tumor was positive for thyroid transcription factor-1.

P-99

Atypical localization of lymphoblastic lymphoma-case report

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Background. Lymphoblastic lymphoma with uterine localization is a rare malignant neoplasm, present in 1% of women with extranodal lymphoma. 25% of malignant lymphomas have extranodal sites and only 1% of them have uterine localization. Uterine malignant lymphoma spreads frequently in cervix and vagina. Beside ovarian lymphoma, uterine lymhoma affects females of all ages, especially the ones in the 4-th decade. The cytological diagnosis of uterine lymphoma becomes more difficult because of the infiltrative evolution in the muscular layer.
Method. This paper reports a case of uterine lymphoma diagnosed in a 36 years old patient hospitalized in Gynecology Clinic of Clinical Emergency County Hospital, Constanta. The clinical diagnosis was massive pelvi-abdominal tumor, myelodisplastic syndrome and secondary anemia.
Results. The specimen of uterine corpus (9/6/6 cm) presents on cut surface a region with firm consistency, infiltrative character and whitish-gray with hemorraghic zones apparance. The microscopic examination reveled: malignant difuse lymphoma with small and large cells associated with lymphoblastic Burkitt-like lymphoma. Immunohistochemical exam reveal CD20 positive which confirm the diagnosis. CD20 is a specific marker for differentiation and proliferation of B lymphocytes. CD10 is a specific antibody for Burkitt lymphoma, diffuse large cell lymphoma, relevant for our diagnosis. Also we examined CD3 and CD68, that gives negative reaction because of their specificity for T lymphocytes. We made the differential diagnosis between T cell lymphoblastic lymphoma, undifferentiated carcinoma, histiocitary and anaplastic lymphoma.
Conclusions. Uterine lymphoma is a rare pathological entity. Because of high malignity lymphoblastic components, the evolution of the patient was unfavorable. The immunohistochemical tests confirms the morphological diagnosis. As a consequence of high malignity lymphoblastic lymphoma the patient's evolution was worse and she died because of leucemic convertion. Necroptic exam reveled macroscopic metastasis on skin, liver, stomach, cardiac papillary muscles.

P-100

Expression of TWIST is frequently observed in epithelial ovarian cancer.

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BACKGROUND: Ovarian carcinoma is the most important cause of gynaecological cancer-related mortality in the western world with a poor 5-year survival. TWIST, a basic helix–loop-helix transcription factor, has been reported to be associated with the development and progression of human cancer. The aim of this study was to analize the distribution and expression of TWIST in ovarian carcinomas and to examine its clinical significance.
MATERIALS AND METHODS: The study was conducted on 65 ovarian carcinomas treated by surgery followed by paclitaxel plus carboplatin-based chemotherapy. The clinicopathologi factors evaluated were histological type (serous vs non-serous), tumor grade, FIGO stage, response to treatment (surgery and chemotherapy), progression-free survival and overall survival. Tissue samples were formalin fixed and paraffin embedded and were immunostained against TWIST antibody (Abcam, 1:100) using the Envision (Dako) method. Staining for the TWIST protein was nuclear. The intensity and percentage of cells that exhibited immunostaining was evaluated.
RESULTS: Overall, 46.15% (n=30) of ovarian carcinomas showed expression of TWIST. When categorized into negative vs positive expression (>5% of tumor cells), TWIST was not associated with any of the clinicopathological parameters examined.
CONCLUSION: Expression of TWIST is frequently observed in ovarian carcinomas and has been recently described as a useful marker in these patients as it appears to increase the risk for recurrence and for poor survival. However, these results could not be confirmed in our series of epithelial ovarian carcinomas. Thus, additional studies including larger patient samples seem necessary to validate the prognostic significance of TWIST expression in ovarian carcinomas.

P-101

Lymph node involvement in deep infiltrating endometriosis

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Background: Lymph node involvement by endometriosis is considered to be uncommon and the status of lymph nodes in endometriosis remains obscure because at evidently lymph node dissection is usually not performed for a so-called benign disease.

Methods: In the present study, we have analyzed the lymph node involvement by endometriotic foci in surgical specimen from rectosigmoid endometriosis (n=26) where the lymph nodes are easily accessible.The lymph node involvement was correlated with the size and the wall layers affected by endometriotic lesions, the number of lymph nodes retrieved, and the presence of lymphovascular invasions as demonstrated by CD 31 and D2–40 antibodies.

Results: Lymph node involvement by endometriosis was observed in 11 of the 26 patients (46,3%) and correlated with the size of the lesions, the number of lymph nodes retrieved and the presence of lymphovascular invasions.

Conclusions: Our data confirm that lymph node involvement by endometriotic foci is a frequent event in rectosigmoid endometriosis and may result at least partially from a lymphatic spread of the disease.

P-102

IS THERE A RELATIONSHIP BETWEEN THE VIRAL LOAD IN CYTOLOGY SAMPLES AND P16 IMMUNOREACTIVITY IN BIOPSY SAMPLES?

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OBJECTIVE: To assess the possible relationship between viral load and p16 immunoreactivity in cytology and biopsy samples, in cervical dysplasia cases.

MATERIALS AND METHODS: 82 cervical biopsies diagnosed as a cervical dysplasia obtained between 2 and 6 months posterior to cytologic diagnosis. An immunohistochemical study of p16 in each case. Review of previous diagnostic cytology in order to know the viral load determined by Hybrid Capture II

RESULTS: 54 cases were mild dysplasias and 28 cases were moderate/severe dysplasias.

11 of 54 mild dysplasia cases showed p16 immunoreactivity (20.4%). Of these, 10 showed RLU greater than 1 (Considered as positive) in previous cytology, with a mean of 1472. These 10 cases were the 23.8% of all RLU positive cases, thus 76.2% of RLU positive cases, didn't show p16 immunoreactivity in biopsy samples. The mean RLU of these cases was 820.

18 of 28 moderate/severe dysplasia cases showed p16 immunoreactivity (64.3%),. Of these, 17 had positive RLU with a mean of 1595. These 17 cases were the 62.9% of all RLU positive cases, thus the 37.1% of RLU positive cases didn't show p16 immunoreactivity in the corresponding biopsy samples. The mean RLU of these cases was 972.

CONCLUSIONS: Apparently doesn't exist correlation between viral load and p16 immunoreactivity but if we consider the biopsy diagnosis there is an increase of correlation in moderate/severe dysplasia cases related to mild dysplasia cases. Also a RLU increase is observed in p16 positive cases, in both groups.

P-103

Concurrent pregnancy and endometrial carcinoma

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Introduction: Endometrial carcinoma in women of reproductive age is rare. We describe a case of accidental diagnosis of endometrial adenocarcinoma in a curretage done because of a missed abortion.

Case report: A 35-year patient with established pregnancy of 9 weeks gestation was admitted to the hospital because of bleeding. A missed abortion was diagnosed, D&C was done and the tissue was sent for histopathological examination. Abundant material was received and was examined completely. On paraffin embedded, H-E stained tissue sections the remains of gestational sac surrounded with immature, partially hidropic chorionic villi were found. In the decidua, there were scarce, confluent and cribriform endometrial glands showing atypical endometrial cells with mitotic activity. The same cells covered the surface of decidual fragments. The diagnosis of endometrioid adenocarcinoma of endometrium was made. After that, the patient underwent a series of imaging diagnostic procedures (US, 4D-US, Doppler, MRI), none of which showed suspicious findings. The patient had menarche at the age 14, she claimed that her periods were always extremely regular, that she has never taken any hormonal preparations, and was not treated because of infertility. However, she had a spontaneous abortion 1,5 year ago, but it has happened at

home, and no histopathological analysis could be done. Since she wants to become pregnant again, she was adviced to control regularly.

Discussion: Women of reproductive age who develop endometrial adenocarcinoma are rare, and usually have a history of infertility and hormonal treatment. It was not the case in this patient, and according to histopathological and imaging studies we can assume that the carcinoma was probably limited to endometrium and caused the failure of pregnancy development.

P-104

THE RELATIONSHIP OF C-ERB B-2 EXPRESSION WITH ESTROGEN RECEPTOR, PROGESTERONE RECEPTOR AND PROGNOSTIC PARAMETERS IN ENDOMETRIAL ADENOCARCINOMAS

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BACKGROUND: The transmembrane receptor encoded by the HER-2 cellular oncogene is amplified in several types of human carcinomas. There are prognostic and therapeutic implications associated with the overexpression of this transmembrane protein. HER-2/neu, the transmembrane receptor encoded by the c-erbB2 gene, is overexpressed by immunohistochemistry in <10% of endometrial cancer. The aim of this study was to investigate the expression of cerbB2 in endometrial cancer with attention both to membranous and cytoplasmic staining, to study its correlation to established prognostic parameters and to elucidate the significance of cytoplasmic signaling.

METHODS: This study is performed on 73 cases between 2004–2007 at Dr Lutfi Kirdar Training and Educational Hospital Pathology Clinic. Parafine embedded tumoral tissue with endometrial adenocarcinoma are examined immunohistochemically (IHC). Immunoreactivity of c-erbB-2 is localized in the cell membrane and is scored semiquantitatively using the following Food and Drug Administration (FDA) approved scoring system: 0, no immunostaining; 1+, incomplete membranous immunostaining of <10% of tumor cells; 2+, weak complete membranous immunostaining of >10% of tumor cells; 3+, strong complete membranous staining of >10% of tumor cells. Scores of 0 or 1+ indicate a negative result, while scores of 2+ and 3+ are regarded as positive c-erbB-2 expression. The relation between CERB-2, estrogen – progetsreone receptor status, histological grade, myometrial invasion depth and vascular invasion is investigated. Estrogen receptor (ER) and progesterone receptor (PR) status are analysed based on the percentage of stained cells and the intensity of nuclear stain. The percentage of positive cells is graded as follows: 1=0 to 25% of the nuclei stained; 2=26 to 75%of nuclei stained, 3= more than 76% of the nuclei stained. The staining intensity is scored as follows: 1= absent or weak, 2= strong, 3= very strong. The sum of both parameters give the IHC score. Tumors are divided into three categories depending on the IHC score. Category I corresponds to a score of 2, category II to a score of 3 or 4, and category III to a score of 5 or 6. Category I tumors are considered as immunonegative, whereas category 2 and 3 tumors are considered as immunopositive. SPSS (Statistical Package for Social Sciences) for Windows 10.0 software is used for the statistical analysis. For the comparison of quantitative data, Qui-square and Fisher's Exact test are used. Results are analysed at $p<0.05$ significance and 95%confidence interval.

RESULTS: In this study, we found a statistically significant relation between C-erb B2 and progesterone receptor ($p<0.05$). In progesterone receptor negative cases, expression of C-erb B2 was statistically significantly high. Membranous C-erb B2 statining and estrogen receptor did not show a statistically significant relation ($p>0.05$). However, it is noteworthy that, in estrogen receptor negative cases, C-erb B2 expression was high. There was no significant difference between C-erb B2 and histological grade ($p>0.05$). The highest levels of C-erb B2 was found in Grade 2 cases. There was not observed any statistically significant relation between C-erb B2 and myometrial invasion depth or vascular invasion ($p>0.05$).

CONCLUSION: In our study, there is found a significant relation between C-erb B2 and progesterone receptor. We conclude that, to observe a negative correlation between progesteron receptor positivity which is considered as a favorable feature in endometrial adenocarcinomas and c-erb B 2 expression will lead us in studies with larger series and will play an important role in therapeutic approach to patients.

P-105

THE RELATIONSHIP OF COX-2 EXPRESSION WITH ESTROGEN RECEPTOR, PROGESTERONE RECEPTOR AND PROGNOSTIC PARAMETERS IN ENDOMETRIAL ADENOCARCINOMAS

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BACKGROUND: In recent years, the design of new antineoplastic agents that can halt the progression of human

malignancies with minimal systemic damage has been at the forefront of cancer research, with cyclooxygenase-2 (COX-2) as a major target molecule. Cox-2 and its role in the pathophysiologic processes is well known. In this study immunohistochemical expression of COX-2 in the endometrial adenocarcinomas (EC) and its relationship with estrogen receptor, progesterone receptor and other prognostic parameteres are investigated.

METHODS: The study is performed on 73 endometrial adenocarcinoma cases diagnosed between 2004–2007 at Dr Lutfi Kirdar Training and Educational Hospital Pathology Clinic. Parafine embedded tumoral tissues are examined immunohistochemically (IHC). Staining intensity is evaluated as negative (0), weak (1), moderate (2), strong (3), and the percentage of positive tumor cells is categorised as follows: 0=0% to 5%, 1=6% to 25%, 2=26% to 50%, 3= 51% to 75%, and 4=76% to 100%. The sum of the intensity and extend score is used as the final staining score (0–7) for COX-2. Tumors having a final staining score of ≥4 are considered as showing strong expression. Estrogen receptor (ER), and progesterone receptor (PR) examinations are done based on the percentage of stained cells and the intensity of nuclear stain. The percentage of positive cells is graded as follows: 1=0 to 25% of the nuclei stained; 2=26 to 75%of nuclei stained, 3= more than 76% of the nuclei stained. The staining intensity is scored as follows: 1= absent or weak, 2= strong, 3= very strong. The sum of both parameters gives the IHC score. Tumors are divided into three categories depending on the IHC score. Category I corresponds to a score of 2, category II to a score of 3 or 4, and category III to a score of 5 or 6. Category I tumors are considered as immunonegative whereas category 2 and 3 tumors are considered as immunopositive. The cases are grouped according to FIGO histological grade as Grade I, Grade II and Grade III and besides they are investigated in three groups according to myometrial invasion as limited to endometrium, invasive in less than 1/2 of myometrium and invasive in more than 1/2 of myometrium. These prognostic parameteres as well as estrogen and progesterone receptor existance are compared with COX-2. SPSS (Statistical Package for Social Sciences) for Windows 10.0 software is used for the statistical analysis. For the comparison of quantitative data, Qui-square and Fisher's Exact test are used. Results are analysed at $p<0.05$ significance and 95% confidence interval.

RESULTS: When compared with Cox 2 scores, histological grade, myometrial invasion level and estrogen-progesterone receptor status of the cases did not show any statistically significant difference ($p>0,05$).

CONCLUSION: Since we could not find any statistically significant relation between COX-2 and estrogen–progesterone receptor status and other parameteres, we conclude that COX-2 can be used as an independent prognostic factor.

P-106

Uterine müllerian adenosarcoma with massive ovarian stromal sex cord-like component in two tamoxifen treated patients

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Introduction: To date, 68 cases of uterinetamoxifen-related uterine sarcomas have been reported. Of these, 13 corresponded to adenosarcomas. We present two further cases of adenosarcoma of the uterus in patients with breast cancer treated with tamoxifen, both of them presenting the particular feature of having a massive stromal component composed by an ovarian sex cord-like proliferation. To our knowledge, this is the first time that this association is reported in tamoxifen treated patients.

Material and methods: Two 71 and 64 year old postmenopausal patients previously diagnosed with infiltrating ductal carcinoma were treated with mastectomy, chemotherapy and tamoxifen (20 mg/day for a period of 5 and 3 years respectively). Presently, both were admitted for vaginal bleeding and subsequently treated by total abdominal hysterectomy with bilateral salpingo-oophorectomy. Follow up is uneventful.

Pathology Results: Macroscopy revealed large intracavitary polypoid tumours of 2.5 and 5 cm in diameter respectively, conformed by a foliaceous, translucent tissue with soft consistence where multiple yellow streaks were present. Myometrial limits were lineal. Microscopically, benign endometrioid, mucinous and ciliated epithelia lined dilated tubulo-cystic glandular spaces often with intraluminal phyllodes-like projections. The mesenchymal component had CD10 positive endometrial stromal periglandular cuffs present as a subepithelial cambium layer. The remaining stroma, comprising more than 80% of the mass, was of ovarian sex-cord type with luteinized cells, luteinized sertoliform tubules andepithelioid trabecular cords that were positive in different proportions and intensity for CAM5.2, Actin, Desmin, Calponin, CD 10, Inhibin,Calretinin.

Conclusions: This is the first report of adenosarcomas containing a predominant sex cord-like elements in patients under tamoxifen treatment. Adenosarcomas with stromal sex-cord elements have been reported in only three previous papers in Endometrium1 (8 cases), cervix2 (1 case) and ovary3 (6 cases) none of them associated with long term tamoxifen treatment. Both our cases had a massively luteinized sex-cord componentand only a minor

periglandular endometrial stromal component. Immunohistochemistry was characteristic of uterine tumours resembling ovarian sex cord tumours. Clinically, both behaved in a benign fashion.

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P-107
ATYPICAL POLYPOID ADENOMYOMA OF THE UTERUS. A REPORT OF ELEVEN CASES.

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Background: Atypical polypoid adenomyoma (APA) is a rare uterine polypoid tumor occurring in women in their reproductive age.
Method: Eleven cases of atypical polypoid adenomyoma have been diagnosed at our department in the last five years (2003–2007). The mean age of the patients was 37 (range 26–46 years). The most common clinical diagnosis was abnormal uterine bleeding, leiomyoma or endometrial polyp. In one patient the clinical diagnosis was endometrial hyperplasia, while two were examined for primary sterility. In one case the diagnosis was established in a curettage material from a spontaneous abortion. Apart from the conventional hematoxylin and eosin, additional histochemical and immunohistochemical stainings were also performed.
Results: Macroscopically, white-gray polypoid fragments measuring 0.5–2 cm were found in the curettage materials. Histologically, the APAs were composed of atypical endometrial glands surrounded by smooth muscle stroma. Squamous morules were a common finding. In three cases, the APA was accompanied by foci of invasive endometrial adenocarcinoma. The immunohistochemical stains confirmed the smooth muscle nature of the stroma (alpha-smooth muscle actin, desmin and caldesmon positive). The morules were positive for CD10 marker, whereas Ki-67 proliferative index was low (5–15%), except in the foci of invasive adenocarcinoma. All tumors were hormone responsive. One of the patients was surgically treated for advanced endometrial adenocarcinoma in FIGO IIIC stage. Complete regression of the disease after tumor extirpation and consecutive high dosage progesterone therapy was achieved in four of the patients, in one the disease persisted after two years due to suboptimal therapy, whereas five were lost to follow up (four of which diagnosed in the last 10 months).
Conclusion: APA is considered to be a benign form of mixed epithelial and mesenchymal uterine tumors. This tumor can be associated with sterility and rarely with endometrial carcinoma, therefore recognition and correct diagnosis of this entity is important.

P-108
EVALUATING THE THE MALIGNANCY POTENTIAL IN ENDOMETRIAL HYPERPLASIA THROUGH PROLIFERATION MARKERS KI-67 AND PCNA AND TUMOR SUPPRESSOR GENE DESIGNATED PTEN

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Introduction: Endometrial Hyperplasia (increase in gland/stroma ratio, disorder number and shape of the glands like modifications of the glandular epithelium: exhibits loss of nuclear polarity, stratification, etc) is a lesion frequently associated with the uterine leiomyomas
Purpose: The Study was realized at the Universitary Emergency Hospital Bucharest, Romania on 294 endometrium biopsies taken in the period 2004–2006 from women with ages between 45 and 55.
Material and methods: Hematoxylin –eosin stained slides of endometrial formalin fixed, paraffin embedded tissue has emphasized in 143 cases the simple hyperplasia (SH), in 101 cases the complex hyperplasia (CH), in 19 cases the simple atypical hyperplasia (SAH) and in 35 cases the complex atypical hyperplasia (CAH). We performed the indirect tristadial ABC method of IHC for 3 antibodies: PTEN, Ki-67 and PCNA on formalin fixed embedded tissue taken by biopsies from 80 cases (14 SH, 12 CH, 54 SAH and CAH).
Results: PTEN was focal positive for SAH, diffuse for CAH and for 1–2 cases of SH and CH. Ki-67 and PCNA were also very frequent in group SAH and CAH.
Conclusion: PTEN, Ki-67 and PCNA take part in the process of endometrial carcinogenesis following probably molecular pathways and determine the malignity potential of atypical hyperplasia of endometrium.

P-109

Struma ovarii with peritoneal implants.

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Background: Struma ovarii is an ovarian monodermal teratoma composed entirely or predominantely of thyroid tissue. It is a rare form of ovarian neoplasm which represents less than 3% of ovarian teratomas. We have experienced a rare case of struma ovarii complicated by peritoneal implants and report it with a review of the main histological findings and differential diagnosis of this entity.
Methods & Results: A 26-year-old female, with no significant medical history, complained for abdominal pain and fever. The pelvic ultrasound showed a left ovarian cystic mass. Surgical removal of this mass and of multiple peritoneal nodules was achieved.
The pathological examination of the cystic mass was consistent with a mature teratoma in which the thyroid tissue had overgrown all other tissues. No atypia or malignant features was identified. The peritoneal deposits were composed of benign thyroid tissue. This condition is termed "benign strumosis".
Conclusion: Struma ovarii is a rare ovarian tumour that is characterized by the presence of at least 50% of thyroid tissue on histologic examination. This tumour is generally benign and has an excellent prognosis. Its association with peritoneal implants is very uncommon and has no side effects.

P-110

Massive chronic intervillositis. A clinicopathological study of 21 cases

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Background: Massive chronic intervillositis (MCI) is an infrequently recognized placental lesion thought to be of immunologic origin that has been associated with poor fetal outcome. It is characterized by a proeminent histiocytic infiltrate in the intervillous space. Here in, we describe clinical and pathologic aspects of 21 cases of MCI.
Methods: In this retrospective study we evaluated 21 cases of MCI diagnosed between 2004 and 2006. Perinatal outcome, other associated clinical features, histopathology and immunohistochemical aspects were assessed.
Results: Overall incidence of MCI among placental specimens submitted during the 3-year period of this study was 1%. Overall perinatal mortality rate was 28%. Six of 21 patients had spontaneous abortions (28%). Intrauterine growth restriction complicated 5 pregnancies (23%). Most case (71%) occurred in the third trimester. Characteristic placental histology included infiltration of intervillous space and occasional villi by mononuclear inflammatory cells withfibrin. Immunohistochemical staining showed an infiltrate composed predominantly of histiocytic, CD68 positive cells.
Conclusion: The mononuclear nature of the inflammatory cell infiltrate and its association with increased fibrinoid material suggests an immunological origin. Although uncommon, MCI is an important cause of recurrent spontaneous abortion and in some cases, loss at later gestational ages.

P-111

Bilateral ovarian serous borderline tumour in a four-year-old girl

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Background: Epithelial tumours of the ovary account forabout 15% of paediatric ovarian masses. Such tumours are extremely rare before menarche. The purpose of this report is to describe clinical and pathological aspects of bilateral ovarian serous borderline tumour (SBT) in a premenarchal girl and to discuss the approach to both treatment and outcome of this type of tumours.
Methods: We describe bilateral ovarian SBT in a girl aged 4 years. Data regarding clinical presentation, treatment, pathology, and outcome were collected from the medical record.
Results: The child presented with abdominal distension and discomfort. Ultrasound and CT scan revealed multiloculated cystic masses in both ovaries. Cysts were surgically removed, with preservation of normal ovarian tissue, and histopathological findings showed a SBT of both ovaries. The girl is well with no evidence of recurrence at a follow up period of 19 months.
Conclusion: Epithelial ovarian neoplasms are rarely seen in the paediatric population, especially in the first decade of life. These tumours, nearly always, are of low grade and stage despite their large size. To preserve fertility, conservative treatment,whenever feasible, is recommended.

P-112

Appendiceal metastasis from ovarian cancer

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Ovarian cancer has a well-established widespread peritoneal dissemination. Although the surface of the appendix constitues less than 1% of the peritoneal cavity, it is suggested as a frequent metastatic site. The aim of this study was to detect microscopically appendiceal metastasis from ovarian cancer as a part of surgical staging and to evaluate the prognostic of these tumors. A total of 13 patients with ovarian carcinoma and appendiceal metastatis who had undergone appendectomy were retrospectively evaluated. We detected microscopic metastasis in only 8 cases. In conclusion, according to our findigs, the identification of the gross and microscopic appendiceal involvement, offers new clinicopathologic data used for surgical staging and routine appendectomy may be justified in all ovarian cancer patients.

P-113

A NEW CASE OF SPONTANEOUS COMPLETE HYDATIFORM MOLE COEXISTENT WITH TWIN NORMAL FETUS

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BACKGROUND The association of Complete Hydatiform Mole (CHM) and normal twin fetus is a rare phenomenon. Its incidence is between 1/10 000 and 1/100 000 pregnancies. The aim of this report is to discuss the difficulty of clinical management, pathological diagnosis and the main differential diagnosis.

METHODS AND RESULTS A 21-year-old woman, gravida 2, para 1 (G2P1) with a history of spontaneous abortion, consulted at 21 weeks of spontaneous pregnancy. The ultrasound scan performed identified a normal viable fetus and normal placenta in association with a molar placenta. A medical interruption of pregnancy was indicated. Pathological findings of the placenta addressed to our department of pathology showed two separate placentas. The first was a normal placenta. The second was molar with obvious grap-like pattern. The main differential diagnosis was partial hydatiform mole; this hypothesis was rejected for two criteria: first the sharp separation between molar mass and normal placenta, second the absence of fetal malformation.

CONCLUSION The association CHM and normal twin fetus necessitate a precise prenatal diagnosis. The management of such pregnancy must be the result of possibility of further pregnancies, risks of medical complications of the molar pregnancy and the hope of the mother.

P-114 WITHDRAWN

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PROLIFERATING TRICHILEMMAL TUMOUR AND EPIDERMOID CARCINOMA OF THE VULVA

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BACKGROUND The case of a patient with a proliferating tumour of the vulva (PT) with an exceptional location of the lesion is presented here. Its association with a metachronic epidermoid carcinoma (EC) in the same place suggests a possible etiopathogenic connection between the two processes.

METHOD The tissue samples from the biopsy, fixed in 4% formalin, were subjected to processing, inclusion in paraffin and usual sectioning, and stained with hematoxiline-eosine. The presence of DNA in the HPV 6, 11, 16 and 18 was evaluated by the PCR technique.

RESULTS A 78 year old patient presented a vulvar PT which developed into an EC in the same location four years later. The initial lesion histochemically consisted of a cutaneous structure with a multicystic formation without connection to epidermis, which showed a parietal epithelial proliferation with an abrupt intracystic keratinization, similar to that present in the external radicular sheath at the level of the isthmus of the hair follicle. Some koilocytic vacuolization cells of a viral appearance were observed, as well as similar changes to those focally observed in the posterior EC.

CONCLUSION The fourth case of PT of the vulva is reported here, which later developed into an EC, a connection which we do not have any previous experience, therefore suggesting a possible etiopathogenic relationship with the HPV.

P-116

Immunohistochemical profile of high-grade endometrial adenocarcinomas. Expression of p53, Ki67 and hormonal receptor status in hysterectomy specimens.

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CENTRE DIAGNÒSTIC UDIAT. CORPORACIÓ PARC TAULÍ. SABADELL. SPAIN

Background: High-grade endometrial adenocarcinomas include different histological subtypes. We analyzed their immunohistochemical profile in order to better characterize them and to detect additional prognostic factors.

Method: Immunohistochemical determination of Progesterone (PR) and Estrogen (ER) receptors, p53 and Ki67 in 25 high-grade endometrial adenocarcinomas, with clinicopathological data. The immunoreactive index (IRI) = intensity of staining (0 to 3) x proportion of positive tumor cells (0, 1:1–10%, 2:11–50%, 3:51–80%, 4:>80%): IRI= 3:7–12, IRI=2:4–6, IRI=1:<4.

Results: We collected 9 grade (G) 3 and 2 G2 FIGO endometrioid (EC), 9 serous (SC), 3 clear cell (CCC) and 2 mixed adenocarcinomas (MC) (1 EC/CCC and 1 EC/SC). 1 (G2 EC) and 5 adenocarcinomas (one G2 and one G3 EC, one SC, and the EC component of the MCs) presented an IRI RE and RP=3, respectively. Ki-67 was highly expressed in 11 adenocarcinomas (5 SC, 2 CCC, 3 G3 and 1 G2 EC) and in the serous component of one MC. p53 was highly expressed in 8/9 SC, in 2/3 CCC and in 2/9 G3 EC. 4/11 patients with EC died with disease (DWD) (one G2, pT3aN0, IRI ER 0 and PR=2; and 3 G3: one with with vascular invasion (VI), one IRI Ki67 and p53=3, and one with all IRI=1, pT3aN1 and VI), all with recurrences. One G3 EC alive with disease (AWD) presented recurrence, VI and an IRI Ki67=3. 6/9 patients with SC DWD (all IRI p53= 3 and 4 with recurrences). Two patients were alive without disease (one IRI p53=1 and one pT1aN0) and one AWD (IRI PR=3). 2 of 3 patients with CCC DWD (with recurrence, VI and IRI Ki67=3). The patients with MA showed no recurrences, one alive and one death, both without disease.

Conclusions: Additional immunohistochemical detection on high-grade endometrial adenocarcinomas can better subtipify them and could have prognostic significance.

P-117

THE HISTOPATHOLOGIC PROFILE OF MIXED TYPES OF ENDOMETRIAL CARCINOMA

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Background. Endometrial carcinoma may present combinations of two or more such neoplasms of the endometrium. A mixed carcinoma has at least one other component comprising at least 10% of the tumor. Method. Our study presents 7 cases of mixed endometrial carcinomas in women with age between 47 and 64 years old. All patients presented clinically abnormal vaginal bleeding and all were treated with total abdominal histerectomy and bilateral salpingo-oophorectomy. The specimens were fixed, paraffin-embedded and routinely stained with H&E. Immunohistochemical techniques were performed for p53 and BCL-2. Results. The histopathological diagnostic was: mixed endometrioid and serous carcinoma of the endometrium (4 cases), mixed endometrioid and serous carcinoma with areas of clear cell carcinoma (2 cases), and mixed endometrioid and clear cell carcinoma (1 case). All these tumors presented more than 25% of the serous component. The clear cell carcinoma was more than 10% in 2 cases and more than 25% in 1 case. The endometrioid component presented in typical form or as villoglandular carcinoma, was well-differentiated, FIGO grade 1. Because of the clear cell and serous components, the endometrial carcinomas were overall high grade, deeply invasive, and in an advanced stage. Immunohistochemically, the tumors presented less frequent expression of p53 in clear cell component than in serous carcinoma. The BCL-2 protein expression was higher in endometrioid than in serous and clear cell components. Conclusions. All mixed types of endometrial carcinomas presented in this study behave as aggressive carcinomas because of the significant percent of serous and clear cell components. The loss of BCL-2 expression was associated with other features of poor prognosis as depth of invasion, FIGO stage, and aggressive cell types.

P-118

MALIGNANT VAGINAL NEOPLASMS

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INTRODUCTION AND OBJECTIVE: Malignant vaginal neoplasms (MVN) are uncommon lesions, that has been reported as representing a range between 2 and 3% of all genital malignancies. We analysed histological type, origin, and the overall result of our revision were compared with those reported previously.

MATERIALS AND METHODS: We reviewed cases of patients with complete clinical data and slides for revision wich includes pathological evaluation, correlation with clinical data, personal history, and vaginal citology when available.

RESULTS: We studied 47 cases of MVN. As already described, bleeding was the most frequent clinical finding. Macroscopically, neoplasms appeared either as vegetant polypoids or pigmented lesions, blind fistulas or ulcers. Frequently neoplasms emerged as tumors at the vaginal cul-de-sac.

In 43 cases lesions were secondary (91.5%) and 4 primary (8.58%). Fifteen cases were squamous carcinomas, 1 clear cell carcinoma, 19 adenocarcinomas, 1 choriocarcinoma, 7 poorly differentiated carcinomas, 1 primary melanoma, 1 mixed sarcoma and two primary pure sarcomas. The most frequent primitive squamous origin was the cervix whereas the vulva was identified as the second most common origin. Adenocarcinomas were identified as secondary tumors being the uterus, the cervix, the ovary and the rectum the location of the primary lesion.

The primary MVN were rare (malignant melanoma, poorly differentiated carcinoma lymphoepithelioma like, and two sarcomas). Dissemination can be due to a spread by direct extension (upper third), vascular embolism (lower third), or by directly implantation (often in anterior wall).

CONCLUSIONS: Among primary genital tumors, MVN are very infrequent lesions. Therefore, the possibility of a secondary origin must be first ruled out. The vaginal topography of tumors is related with to the its origin as well as with its way of dissemination.

P-119

PIK3CA, p53 and E-cadherin alterations in high grade endometrial carcinomas

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Background: Mutations in the *PIK3CA* oncogene are frequently identified in endometrioid endometrial carcinomas (EEC). We have recently confirmed coexistence of *PIK3CA* mutations with MI and mutations in *PTEN, CTNNB1, K-RAS*, and *B-RAF* in EEC, providing evidence that tumors carrying *PIK3CA* mutations are often high-grade carcinomas associated with myometrial invasion. We wanted to determine the impact of *PIK3CA* mutations in high-grade carcinomas (EECs, mixed, and NEECs).

Design: Genomic DNA was obtained from 59 high-grade endometrial carcinomas (29 grade 3 EEC, 6 serous (SC), 4 clear cell (CC), and 20 mixed (9 EC-CC, 7 EC-SC, and 4 CC-SC). Mutational screening for *PIK3CA* (exons 9 and 20), p53 (exons 5–8), *PTEN*, and *K-RAS* were performed. Microsatellite instability (MI) for five different loci (BAT25, BAT26, D2S123, D5S346, and D17S250) was also done. Tissue-arrays were performed for immunohistochemical analysis of p53 and E-cadherin. Clinicopathologic data was obtained.

Results: *PIK3CA* mutations were detected in 34% (20/59) of cases distributed in all histologic groups. Exon 20 mutation occurred in 90% (18/20) of cases. p53 overexpression was detected in 35% (18/51) being more frequent in NEEC, and mixed tumors ($p=0.037$). *PIK3CA* mutations coexisted with p53 overexpression in 11% (6/51) of either pure EEC or mixed carcinomas with endometrioid component. We found that *PIK3CA* mutations coexisted with *PTEN, K-Ras* mutations, and MI in pure EEC but not in mixed carcinomas with endometrioid component. Lack of immunoexpression of E-cadherin was detected in 14% (7/48) of cases and coexisted with p53 alterations more frequently in NEEC and mixed tumors.

Conclusions: Our results show that exon 20 PIK3CA mutations are frequent in high grade endometrial carcinomas of any histological type, whereas p53 overexpression and loss of E-cadherin immunoexpression are more frequent in NEEC, and mixed tumors. Our results confirm the role of PIK3CA as an alternative pathway in high-grade endometrial carcinomas.

P-120

Deregulation of Stathmin/OP18 in ovarian carcinomas is not due to its direct upstream regulator PAK1.

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Background: Rho GTPases play an important role in cancer by regulating cell motility and invasion. PAK1 and Stathmin control the microtubule polymerization and filopodia emission. The role of these two proteins in ovarian metastasis deserves further research.

Design: Tissue-microarrays (TMA) from 70 FIGO stage II-IV ovarian carcinomas (51% serous, 12% endometrioid, 13% poorly differentiated, and 24% clear cell carcinomas) treated with CBCDA-Paclitaxel. TMAs included normal tissue, primary and metastatic tumor. Tumors were stratified into 2 groups according to onset of recurrence after chemotherapy: sensitive > 6 months; and resistant < 6 months. Immunohistochemistry (IHC) for PAK1 and OP18/Stathmin were performed.

Results: We found a statistically increase of Stathmin protein levels in tumor ($p<0.001$) and metastasis ($p<0.001$) in comparison with normal tissue. Stathmin and PAK showed low correlation only in metastatic tumors ($p=0.05$, $r=0.26$). We found a statistical increase in Stathmin levels in resistant metastasis ($p=0.05$), however no signif-

icant differences between resistant and sensitive tumors (p= 0.18) nor metastasis (p=0.45) were found in PAK1.
Conclusion: Deregulation of Stahmin in ovarian tumors and metastasis is not due to its direct regulator PAK1, other collateral proteins may be affecting Stathmin/OP18 protein levels in ovarian carcinogenesis/metastatization.

P-121

Epithelial-mesenchymal transition (EMT) in early stages of endometrioid endometrial carcinomas

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Background: Epithelial-mesenchymal transition (EMT) is involved in the development of invasion and metastasis. Twist is a transcription factor that regulates the EMT program. We investigated some of the EMT genes and other molecular alterations in early stages of endometrioid endometrial carcinomas (EEC).
Design: Genomic DNA was obtained from 26 early stage EEC (8 Ia, 4 Ib and 14 Ic). Ic samples both from surface and myoinvasive fronts were taken. Mutational analysis for *PIK3CA* (exons 9 and 20), *PTEN*, *K-RAS* and *CTNNB1* were performed. Loss of heterozygosity (LOH) at the PTEN locus was measured by analyzing 3 different microsatellite markers (D10S579, D10S532, D10S2491). Microsatellite inestability (MI) for 5 different loci (BAT25, BAT26, D2S123, D5S346 and D17S250) was also done. Tissue-arrays for immunohistochemical analysis of Twist, E-cadherin and vimentin was done. Expression of Twist, E-cadherin, Snail, and HMGA2 was evaluated by RT-PCR. Clinicopathologic data was obtained.
Results: LOH of *PTEN* (5%), MI (38%) and mutations of *PTEN* (62,5%), *KRAS* (12,5%) and *PIK3CA* (exon 20) (12,5%) were found in Ia carcinomas. LOH of PTEN (14,5%) and mutations of *PTEN* (58%), *KRAS* (14,3%), *CTNNB1* (7,15%) and PI3KCA (exon 9 and 20) (14,5%) were detected in Ic cases both in surface and myoinvasive fronts of the tumor. Exons 9 and 20 *PIK3CA* mutations (50% each) were found in Ib tumors. *KRAS* mutations coexisted with exon 8 *PTEN* mutations in all cases (p= 0.00). Twist, vimentin, HMGA2, and SNAIL expressions were found to be increased in the myoinvasive front compared with normal tissue (p<0,05).
Conclusion: Although molecular alterations were similar in Ia, Ib, and Ic carcinomas. The expression of EMT markers was higher in the myoinvasive front.

Infectious diseases

P-122

Intralesional IgA in mice infected with Leishmania mexicana

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Background The presence of immunoglobulin deposits in leishmaniasis lesion is a striking histological feature in man and experimental animals infected with *Leishmania* parasites. Previously, we described the immunocytochemical pattern of intralesional antibody isotype profiles (IgG1–4 and IgA) and plasma cells in human patients with localized American cutaneous leishmaniasis from Mérida state in Venezuela. To further characterize the presence of IgA in the lesion, we have evaluated this isotype in lesions of mice experimentaly infected with *Leishmania mexicana* (MHOM/VE/72/AZV).
Methods The presence and distribution of IgA immunostained deposits, in skin biopsies from inoculated footpads of infected resistant C57BL/6 and susceptible BALB/c mice were compared to the corresponding contralateral non-inoculated footpad's skin from the same animal, after 8 weeks of infection, using two different antibodies directed against mouse IgA by immunocytochemical procedures.
Results IgA deposits were detected in all inoculated mice footpads active lesions, from both mice strains, but they were absent in the skin of the control contralateral non-inoculated footpads. The pattern of IgA distribution in the lesion, of both mouse strains, with the two distinct IgA antibodies was the same. IgA was found in different components of the infiltrated dermis (connective tissue, macrophages, plasma cells, giant cells, granuloma, blood vessels and parasites) and in the ulcerated epidermis of BALB/c mice.
Conclusion The domonstration of IgA immunostaining in plasma cells of mice with active lesions together with the absence of IgA deposits in non-infected skin gives further support to the concept of local production of IgA. The nature of the antigen recognized by IgA in the leshmaniasis lesion appears to be non-specific, since the immunoreactivity observed with rat or goat anti mouse IgA, albeit amastigotes localization in other components of the skin lesion.

P-123

Hpv prevalence in hyperplastic tonsillary and adenoid tissues in children and young adults in Turkey

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Background: The aim of the study was to determine the frequency of HPV and genotypes in children and young adults which suffered for upper airway obstruction and treated with tonsillectomy and/or adenoidectomy.
Method: Archival resection materials of 258 patients threated between 2003–2007 were retrived for HPV detection and typing. Six patients were excluded because of inadequate quality of DNA extraction. Age range of remaining 252 patients was 5 to 21 (11.94±4.20). MY/GP consensus primers were used for screeing PCR. Four different set of primers covering 18 HPV types were used for M-PCR HPV typing.
Results: Sixteen of 252 samples namely, 9 of 114 tonsillectomy, 4 of 87 adenoidectomy and 3 of 51 tonsiloadenectomy tissues were found to be containing HPV virus genome by screeing PCR (6.34%). HPV-16 was dominant genotype and found in 8 tonsillectomy, 2 tonsiloadenectomy and, and 1 adenoidectomy. HPV-6 was detected in 3 tonsiloadenectomy and 1 tonsillectomy materials. HPV-31 genome was found only in 1 samples. None of the samples in the series were found to be containing multiple HPV genotypes.
Conclusion: HPV prevalence in children and young adults which suffering upper airway obstruction because of non-tumoral tonsillary and/or adenoid over growth were found 6.34% in Turkish population. HPV-16 was encountered as the most frequent genotype.

P-124

HISTOLOGIC AND ELECTRONMICROSCOPIC STUDIES IN HIV-1 ENCEPHALOPATHY

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The HIV-1 retrovirus attacks directly CNS in HIV-1 patients. The light- microscopic studies of the brains among 52 mortal cases with AIDS exhibit brain cortex atrophy, perivascular mononuclear cell infiltrates and single foci of neuronophagia. Parallel ultrastructural findings in 10 cases proved to be macrophages containing viral particles in their surface and extracellular spase. The virions measuring 100–120 nm in diameter reveal circular envelopes transected by inner lucid cores. Subplasmalemmal linear densities are prominent featuring the virus-associated cells. The visualized myelin-like bodies in the cytoplasm of the activated macrophages is a sign of brain degeneration. Giant mononuclear cells are identified in adition.

P-125 WITHDRAWN

P-126

Seroprevalence of Blood-Borne Infections Among Blood Donors in Venezuela, 2001–2002

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Objectives: From the data collected at the Ministry of Health from whole nation public and private blood banks (BB) find out the proportion of blood units discarded for being seropositive HBV, HCV, HIV, syphilis and *Trypanosoma cruzi*, and the seroprevalence of these infections among their donors. *Methods*: ELISA serological testing was done with 715,393 donors seen at 535 blood banks between 2001 and 2002. Samples that were repeat reactive (RR) with the ELISA underwent supplementary Western blot (WB) testing. *Results*: Of the 715,393 blood banks donors, 9,294 of them (1.3%) were positive for syphilis (VDRL), 6,107 (0.85%) for HBV (anti-HBc), 4,825 (0,67%) for *T. cruzi*, 3,859 (0.54%) for HCV and 1,870 (0.26%) for HIV. *Conclusions*: Although syphilis or *Treponema pallidum* infection was the most important blood-borne disease found in this study, the seroprevalence found is lower than others reported in other countries in the region (e.g. Goiânia, Brazil, 1989, 4.1%). For HBV and HCV, the seroprevalence estimates were also similar than those found in other countries in the region (such as Río de Janeiro, Brazil, where in 2005 the anti-HBc was 2.05% and 0.79% in 2004 for HCV), indicating high rates of infection by HBV and HCV and a persistent risk of HBV and HCV transmission by transfusion. For Chagas disease is also lower than others reported in other countries in the region (e.g. Goiânia, Brazil, 1989, 3.3%). Finally for HIV the found seroprevalence is similar to that reported in Mexico (Irapuato, Mexico, 2003, 0.24%). But as seen herein, those seroprevalences are regional, and our report is nationwide. Further epidemiological research is expected.

P-127

Prevalence of Chlamydia penumoniae in paediatric populations of Alto Tiete region (Brazil) and the clinical implications of these infections.

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Introduction: Chlamydia pneumoniae is a human respiratory tract pathogen. Seroepidemiological studies indicate that C. pneumoniae infection is most common in school-aged children and infrequently detected in younger children.
Objective: The aims of this study were to further elucidate the prevalence of C. pneumoniae in paediatric populations and to describe the clinical implications of these infections.
Patients and Methods: The study population consisted of 154 children of Alto Tiete region (Brazil) with respiratory tract diseases, 231 presumed healthy children at day-care, 44 children undergoing adenoidectomy and 886 children from a population based cohort. Family members to infected day-care children were investigated. The laboratory methods used were polymerase chain reaction (PCR) on specimen from upper respiratory tract, serology by micro-immunofluorescence (MIF), and immunohistochemistry (IHC) on adenoid tissue specimen. Personal data and medical history were obtained by the means of questionnaires and by the study of patient records.
Results: In children younger than five years, the prevalence of C. pneumoniae was 11% as detected by PCR. This prevalence started to increase with increasing age from two years of age. The corresponding increase in serology as detected by MIF started at the age of four years. The prevalence at day-care centres varied from 8 to 33%. Both PCR and MIF underestimated the prevalence of C. pneumoniae detected by IHC. Families to infected children were investigated: mothers were more often infected than fathers were.Most C. pneumoniae infections in small children were confined to the upper respiratory tract. These infections were usually mild or asymptomatic. Symptomatic disease may be of prolonged nature. No subsequent illness after C. pneumoniae infection was detected at follow-up after four years. In general, no association between C. pneumoniae and asthma was found, but C. pneumoniae may be of importance for asthma in some susceptible individuals. Previous C. pneumoniae infection reduced the risk for later atopy.
Conclusion:The C. pneumoniae is a common finding in small children and most often causes relatively mild disease. If the acquisition of this infection early in life will have any implications for future health remains to be investigated.

P-128

Sinonasal mucormycosis: A report of 3 cases

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Background: Mucormycosis, also known as "zygomycosis" or "phycomycosis" is a rare opportunistic infection caused by fungi that are commonly found in soil and among decaying vegetation. It is an aggressive and often fatal disease which occurs mainly in patients with immune disorders. It involves the rhinofacial-cranial area, lungs, gastrointestinal tract, skin and less commonly other organs.
Aim of study: To highlight clinicopathologic features of this opportunistic infection with special emphasis on prognosis and differential diagnosis.
Case Reports: we report 3 new cases of sinonasal mucormycosis that occurred in 3 female patients aged between 40 and 68 year old with a medical history significant for diabetes mellitus, who presented with complaints of nasal obstruction, purulent rhinorrhea and postnasal drip. The biopsy from the nasal mucosa revealed the presence of aseptate, broad and pleomorphic hyphae with right-angled branching which are typical of mucormycosis evidenced by special stains for fungi. In all patients, CT scan showed involvement of ethmoid and maxillary sinuses along with retro-orbital extension. The three patients were managed with lipid complex amphotericin B coupled with repeated surgical debridement. During the 3-month follow-up period, one patient died whereas, the other patients are still alive.
Conclusion: Greater awareness of this opportunistic infection and rapid clinical intervention have resulted in improved prognosis. Aggressive treatment with antifungals, antibiotics and wide debridement are the key to successful management of sinonasal mucormycosis.

P-129

Bone marrow in acquired immunodeficiency syndrome: a histologic, hematologic and microbiologic study

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Background: Bone marrow (BM) examination in HIV infected patients is performed for a variety of indications including pyrexia of unknown origin, diagnosis of cytopenia and for diagnosis or staging of lymphoma. BM studies offer the possibility of providing relatively rapid information about the presence of opportunistic infections in

addition to information about co-existent hematologic abnormalities.

Materials and Methods: We retrospectively evaluated 52 bone marrow trephine biopsies from HIV-infected persons in last five years. Fever of unknown origin, unexplained anemia or cytopenia were main indications for bone marrow studies.

Results: Seventy six percent marrow biopsies were normocellular or hypercellular; 24% were hypocellular. Marrow plasma cells were increased in 67% of patients. Histopathological examination of BM alone led to the diagnosis of specific condition in 17 (32%) patients (granuloma 11 cases, 2 cases of Histoplasma capsulatum and megaloblastic anemia and 1 case each of high grade B cell lymphoma and Idiopathic thrombocytopenic purpura). Ziehl -Neelsen stain for acid-fast mycobacteria was positive in 5 trephine biopsies. In 20 (38%) cases with pyrexia of unknown origin infectious pathology could be identified on histopathologic and microbiologic studies. The commonest being Mycobacterium tuberculosis in thirteen cases and Mycobacterium avium intracelluare in three cases, two cases of Histoplasma capsulatum and one case each of Cryptococcus neoformans and Candida species. Ninety four percent patients were anemic with iron studies showing a pattern consistent with anemia of chronic disease in majority of these. Forty four percent patients showed leucopenia, 51% had thrombocytopenia and 94% showed markedly reduced CD4+ T lymphocytes

Conclusion: Bone marrow biopsy may be a useful method for early diagnosis of opportunistic mycobacterial and fungal infection in patients with HIV infection presenting with persistent pyrexia. This may allow the treatment to be initiated before microbiological confirmation especially in Indian scenario.

P-130

Resolution of refractory TTP after successful treatment of a fulminant colitis due to Entamoeba histolytica.

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Background We report the case of a 33-years old female patient with history of long standing refractory TTP who recently presented with an acute abdomen.

Case description The patient was most recently hospitalized to evaluate new purpuric marks on her legs, with no additional symptoms. Initial examination revealed serum platelets lower than 5000. She was started on plasmaferesis and steroid therapy.

A week later, the patient suffered a sudden thoracic pain and dyspnea with haemoptysis. Thoracic plain X-ray showed bilateral infiltration that was interpreted as TRALI (Transfusion related acute lung injury) and treated with oxygen therapy. During her treatment the patient presents with acute abdominal symptoms indicative of severe peritonitis, potentially due to cecal perforation, and required emergency surgery. The presumptive diagnosis was that of steroid induced colitis with cecal perforation. Pathology examination of the right hemicolectomy specimen, revealed the presence of numerous amoebic forms, compatible with *E. histolytica, associated* with multifocal ulceration and perforation of the bowel wall. She received treatment with metronizadol and paromomicina with complete recovery. Furthermore, at ten months of follow up, there is no evidence of TTP.

Conclusion This is a very interesting and challenging case, since is the first described of a refractory TTP which resolves after treatment for E. histolytica infection. Amoebic infection needs to be considered as a potential cause for TTP. The pathobiology of this association requires additional studies.

P-131

Primary ovarian carcinosarcoma: a case report and review of the literature

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Background: Ovarian carcinosarcoma is a rare malignant neoplasm characterized by an admixture of malignant epithelial and stromal elements. This tumor grow very rapidly and is usually in advanced stages when diagnosed. Most patients are postmenopausal. About 77 per cent of the patients are dead of their disease within 1 year.

Method: We report a case of a primary ovarian carcinosarcoma in a 74 years old woman and we review the clinicopathological features and prognostic factors in ovarian malignant mixed Mullerian tumors.

Results: The patient presented with acute abdominal pain. Clinical examinaion and computed tomoraphy revealed a left-side pelvic mass. The patient underwent an exploratory laparotomy and a right ovariectomy. Histological examination of operative specimen revealed malignant epithelial and mesenchymal components. The patient had than total hysterectomy with left annexectomy. Despite postoperative chemotherapy, she died from the disease 8 months after surgery.

Conclusion: Ovarian carcinosarcoma is a distinct entity with a poor overall prognosis. The management of this tumour is difficult and randomised trials are needed to

accrue sufficient patient numbers and demonstrate optimal therapy.

P-132

SIMULTANEOUS EVALUATION OF T LYMPHOCYTE SUBPOPULATIONS IN THE LIVER AND IN PERIPHERAL BLOOD CELLS OF HCV, HBV-CHRONIC INFECTED PATIENTS

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T lymphocytes population are believed to be involved in the immunopathogenesis and evolution of chronic viral hepatitis.

In this study, we evaluated both intrahepatic (by immuno-histochemistry) and peripheral blood (by flow cytometry) T lymphocytes CD3+, CD4+ and CD8+, in 15 HBV and 38 HCV chronic infected patients, serologically proven. Simultaneously, histological lesions were assessed.

In peripheral blood, CD4+ T lymphocytes had increased values in HBV infection, while in HCV infection the values were normal/slightly increased. Compared to the average of normal values (41.5%) of CD4+ lymphocytes from healthy donors (HD) we have observed an increased tendency (~10%) of this subpopulations in case of HBV infection, while for the HCV infection, the values were almost normal. CD8+ lymphocytes presented a significant decrease in both types of chronic viral infections. Compared to the normal average values of 35.5% of CD8+ T cells from HD we have noticed a decreased value (~10%) for this subset in HBV and HCV infection.

At hepatic level,comparative statistic analysis between CD4+ and CD8+ T cells, in case of HBV chronic infection demonstrated a reverse proportion statistically significant ($r=-0.7$, $p=0.05$), the increasing number of CD4+ T cells being associated with the decreasing number of CD8+ T cells. In HCV chronic infection approximate 80% of cases have shown a low positive percentage of CD8+ T cells (no more than 5–15% cells from inflammatory infiltrate); no correlation between T cells subsets was noticed at hepatic level. It was found a correlation between intrahepatic CD8+ T lymphocytes and the severity of histological lesions. The imbalance of T lymphocytes subpopulations, especially CD8+ cells, may be associated with failure to clear the virus and a chronic course of disease, facilitated the liver damage.

P-133

Fine needle aspiration biopsy in the Hospital Central de Maputo, Mozambique

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Background: Fine needle aspiration (FNA) biopsy is an important adjuvant to the diagnosis of a palpable lesion which often permits the complete assessment of patients. There is limited information on the use of this technique in sub-Saharan Africa. The main objective of this study was to determine the clinical benefit of a FNA service at a tertiary referral hospital in Maputo, Mozambique.

Methods: All FNA biopsies performed at the Hospital central de Maputo during a whole year (January-December 2006) were retrospectively reviewed and analyzed.

Results: A total of 2479 FNA biopsies were performed during this period. 1599 (65%) were females and 874 (35%) males. Most biopsies were performed in children (14.5%) or young to middle aged adults (56.8%). Lymph nodes (43%), breast (23%), soft tissues (15%), thyroid (4%), salivary gland (3%) and liver (3%) were the most frequently biopsied organs. In the lymph nodes, granulomatous inflammation/tuberculosis (38%) and reactive hyperplasia accounted for 65% of the diagnoses, whereas only 2% of metastatic carcinomas were identified. Breast lesions were predominantly benign (88%). Overall, only 10% of FNA-biopsy specimens were malignant.

Conclusions: Benign conditions and particularly granulomatous diseases/ tuberculosis, probably related to HIV infection/AIDS, account for a significant number of biopsies, whereas malignant diseases represent only 10% of the aspirations. Our study shows the clinical benefit and cost-effectiveness of FNA services in a tertiary hospital in sub-Saharan Africa.

Molecular Pathology

P-134

Gene networks common to cancer stem cell status and malignant progression

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Malignant progression in cancer is closely linked to the emergence of populations of cancer stem cells (CSCs) endowed with capabilities for unlimited self-renewal, survival under stress, and establishment of distant metastases. By high-throughput transcriptome analysis, we have observed that cancer progression and CSC-like populations are associated with a limited number of tightly clustered gene/protein networks. The most significant progression-associated networks control (a) mitotic transitions and checkpoints, (b) DNA replication, and (c) DNA damage recognition and repair. The activation of these networks is associated with malignant progression and poor prognosis in most, if not all, solid tumors, independent of their tissue of origin. Inappropriate activation of these networks may provide unifying molecular and mechanistic explanations for the emergence and maintenance of metastatic cancer stem cells, and thus the aggressive phenotype of the majority of tumors.

P-135

Comparison of Formalin and Finefix in Preserving DNA Material in Small Biopsies.

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Aim: Formalin is a routine fixative in surgical pathology wards. Although morphologic assessment is very desirable in formalin fixed tissues but DNA and RNA are not preserved well and molecular studies can not be performed with reliable results. In this study we wanted to compare DNA preservation of a newly introduced alcohol based fixative with formalin in small biopsies.
Materials and Methods: Seventy small gastric biopsies taken in endoscopic ward was put in formalin and finefix in equal number. Standard processing was performed for two groups. DNA extraction and conventional PCR for Beta globulin gene (amplicon length: 256 bps) was performed for each case. Statistical analysis with Chi – square test for comparison of two groups was performed.
Results: In formalin fixed group, 20 of 35 specimens show Beta globulin band, but 30 out of 35 finefix group revealed this band. The difference between these two groups in preserving DNA for this gene is statistically significant (p value: 0.008).
Discussion: Although formalin is a good preservative for morphological purposes but molecular studies especially in small biopsies is not desirable and other fixatives such as finefix may be substituted.
Keyword: formalin, finefix, fixative, molecular studies, small biopsies.

P-136

The role of NKG2d and MICA genes in susceptibility to cervical cancer

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Cervical intraepithelial carcinoma represents the precursor of invasive neoplasias.This concept is of great importance because it represents morphological abnormalities and molecular characteristics. These are typical changes in malignant neoplasias when they have been associated with mutational viral lesions, even though these high grade types are not clinically apparent.The MICA gene expression has a restricted tissue distribution, and it is transcribed in epithelial cells when the protein is constitutively expressed in surface cervical cells.
MATERIAL AND METHODS: We have studied 167 patients in the hospital`s displasia clinical department.These patients were subjected to colposcopy, citology and biopsy. Of the total number of patients, fifteen were diagnosed using PCR and by immunocytochemically P16 as having papova viral lesions.
In these fifteen patients, MICA gene polimorphism was studied with PCR.SSCP technique which encoded allelic variants of MICA by polimorphisms in exons 2,3,and 4. This encoded the extracellular protein domains. This has led to the concept that MICA is a product of a marker of epithelial cells stress. This is consistent with induced virus where MICA proteins are incremented in the cell's surface. A total of 20 combinations of sense and antisense primers were selected for the analysis of MICA alleles 001–016. The putative MICA alleles was recognized, and the predicted size of PCR products studied in the 15 patients with VPH lesion.
The polymorphism of NKG2D gene was studied in these fifteen patients to determine the function of CD8 and NK cells and the capacity to destroy the host cells that express MICA.

P-137

Obtention and typification of prostate carcinoma explants and primary cultures

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Background In vitro studies on prostate cancer are mainly based on commercially available cell lines obtained from metastatic implants or artificially transformed normal

prostate epithelium such as PC3, LNCap, RWPE1 and RWPE2. Therefore, extrapolation of results obtained from such material to hormone-dependent primary tumours can be questionable. Thus, obtention and use of tumour explants and derived cell lines could be and important tool in molecular studies on prostate carcinoma.

Method Thin fresh tissue sections from radical prostatectomies with identifiable tumours were washed and cultured in serum-free medium supplemented with androgens. Of them, 10 primary cultures of epithelial cells survived more than one month and were subjected to several culture passes. RNA and DNA were extracted and CGH and 8 K Affymetrix Human Focus microarray analyses were performed. Further typification was carried out with immunocytochemistry for several markers including CD133.

Results Unsupervised cluster analyses of our primary cultures showed a relatively common profile with overexpression of basal/stem cell markers, which was very different from that of commercial cell lines and primary source tumours. Immunohistochemical expression of stem cell markers such as CD133 was also observed.

CGH analysis did not show significant structural abnormalities in 4 cases analysed.

Conclusion Contrarily to initial expectations, the primary cultured obtained from prostate cancer did not show evidence of malignant transformation, with scarce or no structural abnormalities, but and overall resemblance to basal/stem cells, whose RNA profile is very different to that of commercially available malignant prostate cell lines. We believe that the cell lines here presented have a great potential for future uses in *in vitro* models in the study of prostate cancer.

P-138

CDKN2A and TP53 mutational analysis in metastatic squamous cell carcinoma of the skin

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Background: At present, prognosis of cutaneous squamous cell carcinoma (CSCC) is mainly determined by TNM stage and certain histological features as poor differentiation grade. So far, molecular features of metastatic CSCCs are relatively unexplored. In non-metastasized sporadic CSCCs, the *TP53* gene is mutated in up to 50% of cases. More recently, also mutations in the *CDKN2A* (*INK4A-ARF*) locus were reported in up to 24% of CSCCs, while combined *TP53/CDKN2A* mutations are rare (5%). In addition, in skin carcinogenesis, several studies have suggested an oncogenic role for cutaneous and mucosal HPV types.

Material and methods: The aims of this study were to assess a possible role for *CDKN2A* and *TP53* in metastatic CSCCs, by performing mutational and immunohistochemical analyses on 35 metastases and their corresponding primary tumors, and to detect persistent beta-PV and mucosal HPV infections, by using PCR based assays.

Results: We detected a *CDKN2A* mutation frequency of 37% (13/35) and a combined *CDKN2A/TP53* mutation frequency of 14% (5/35). *TP53* was mutated in 18/35 cases (51%). P53 protein expression proved to be significantly associated with missense type of *TP53* mutation (P=0.001), while effects of *CDKN2A* mutations on p16 and p14 expression were less clear. Finally, no persistent HPV types were detected in the metastases.

Conclusion: Our data demonstrate a higher frequency of *CDKN2A* mutations and combined *CDKN2A/TP53* mutations in metastatic CSCCs, compared to frequencies reported in literature for non-metastatic sporadic CSCC, possibly indicating a higher metastatic potential in CSCCs. Our data do not support a role for beta-PV and mucosal HPV in metastatic CSCCs.

P-139

EXPRESSION OF VEGF-A IN WOUND HEALING BY SECOND INTENTIONAND ITS RELATION TO CELLULAR PARAMETERS

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Introduction: VEGF is the most important mediator of angiogenesis, while both neutrophils and macrophages have a role in VEGF-A transcription.

Pentadecapeptide BPC 157 has beneficial influence on healing process.

AIM: to analyze wound healing by second intention through expression of mRNA VEGF A, number of neutrophils and macrophages and attempt of compare them in control group and treated group.

Methods: Full-thickness excisional wounds were made on the back of adults rats. The animals were treated as follows: BPC 157 10 μg/ kg i.p., control group; equivolumen of saline. Wounds were left uncovered, animals were harvested 4, 6, 12, 24, 48, 72, 120, 168 hrs after wounding. For histology analysis was taken half of the full thickness skin wound area and subcutis of the wound for molecular analysis (RT-PCR).

Results: In control group expression of mRNA VEGFA followed the published data (VEGF levels peaked at 6 and 48 hrs post-injury) and in treated group mRNA VEGF showed a sinusoidal curve with three positive and two negative peaks tending toward basal level. Difference in number of neutrophils and macrophages in different part of skin, at the same post-injury time is evident in control and treated animals. Number of neutrophils was significantly elevated in control group of animals; subcutis: 4, 12, 24, 72 hrs post-injury, low third of dermis: 12, 24, 120 hrs post-injury, middle third of dermis: 24, 120 hrs post-injury, high third of dermis: 24, 48 hrs post-injury. Number of macrophages was significantly elevated in control group of animals; subcutis: 6, 12, 24, 48 hrs post-injury, low third of dermis: 48, 72 hrs post-injury, middle third of dermis: 72, 120, 168 hrs post-injury, high third of dermis: 72 hrs post-injury.
Conclusion: Control group shows that in vivo wound healing by second intention confirms results of in vitro studies. Treatment with BPC 157 results with better controlled healing process through influence on mRNA VEGF-A expression and downregulation of number of neutrophils and macrophages. Correlation between neutrophils, macrophages and VEGF-A expression is evident, in both groups of animals.

Transplantation pathology

P-140
HISTOLOGICAL, IMMUNOMORPHOLOGICAL AND ULTRASTRUCTURAL CHANGES IN A TRANSPLANTED KIDNEY

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The present investigation was carried out on 148 punctural renal biopsies of transplanted kidneys during the period between 1999 an 2006. All materials were investigated with the standard methods of nephromorphology:

1. Histological and histochemical – hemalaun – eozine, PAS-reaction, trichrome – Mason, silver impregnation of Wilder, Congo – rot;
2. Immunomorphological – direct immunofluorescence with antiserums for IgA, IgG, IgM, C1q, C3c, C4c and fibrin;
3. Eighty-six of the biopsies underwent electronmicroscopic investigation.

Using the classification of Banff 1997, we divided the results we obtained into the following groups:

1. Acute transplant rejection – 43 cases;
2. Chronic transplant nephropathy – 88 cases;
3. Cyclosporine lesions of the allograft – 7 cases;
4. Postoperative necrosis in renal allograft – 4 cases;
5. Glomerulopathy recidive – 6 cases.

The results of the electronmicroscopic investigation gave us valuable data for particularization of the morphological diagnosis in some cases of chronic transplant nephropathy, cyclosporine lesion of the transplanted kidney as well as in recidives of the main kidney disorder.
Although the present study is concerned with morphological diagnosis, we have to point out the undoubted role of the clinical data about the state of the patients.

P-141
Cholesterol embolization in cadaveric donor kidneys

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INTRODUCTION Cholesterol embolization (CE) is rarely seen in renal allograft specimens. In contrast to CE in native kidneys, its natural history and prognostic significance are not as well characterized.
MATERIAL AND METHODS Archival samples from 42 preimplant biopsies of cadaveric donor kidneys (November 2006 to November 2007) were reviewed, as well as the clinical charts and additional pathology samples of the patients found to have CE.
RESULTS Three cases with CE were found. In the first two cases, the donors had atherosclerotic risk factors and kidneys were refused. CE were present in the nephrectomy specimen (but not in the preimplant cortical biopsy) together with associated ischaemic changes. The third case was from a 66 year-old male without known cardiovascular risk factors who died of a stroke. A preimplant frozen biopsy from the left kidney showed a unique intravascular CE. The two kidneys were grafted in two different patients. The left one failed on the fourth day after transplant. The nephrectomy specimen showed diffuse ischaemic necrosis with several CE and marked arterial occlusion. The right kidney performed well and a five months protocol posttransplant biopsy revealed borderline acute rejection, without CE.
CONCLUSIONS We have found a 7% incidence of CE in preimplant biopsies of donor kidneys which is higher than previously reported for implanted kidneys. It is probably due to the policy in our center of accepting a more

extensive donor age. Intraoperative preimplant frozen sections appear not to be a suitable method to rule out CE in donor kidneys. The prognostic significance of finding CE in preimplant biopsies of donor kidneys remains unclear.

P-142

Positivi capillary staining for C4d associated with viruses molecular detection in monitoring endomycardial biopsiesof cardiac transplanted patients

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Objectives: The finding of complement deposition in linear pattern by monoclonal antibodies for the activated complement split product C4d by immunofluorescence or immunohistochemistry is recognized to be insufficient for the diagnosis of antibody-mediated rejection.
It has been recognized that early ischemic injury and viral infection can produce a positive capillary staining for C4d. In heart transplant no data are available which correlate C4d capillary positivity with virus infection. Aim of our study was to assess the frequency of C4d positivity with the molecular detection of virus.
Material and methods: 183 endomyocardial biopsies were studied. C4d was performed on paraffin embedded specimens and C4d staining was graded according to a score from 0 to 4. PCR/RT-PCR analysis for detection of viral genome of more common cardiotrophic viruses (CMV, EBV,EV, HSV,PV,B19) in EMB was carried out.
Results: Among the 183 EMB analyzed, 29 EMB of different patients were positive for viral detection (15,8%). Of these, thirteen EMB showed a C4d staining of grade 1 and 2 (44%), according to the score from 0–4 and 3 EMB showed a C4d staining of grade 3 and 4(10%), the other 13 EMB were negative for C4d staining. The viruses detected were CMV in 8 EMB with grade 1–2 of C4d staining, EBV in 5 EMB with grade 1–2 and, EV in 3 EMB with grade 3–4 of C4d staining.
Conclusions: C4d positivity on EMB can be detected during viral infection. In our population EV was the most frequent virus associated with severe C4d staining suggesting that a capillary positivity for C4d should be further assessed for cardiotrophic viruses myocarditis

Posters
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Paediatric Pathology

P-143

Detection of fusion gene trasncripts in formalin-fixed paraffin-embedded tissue sections of rhabdomyosarcoma after reverse transciptase-polymerase chain reaction and fluorescence in situ hybridization

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Introduction: Rhabdomyosacoma is the most common soft tissue sarcoma in childhood. The histology of rhabdomyosarcoma correlates with prognosis; alveolar rhabdomyosarcoma (ARMS) has a survival of 55%. ARMS have one of two chromosome translocation detected in 85% of the cases, t(2;13)(q35;q14) more common and t(1;13)(p36, q14). RT-PCR and FISH are powerful tools for the retrospective analysis or formalin-fixed paraffin-embedded tissue.
Methods: We designed a descriptive, cross sectional, comparative studies. The study population consisted of 30 cases of ARMS from archival paraffin-embedded biopsy samples. The negative controls were consistently in all cases. For RNA extraction from paraffin-embedded tissue was made using the acid guanidinium-phenol-chloroform method. Each RNAsample was studied using RT-PCR for three differente primer pairs: PGK fwd/rev (quality of theRNA), PAX3-FKHR, PAX7-FKHR. Amplified products were separated on 2% agarosa gel electrophoresis. For FISHassay we used bacterail artificial chromosome (BAC) probe sets. Two color FISH studies were performed. Probeswere directly labeled for nick translation with either spectrum green (FKHR) or spectrum orange PAX7 and PAX3).Images were adquired by use of CytoVision Applied Image.
Results: ten cases of PAX3-FKHR were found, one case of PAX7-FKHR and ten cases with no translocation. In nine cases we did not get RNA. We also compared the results between the two techniques. The oldest paraffin-embedded tissue had 14 years old.
Conclusions: There are a number of methodologies that can be used for the detection of tumor specific fusion trsncripts from archival paraffin-embedded tissue. In our hospital is posible to do it in retrospective studies, prospective studies and for the analysis of low incidence diseases. The fusion status is an essential molecular marker

in the prognosis or ARMS and makes the molecular biology methods an invaluable tool in the treatment assignment.

P-144

Cryptosporidium Infection in Pediatric Patients with Lymphohematopoietic Malignancies

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Objective: Cryptosporidium parvum is a common protozoan pathogen with worldwide distribution. It localizes on the intestinal cells and prolonged diarrhea in immunocompromised patients. The aim of this study was to estimate the prevalence and the clinical features of enteric cryptosporidiosis in pediatric patients with lymphohematopoietic malignancies.
Material & Methods: In this cross-sectional study stool samples were collected from 100 children (67 boys, 33girls) with lymphohematopoietic malignancies who underwent chemotherapy between the ages of 6 months and 17 years (mean age 7.5 years). All of the specimens were examined for the oocysts of C. parvum by modified Ziehl Neelsen (MZN) staining technique and coproantigens of C. parvum by ELISA.
Findings: Cryptosporidium infection was detected in 22 patients. 16 (72.7%) of the infected patients were male and 6 (27.3) female. 7 (31.8%) patients were <5 years, 8 (36.4%) 5–10 years and 7(31.8) >10 years old. Parasites were detected in 19/85 (86.4%) patients with ALL, 2 of 5 (9.1%) with AML, and 1 of 10 (4.5%) with NHL. Clinical symptoms were found in 11 (50%) of the patients. We found longer duration of chemotherapy in patients who were positive for cryptosporidium infection (Mean= 2067 days) in comparison to negative group (Mean= 258.5 days) (ANOVA, f=2.82, P=0.04).
Conclusion: The incidence of cryptosporidium infection was 22% among pediatric patients with lymphohematopoietic malignancies. We recommend evaluation of these patients with at least two different diagnostic methods in order to prevent possible life threatening outcomes.

P-145

Immunohistochemical expression of P53 protein in histologically favorable Wilms tumor and its relationship to tumor stage at presentation

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Objective: Wilms tumor, as the most common renal tumor of children, has been associated with chromosomal abnormalities. Although a correlation between anaplasia and mutations of P53 tumor suppresser gene has been found in Wilms tumor, significance of these mutations in different clinical stages of favorable-Wilms tumor, remains largely unresolved. The goal of this study was to determine the frequency of P53 expression in histologically favorable Wilms tumors and its correlation to tumor-stage at presentation.
Materials and Methods In this retrospective study, 48 cases of confirmed Wilms tumor with favorable-histology were retrieved from the files of departments of pathology in three hospitals in Mashhad University of Medical Sciences between 1990 and 2004. Histological characteristics and clinicopathological staging were in accordance with National Wilms Tumor Study guidelines. P53 expression was determined by the immunohistochemical method. For each section, the proportion of neoplastic cells exhibiting nuclear positivity was broadly quantified and their intensity of staining was charted, based on visual impression, elever by two pathologists.
Results A total of 48 cases of histologically favorable Wilms tumor were assessed. Eleven cases (23%) showed positivity for P53 which were 3 (27.3%) with stage II, 3 (27.3%) with stage IV, 2 (18.2%) with stage I, 2 (18.2%) with stage III and 1 case (9.1%) with stage V. The P53 immunopositivity was seen in 1–25% of tumor cells in 9 cases (18.8%), in 26 to 50% of tumor cells in 1 case (2.1%) and in >75% of tumor cells in the other one case (2.1%). The intensity of staining was moderate in 6 cases (12.5%), weak in 4 (6.3%) and strong only in one case (4.2%). The most common component with P53 immunoreactivity was blastemal in 11 cases (100%). In 4 cases (36.4%) there was also positivity in epithelial and in 2 cases (18.2%) in mesenchymal components.
Conclusion We found no correlation of P53 immunoreactivity and its intensity to tumor stage at presentation in individuals with histologically favorable Wilms tumors (p= 0.66, p=0.52 respectively).

P-146

Ameloblastic fibrosarcoma of the maxillary sinus in an infant: a case report with long-term follow up

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Ameloblastic fibrosarcoma (AFS) or ameloblastic sarcoma is an extremely rare odontogenic neoplasm. The authors report ameloblastic fibrosarcoma (AFS) in the maxillary sinus of a

4-monthold boy. The tumor composed of odontogenic epithelium, resembling that of ameloblastoma and a mesenchymal part exhibiting features of fibrosarcoma. We also found some areas with deposition of dentinoid material closely adjacent to ameloblastic epithelium. Although AFS has been occurred in a wide age range, this is the first report of this tumor in infancy with long term follow up.

P-147

Sialolipoma of the parotid gland with diffuse sebaceous differentiation in a female child

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Sialolipoma of the salivary gland is a tumor with ambiguous histogenesis.Histologically,this lesion is composed of mature adipose tissue and salivary glandular components.To the best of our knowledge,only twelve documented cases of sialolipoma have been reported in the literature.Except for one congenital case,all of the cases were found in adult patients.In this report,we present a unique case of sialolipoma with diffuse sebaceous differentiation in a 3-year-old female child.The differential diagnoses are discussed.

P-148

SIRENOMELIA IN SIAMESE TWINS. FIRST CASE REPORT OF THE LITERATURE

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Background. The sirenomelia as the presentation of Siamese twins are extremely rare birth defects, and its presentation as a whole is even more unusual.

Objective. To describe and make a discussion of the morphological features of a case of Siamese twins in which one of them had sirenomelia.

Case report. Thoracopagus Siamese Twins, with a gestational age of 15 weeks, product of the fifth pregnancy of a 26 years old woman, who arrives to the obstetrics service with clinical symptoms of uterine activity and genital bleeding. Obstetrical ultrasound showed Siamese twins pregnancy. Subsequently presents delivery of thoracopagus Siamese fetus.

Autopsy. We received two male fetuses bodies, weighing both 320 grams, each with a length crown to ankle 13 centimeters, cranial circumference of 11.5 centimeters and a length of the sole of 20 millimeters, with multiple external malformations common to both fetuses as broad defect in the abdominal and thoracic anterior wall with exposure of the heart and abdominal viscera, agenesia of the lumbar spine and anal atresy; also one of the fetuses showed fusion of the lower limbs by the middle line to the ankles. With the above findings, diagnosis of Thoracopagus Siamese Twins and Sirenomelia type 1 was made.

Conclusions. We present a case of an unusual combination between two rare congenital anomalies; in a search in MedLine literature from January 1964 to November 2007 there where no citations. This mixture is characterized by submitted in conjunction with other alterations of the thoracic wall, vascular, renal, gastrointestinal and spinal system that make it incompatible with life.

P-149

Ezrin immunohistochemical expression in pediatric tumors by tissue array

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Background Ezrin is a cytoplasmic cytoskeleton-linker protein. In normal cells, ezrin is involved in polarity and cell-to-cell signal transduction, whereas in some tumor cell types it is believed to play a role in regulating growth and metastatic capacity. Its prognostic significance has been described in some adult neoplasms and osteosarcoma. The aim of this study was to analyze ezrin expression in several pediatric tumors and to determine if ezrin expression in the primary tumor correlates with a higher metastatic rate.

Methods Tissue microarray blocks were constructed, including 56 neuroblastomas (NB), 23 Ewing's sarcomas (ES), 12 synovial sarcomas (SS) and 19 fibroblastic proliferations (FP). Whole-tissue sections were studied whenever needed. A commercially available antibody (Ab1; 3C12 clone; Neomarker, Lab Vision Corp, CA) at 1/100 dilution was used. The intensity and localization of the immunohistochemical expression were recorded.

Results and conclusion Out of the 56 NB, 42 were evaluable and all showed positive staining (100%) with no definite pattern. Ten out of the 12 SS could be evaluated and they were all positive (100%). The fusiform and epithelial portions showed cytoplasm labeling, being the epithelial component the more intensely stained. The FP tissue array included 19 cases, of which 13 could be evaluated and displayed positive cytoplasm immunoreactivity. Twenty two of the 23 ES evaluated were positive (95.6%). Fourteen exhibited membrane reinforcement and eight showed cytoplasm diffuse positive staining alone.

There were no differences in the metastatic rate and outcome in these two subsets of ES patients.
In conclusion, intense and widespread ezrin immunoreactivity was observed in several pediatric tumors, including NB, SS, FP and ES. Further investigation is warranted to determine its clinical relevance.

P-150
INTRARRENAL TERATOMA OCCURRING IN INFANCY. REPORT OF A CASE WITH DISCUSSION OF EVALUATION AND DIAGNOSIS

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BACKGROUND: Germ cell tumors are neoplasms that occur relatively infrequently and are most commonly encountered in the gonads. Nonetheless, such tumors have also been described in other locations, such as the pineal gland, retroperitoneum, mediastinum and sacral area. Very rarely teratomas and tumors derived from germ cell can arise within the kidney.
METHODS: The clinical and radiological features a three month old female infant with abdominal mass and review of the literature.
RESULTS: This cases presented during the year 2000 represent the casuistry of the University Hospital of The Andes (IAHULA), Mérida, Venezuela. The infant was asymptomatic with progressive abdominal distention. Serum α-fetoprotein, routine hematological and serum chemical results were normal. The X-ray and ultrasound study showed a retroperitoneal tumor of left kidney heterogeneous and amorphous calcifications. During the surgery, the tumor was excised completely and the specimen weighed 420 g. Macroscopically, the tumor was described within the kidney parenchyma. The tumor multicystic with of fat, sebaceous and mucinous materials, hair, and blood. The histological examination revealed the presence of neoplastic cells derived from more that one germined status.
CONCLUSIONS: The teratoma renal is an uncommon neoplasm with a benign clinical course and good prognosis. The suspicion clinical is based in the anamnesis, examination physical and imaging technology. Surgery should thus be the first choice for treatment with excised completely of tumor. Histopathologic differential diagnosis with Wilms' tumor with teratomatous areas is mandatory. Twelve years later, there was no evidence of tumor recurrence.

P-151
Pathology of the thymus and immunodeficiency in pediatric mortality and experiments

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Severe or generalized infections are one of the main causes of death in pediatric mortality and associate with congenital or acquired immunodeficiency (ID). The actual problem is post-mortem diagnostics of ID with comparison to clinical and laboratory data. *The aim of investigations* is evaluation of thymus pathology and ID in pediatric mortality from infectious diseases and experiments.
Methods. Diagnostics of ID was based on retrospective analysis of pediatric autopsy in comparison to manifestations of infections, in some groups - with symptoms of SIRS. Experimental model of poststress accidental involution (AI) was worked out to study the thymus pathology and mechanisms of acquired ID (Volkova L.V., 1996). H&E/Van Geison methods, morphometry, correlation and systemic analysis were used.
Results. The main variants of post-mortem histological diagnosis were following: *accidental involution of thymus* of different degree (1–2-"starry sky", 3- inversion of cortex and medulla, 4–5 - atrophy of thymus), *thymomegaly, dysplasia* and *hypoplasia* of the thymus. It was revealed the high rate of AI and thymomegaly. AI of 3–5 degree was the variant of acquired ID and in some cases associated with pathology of pregnancy, artifical nutrition of newborns, repeated or severe infections in postnatal period. Morphology and mechanisms of AI were examined in experiments with morphometry, systemic and correlation analysis. High synchronism of quantitative alterations of cytological profiles in structural zones of the thymus, spleen, peripheral blood and expressed changes of lymphoid cell population in AI 2–3 were revealed. The facts can be used in post-mortem diagnostics and clinical laboratory examinations of patients with ID and infections. High rate of AI in cases of lethal infectious diseases in pediatric pathology associates with acquired ID and possibilities of prophylaxis.

P-152
Study of respiratory infection caused by Chlamydia pneumoniae in children in the departament of pediatrics - the Ipiranga Hospital, Mogi das Cruzes (Brazil)

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Introduction: *Chlamydia pneumoniae* is a pathogen usually associated with respiratory pathology. It has been estimated

that this agent causes 5 to 15% of all pneumonias but its incidence among hospitalized children with respiratory infections is unknown.
Objective: Characterization of the *Chlamydia pneumoniae* respiratory infections in hospitalized children.
Patients and Methods: Retrospective longitudinal study of the *Chlamydia pneumoniae* respiratory infections in children admitted between January 2005 and June 2007 in the Department of Pediatrics in the Ipiranga Hospital - Brazil. Sex, age, race,socio-economic status, number of scholar siblings, frequency of nursery/school, parents smoking habits, familiar atopy, personal history, clinical presentation, diagnosis,treatment and follow-up were analyzed for each child.
Results: 55 cases were documented, 67% males and 52% whites. The age on admission varied between 14 days and 16 years-old, with a higher incidence below 6 years-old (57%). Twenty-three (40%) of the children were in nursery or in school and 12 (43%) had siblings at school-ages. Parent smoking habits were documented in 21 (56%) and familiar atopy in 21 (48%) children. Respiratory atopy (9 children), gastroesophageal reflux (5), cerebral palsy(1), cleft palate (3) and prematurity (3). Pneumonia was the most frequent clinical presentation (67%), followed by bronquiolitis (29%). The clinical presentation was not specific, with cough (86%), respiratory distress (69%) and fever (66%) beingthe most frequent signs. The most prevalent radiological pattern was the interstitial(49%). Coinfection by other agents occurred in five cases: *S. pneumoniae* (2), *H.influenzae* tipo b (1), *P. aeroginosa* (1) and tuberculosis (1). Complications weredocumented in 32 (58%) children: hypoxemia (24), pleural effusion (11), atelectasis (5) and atelectasis and hypoxemia (5). Macrolids were prescribed in 49% cases.
Discussion: This study calls the attention to the fact that *Chlamydia pneumoniae* infection is an etiology to be considered in children with respiratory infection and hospitalizationcriteria. This infection can occur in all ages. Because the clinical presentation is not specific and complications can occur, a high leveI of suspicion is necessary for its diagnosis.

P-153

THE EXPRESSION OF HISTONE ACETYLTRANSFERASE PCAF IN PEDIATRIC ASTROCYTOMAS, IT'S ROLE TO RESPONSE TO THERAPY AND MORE HIGHLY ACETYLATED HISTONE H4.

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Background. Central Nervous System tumors (CNST) are the third neoplastic disease in childhood and astrocytomas represent 20%. Currently, there is not a tumoral marker that correlates with astrocytoma. Characterisitcs as expression of chromatin modifiers and acetylated histones could be considered as tumoral markers not-described in astrocytomas.
Method. We analyzed the expression of PCAF and p300 by semi-quantitative RT-PCR in CNST surgically obtained. Also we isolated total histones from nuclei and they were electrophoresed. The median of expression of PCAF and p300 was calculated and compared against benign tumors (BT). The correlations were performed with clinical data.
Results. PCAF was not expressed in BT but it was in 100% of CNST. PCAF was expresssed with a median of 134 DU in CNST against 82.4 DU in BT; the differences were significant ($p<0.05$). CNST treated with chemoterapy revealed 110 DU and chemo plus radioterapy 124 DU; comparison to CNST not treated were also significant. 92% CNST presented di- and triacetylated H4 against 0% of triacetylated H4 in BT.
Conclusions. PCAF gene expression could be a molecular marker in CNST. The increased pattern of histone H4 acetylated also can represents a malignant marker correlated to high PCAF expression. Actually we are going to evaluate PCAF as prognostic marker.

P-154

Clinico-morphological criteria for differential diagnosis of septicenia in labor and puerpperal sepsis.

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Background -Pyoseptic desease still rank the third among the causes of maternal death after hemorrhages and gestoses. The purpose of our research was to develop clinico-morphological criteria for differential diagnosis of such special forms of obstetric sepsis as septicemia in labor and puerperal sepsis.
Methods -We studied 24 autopsies of puerperas who died over a 10 year period (1996 - 2006) of pyoseptic diseases in the Rostov Region, and analyzed their placentas and partum histories. Microscopic examination of uteri, spleens and lymph nodes and placentas was performed by standard methods of light microscopy, with hemotoxillin and eosin staining and PAS reaction. We used antibodies of DAKO, Denmark for immunohistological verification of cells from the inflammatory infiltration.
Results - Microscopic and immunohistochemical examination of the uterus in cases of septicemia in labor showed

low-grade infiltration with granulocytes, macrophages, T-lymphocytes in endometry. With puerperal sepsis there was diffuse infiltration with plasmacytes and macrophages in endometry and myometry. Some cells in the infiltrate were positive with TNF.
In cases of septicemia in labor the inflammatory infiltrate in the placental fetal membranes was represented by a great number of granulocytes, plasmacytes, macrophages, and rare T-lymphocytes. Some cells in the infiltrate were positive with TNF. There were no inflammatory infiltrate in fetal membranes in puerperal sepsis.
Conclusion - With septicemia in labor the onset of clinical symptoms was observed within the first 24 haurs after the delivery and in cases of puerperal sepsis the symptoms developed 3–5 days later. With septicemia in labor the focus of infection was in placenta, and in cases of puerperal sepsis the infection developed from the uterus. Our conclusion is confirmed by the presence of the key antiinflammatory mediator TNF (i.e. development of cytokins as a response to the focal damaging effect of microorganisms and their toxins) in placenta or in uterus respectively.

P-155

Medulloblastomas: Review of 51 cases. Study of INI1 to disclose atypical teratoid/rhabdoid tumours

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Background: The aim of this study is to verify histological and immunohistochemical (IHC) markers of medulloblastoma, which can help us in the differential diagnosis and survival correlation.
Method: The study included 51 patients with a diagnosis of medulloblastoma recorded from 1982 to 2004. Histological variants, mitotic activity, microvascular proliferation, pseudopalisading necrosis and neuroblastic rosettes were analyzed. Additionally, a tissue microarray was performed including synaptophysin, NSE, GFAP, NF68, EMA, vimentin, WT1, c-erbB2, p53, INI 1 and ki67 expression.
Results: The average age at diagnosis was 5.6 years old. The overall survival (OS) was 37.5%, although survival improved in recent cohorts. According to the histological parameters studied we found that desmoplastic type and extensive nodularity were histological variants with better prognosis and high OS, whereas tumours with more than 20 mitosis/10HPF were associated with a worse prognosis and low OS. IHC analysis showed expression of synaptophysin in 76.6% of cases, NSE 100%, GFAP 31.9%, NF68 36.2%, EMA 8%, vimentin 34%, WT1 (cytoplasmic) 25.6%, c-erbB2 4.4%, p53 6.7%, INI 1 93.5%. Ki67 expression was >50% in 23.5% of cases, 20–50% in 41.2% and <20% in 35.3% of cases. Lack of INI 1 expression in 3 tumours previously diagnosed as medulloblastoma suggested the diagnosis of atypical teratoid/rhabdoid tumour (AT/RT). This was confirmed by hSNF5/INI 1 gene mutation analysis.
Conclusion: Long survival correlates with desmoplastic and extensive nodularity; likewise with less than 20 mitosis/10HPF. AT/RT can show similar histology and IHC markers as medulloblastoma. Lack of INI 1 expression is only detected in AT/RT tumours. INI 1 study in embryonal tumours in children is essential in order to disclose a possible AT/RT.

P-156

Morphological patterns of the intrauterine

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Background: Epidemiological studies demonstrated a relationship between impaired fetal growth and risk of adult diseases. Animal models support the hypothesis that some pathologies may originate early in gestation.
The aim of present investigation was to find out the relationship between impaired placental growth and restriction in growth of fetal organs and body at late gestation.
Fetal autopsy and placenta study were performed in 23 cases of termination of pregnancy for socio-economic reasons at 23–25 weeks of gestation. Fetal weight (FW), length, body mass index, circumferences of the chest (Cch), head (Ch), abdomen (Ca), and placental weight (PW) were recorded. Besides these, fetal organs weights were found, including liver (LW), kidneys (KW), heart (HW), pancreas (Pn W). Some indices were then calculated: PW/FW; PnW/PW; KW/PW; HW/PW; LW/PW, etc. Results: The high PW/FW index (0.31±0.02) has made it possible to identify group which shows disproportionate growth patterns between the fetus and placenta. (IUGR group). This group had smaller parameters of LW/PW (0.18±0.01), KW/PW (0.03±0.00) and HW/PW (0.02±0.00) than in controls (0.21±0.01, $p<0.04$; 0.05±0.01, $p<0.05$; 0.03±0.01, $p<0.00$ respectively).Conclusion. The results showed that a stressful intrauterine environment at 23 weeks of gestation results in series of placenta-fetal responses including disproportional growth patterns between placenta, fetal kidneys, heart and liver.

P-157

Neuronal maturation in an experimental model of brain tissue heterotopia in the lung

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Neural maturation involves diverse interaction and signaling mechanisms which are essential to the development of the nervous system. However, little is known about the development of neurons in heterotopic brain tissue in the lung, a rare abnormality occasionally observed in malformed babies and fetuses. The aim of this study was to identify the neurons and to investigate its maturation in experimental brain tissue heterotopia both during fetal and neonatal periods. Twenty four pregnant female Swiss mice were used to induce brain tissue heterotopia on the 15th gestational day. Briefly, the brain of one fetus of each dam was extracted, disaggregated and injected into the right hemithorax of siblings. Six of these fetuses with pulmonary brain tissue implantation (PBI) were collected on the 18th gestational day (group E18) and six other on the 8th postnatal day (group P8). The brain of fetuses from dams not submitted to any experimental procedure was collected on the 18th gestacional day (group CE18) and on the 8th postnatal day (group CP8) to serve as a control. Immunohistochemical staining for NeuN was used to assess neuron quantification and maturation. NeuN Labelling Index (LI) was greater in postnatal than fetal period both for the experimental and control groups (P8 > E18 and CP8 > CE18), although there were fewer neurons in experimental than in control groups (P8 < CP8 and E18 < CE18) ($P < 0.005$). These results indicate that fetal neuroblasts/neurons not only survive a dramatic event such as the one secondary to mechanical disaggregation, similar to what happens in human cases, but they keep its development in heterotopia irrespective of local tissue influences.

P-158

Retroperitoneal extralobar pulmonary sequestration

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Background: Pulmonary sequestration is a pulmonary mass anatomically separated from bronchial tree with sistemic blood supply. Intralobar variety shares visceral pleura with normal lung. Extralobar variety (EPS) is covered by its own pleura; it can be thoracic, intradiaphragmatic or infradiaphragmatic, which happens because of the entrapment of developing pulmonary tissue into the infradiaphragmatic space before pleuroperitoneal membranes close (5th-8th gestational week).

Case report: One month-old female newborn. An intra-abdominal mass is detected by ecography at 26th gestational week. Delivery occurs at 40th week. Abdominal ultrasound and sanner reveal an image in left hypochondrio the suggests differential diagnosis between cystic neuroblastoma and pulmonary sequestration. Left adrenalectomy and excision of cystic tumour are performed. The specimen is 7 g and includes adrenal gland of 5 cm and a rounded 2 cm mass, covered by a smooth tissue; cut surface is fleshy and microcystic.

Results: Histologically the nodule is composed by tubular structures that remain pulmary airways, some cystic, with papillary projections into the lumens, covered by a single layer of ciliated cylindrical cells. Arterial vessels with thickended walls, dilated lymphatic vessels, lymphoid aggregates, nerves, striated and smooth mucle fibers and cartilaginous tissue are seen in interstitium.

Comments: EPS may be composed by almost normal pulmonary tissue, may have reactive changes if secondary infection, or may have an image similar to congenital airway malformation. Finding striated muscular tissue in interstitium is usual, although its clinical importance is not already known. EPS can associate with cardiac and gastric anomalies or diaphragmatic hernia. Usually it is diagnosed in prenatal ultrasound controls, until first six months. Diagnosis is adults is rare. Differential diagnosis of a prenatal diagnosed abdominal mass must include neuroblastoma, pulmonary sequestration, adrenal hemorrage, renal tumours and malformations, gastric duplication, teratoma, lymphangioma and hemangioma. Microscopically, pulmonary sequestration, teratoma and rabdomyosarcoma must be considered.

P-159

Congenital paranchymatous diseases of the lungs, incidence, distribution and relation with extrapulmonary malformations in pediatric autopsies and biopsies

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Among the autopsies and biopsies performed between 1977–2005 at Hacettepe University Children's Hospital, Division of Pediatric Pathology, cases diagnosed as congenital paranchymatous diseases of the lung were investigated. In congenital paranchmatous diseases of the lung, incidence, distribution and relation with extrapulmonary malformations were retrospectively evaluated. In this

period totally 44 553 biopsies ve 3483 autopsies were performed. Of the cases, 61 (0.13%) and 85 (2.4%) were diagnosed with congenital paranchymatous disease of the lung in biopsies and autopsies respectively. The most commonly encountered congenital paranchymatous diseases of the lungs were pulmonary agenesis, aplasia and hypoplasia. Less commonly congenital pulmonary airway malformation, congenital pulmonary emphysema, bronchogenic cysts, enteric cysts, pulmonary sequestrations and congenital pulmonary lymphangiectasis were seen respectively. In 83.5% of autopsy cases, one or more extrapulmonary malformations were detected. The most frequent encountered extrapulmonary malfomations were craniofascial and genitourinary system malformations. Except congenital pulmonary lymphangiectasis, other congenital paranchymatous diseases of the lung are commonly associated with extrapulmonary malformations. When a congenital paranchymatous diseases of the lung are detected in a patient, detailed investigations on extrapulmonary malformations should be done. In conclusion, the exact true incidence and mortality of all congenital paranchymatous diseases of the lung are displayed by this study including not only operated on congenital bronchopulmonary malformations but also with autopsy findings of neonates and stillbirths.

P-160

Alobar Holoprosencephaly in Monosomy 45,X0 with a Male Phenotype

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An individual with a karyotype 45,X0 is typically a short female with webbed neck and ovarian digenesis that are the most consistent features of X0 monosomy or Turner syndrome. Maleness in association with karyotype 45,X0 is an infrequent finding. The male-specific region of the Y chromosome, the MSY, differentiates the sexes and comprises 95% of the chromosome's length.The presence of the testicular determinant gene SRY on the short arm of Y chromosome is crucial for the development of male-type genitalia. Apart from the XY/X0 mosaics, in X0 males the maleness is due to a *de novo* Y/autosome translocation derived from the father. Depending on the type of deletion and the autosome involved, the phenotype could show a broad spectrum of congenital malformations.

We report on a case of Y/autosome translocation with loss of 18p and distal Yq material in a holoprosencephalic fetus. The fetus presented with a normal male phenotype and 45, X0 karyotype without evidence of mosaicism or apparent translocation on the routine cytogenetic analysis. The further cytogenetic and molecular studies indicated 45,X, der(18)t(Y;18)(p11.3;p11.3) arisen *de novo*. Parental blood karyotypes were normal showing no evidence of rearrangement involving the short arm of chromosome 18. To our knowledge, only 4 cases of Yp/18 translocation in a 45,X0 holoprosencephalic male have been previously reported.

P-161

Unclassified sex cord/ stromal tumors with spindle cells tumour occurring in a 3 months old boy

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The incidence of prepubertal testis tumours is approximately 0.5 to 2/100,000, accounting for 1% of all paediatric solid tumours. Sex cord-stromal tumours (SCST) account for ~4% of all testicular tumours and ~8–30% of all paediatric testicular tumours. These tumours may be composed of Leydig, Sertoli, or granulosa cell types, existing as either pure or mixed populations. A minority of SCST shows no definitive differentiation. Among the unclassified SCST there is a small, extremely rare group of tumours composed predominantly of spindle cells.

We report a case of a sex cord-stromal tumour with spindle cells occurring in a 3 months old boy. A firm solid nodular mass was noticed by the parents. The serum level of α-fetoprotein was mildly elevated, and other tumoral serum markers were negative. USS was not suggestive of cyst or infection. With the clinical diagnosis of testicular teratoma, the patient underwent radical left orchiectomy.

Microscopic examination revealed a non encapsulated circumscribed tumour composed of interlacing bundles of spindle cells with some compressed trapped tubules and frequent mitoses. There were no germ cells present. Immunohistochemically, neoplastic cells were diffusely and strongly positive for vimentin, inhibin and α-SMA, with focal positive staining for S-100 and calretinin.

To the best of our knowledge only nine cases of Unclassified SCST of the testis composed predominantly of spindle cells are reported. The majority of the cases reported are immunochemically SMA+, S100+, Vim+, CK-. The youngest case reported was 5 months old baby in which limited IHC was performed. A recent case with brain metastasis has been described. The histological features of unclassified SCST with predominance of spindle cells do not predict the biological behaviour of this tumour. As only one case with metastasis has been reported and there are few cases in the literature, long term follow up is recommended.

P-162

Pulmonary neuroendocrine cell hyperplasia of infancy: study of 3 cases

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Introduction: Pulmonary neuroendocrine cell hyperplasia of infancy (NEHI), also known as persistent tachypnea of infancy, is a rare entity presenting as interstitial lung disease (ILD). NEHI affects young children like ILD, with persistent tachypnea, hypoxia, retractions or respiratory crackles, and with nonspecific and nondiagnostic lung biopsy findings.

Material and method: Three infants, aged three weeks, six months and two years, presented as non-filiated ILD. Chest radiographs demonstrated hyperinflation and high-resolution computed tomography, exhibited hyperinflation and ground-glass densities.

Results: The lung biopsies from all patients, showed no significant interstitial involvement or inflammation, but mild and nonspecific changes as increased alveolar macrophages and mild airway smooth muscle hyperplasia. Immunohistochemistry was performed using antibodies to synaptophisin, chromogranin A and bombesin. Immunopositive cells and neuroepithelial bodies in lobular parenchyma and in the bronchioli were observed, in larger numbers than in control samples. The patients were treated with oxygen-therapy and symptomatic bronchodilatador drugs for a long time, with gradual improvement in time.

Discussion: Neuroendocrine cells are specialized epithelial cells, distributed as solitary cells along the conducting airways, and as innervated clusters, the neuroepithelial bodies (NEB), in the lobular parenquima. These cells synthesize and release amines and a variety of neuropeptides. During the early stages of lung development, they act as modulators of fetal lung growth and differentiation, and after birth, as airway oxygen sensors involved in neonatal adaptation. These cells and NEB are related to different pediatric lung diseases as congenital lung disorders, bronchopulmonary dysplasia, disorders of respiratory control, cystic fibrosis, bronchial asthma and NEHI. The precise etiology and pathogenesis of NEHI remains unknown. It is not clear if a relation exists with the idiopathic diffuse hyperplasia of pulmonary neuroendocrine cells described in adult patients.

P-163

Pathological features in 3 cases of venous ductus agenesis associated to hydrops fetalis.

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Introduction. The venous ductus (VD) is a fetal vessel that takes oxygenated blood from the placenta through the umbilical vein (UV) to the fetal heart. Three main patterns of abnormal VD circulation were documented:

– 1) UV bypassing the liver and connecting directly into the right atrium (46% in the reported cases)
– 2) UV bypassing the liver and connecting into the inferior vena cava maintly through one of the iliac veins (25%).
–3) UV connecting to the portal circulation, without developing a true VD (21%)

Material and method. We show three cases which each represent an example of these mentioned patterns of UV drainage in patients with VD agenesis. The three pregnancies ended in postnatal death at 45 minutes, 4 days and 11 days respectively, with severe hydrops fetalis.

Discussion. In two cases, the cause of the fetal hydrops, could have been that most of the umbilical blood was bypassing the liver and drained directly into the heart or into the inferior caval vein, causing a cardiac overflow and under perfusion of the liver. In the patient whose umbilical blood drained directly into the hepatic sinusoids, hepatic hyper perfusion and portal hypertension could have caused damage to the liver cells, and an impaired fetal plasma protein synthesis and secretion. This could be a contribution to fetal hypoproteinemia causing hydrops.

The VD agenesis is a rare malformation and should be investigated in cases of prenatal diagnosis of hydrops fetalis or in hydropic newborns.

P-164

Non-immune hydrops fetalis: autopsic series of 53 cases

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Introduction: 'Hydrops fetalis' is used to describe the presence of generalized edema, together with liquid

accumulation in serous cavities. Despite the fact that it is a well-known pathology, it can be difficult to ascertain the cause, even in detailed autopsy studies. Its incidence has decreased notably since the exhaustive control of Rh isommunization. Although systematic complementary studies are not necessary in all cases, cytogenetic, metabolic and microbiologic studies should be carried out to obtain the etiological diagnosis. The aims of this study were to establish the prevalence of hydrops diagnosis in a recent autopsic series and analyse its causes.

Material and method: The diagnosis of hydrops has been reviewed from the records in autopsies of fetuses and infants up to 1 year of age, carried out at the Vall d'Hebron Universitary Hospital, from January, 2000 to November, 2007.

Results: In 1919 fetal and 179 newborn autopsies, the diagnosis of hydrops was confirmed in 53 cases (43 fetus and 10 newborns), representing 2.5% of the total. Grouped into categories, a metabolic disease was proved in 7 (13.2%) cases, an infection in 7 (13.2%) cases (2 of these - parvovirus B19), a placental pathology in 6 (11.3%) cases (4 of them - feto-fetal transfusion). A variety of congenital anomalies were found in 25 (47.1%) cases, (among these, 8 chromosomal abnormalities, 3 lymphatic dysplasia, 3 ductus venous agenesis, 2 achondrogenesis and 4 polimalformative anomalies). It was impossible to establish the cause in 8 (15.1%) of the cases.

Conclusions: The diagnosis of 'hydrops fetalis' represents 2,5% of cases in a recent autopsic series and its aetiology can be established in 84.9% of them.

P-165
POST-MORTEM EXAMINATIONS IN A SPECIALIZED PAEDIATRIC PATHOLOGY SERVICE 2005–2007

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Between January 2005 and November 2007 a total of 998 prenatal, perinatal and infant autopsies were performed by the Paediatric and Perinatal Unit at The Royal London Hospital's Department of Cellular Pathology. In the vast majority of cases (91.3%) the cause of death or important information was obtained at autopsy.

The post-mortems were divided in groups according to gestational age and neonates.

In the gestational age group less than 17 weeks gestation, 119/150 (79.33%) of the post-mortems performed revealed significant information, in 14/150 (9.33%) not enough clinical information was available to make a diagnosis, and in only 17/150 (11.33%) no relevant condition was identified.

Between 17 and 24 weeks gestation relevant information was obtained in 92% of the cases, only 28/351 of the post-mortems (8%) were unclassified.

The ReDeCo (Relevant Condition at Death) classification was used for babies over 24 weeks gestation. In stillborns (24 to 42 weeks gestation) relevant information was obtained in 327/349 (93.7%). Only 6.30% (22/349) were considered as "unclassified"; of these there was not enough clinical information and/or placenta in 6 of the cases (1.7%), the remaining 16 (4.6%) cases were considered as "No relevant condition identified".

In the group of neonatal post-mortems (less than 1 month old babies) the cause of death was identified in 71/73 (97.3%) of the cases, but relevant information was obtained in all of them.

In post-mortems performed in older babies and children (1 month to 13 years old) the cause of death o relevant information were obtained in 69/75 (92%) of the cases.

Before the use of the ReDeCo classification the percentage of unclassified cases was 16% in 2001–2003 and is now 8.7% in 2005–2007. Post-mortem performed by specialist Paediatric Pathologists offers more information to clinicians to improve prenatal care and is important for parental counselling.

P-166
Immunohistochemical analysis of tissue factor expression and microvessel density in Wilms tumor

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Background: Wilms tumor is the most commonsolid renal malignancy in childhood. Even among patients with favorablehistology and staging, the most significant prognostic factors demonstrated sofar, some recur and eventually die from the disease. Novel prognostic factorscould be especially useful in the management of these children. Tissue factor(TF), a protein linked to the extrinsic coagulation pathway, is highlyexpressed in various tumors, and has been associated to the development ofmetastases and to a poor prognosis. Our study describes, for the first time,the expression of TF in paraffin-fixed specimens of Wills tumors from 41patients treated at Hospital Sao Lucas da PUCRS and Hospital da Crianca Conceicao,Porto Alegre, Brasil.

Method: The immunohistochemical expression of TF and microvasculardensity (MVD) assessed by CD34 underwent morphometric analysis with the digitalimage analysis system Image Pro Plus 4.5 (Mediacybernetics, USA).

Theseresults were compared to other established prognostic criteria, such as age,staging, histology and tumor recurrence, overall and cancer-specific mortality.
Results: Increased TF expression was correlated to a higher chance oftumor recurrence (p=0,01) and death (p=0,02). However, there was no significantcorrelation between TF expression and other established prognostic factors.Likewise, TF expression was not correlated to an increased MVD.
Conclusion: the results strongly suggest that further studies are neededto asses the potential of TF as a prognostic marker. Eventually TF may be testedas a predictive factor, considering the emergence of monoclonal antibody drugstargeting TF.

P-167
Recurrent left atrial myxoma
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BACKGROUND: Cardiac myxoma is the most frequent primary benign cardiac tumor, occuring sporadically in adults. Pediatric cases are rare and familial cases occure in younger adults.
METHOD: We analyzed clinicopathological aspects of cardiac tumors in13 year-old female patient.
RESULTS: A 13 yr-old girl, was admitted at our institution, in May 2006 after cerebrovascular insult. Echocardiography revealed left atrial tumor. Dark red-grey, friable, gelatinous tumor mass, measuring 4.5×4×1 cm, attached by fibrous stalk to the tip of interatrial septum was resected. Microscopically, tumor tissue consisted of capillary-rich myxoid stroma, with foci of hemorrhage and chronic inflammatory infiltration, and superficial necrotic tissue. Tumor cells were spindle and stellatous, dispersed discohesivly, with no mitotic activity. Postoperative neurologic sequela was left hemiparesis. In November 2006, the follow-up ehocardiography revealed the new left atrial tumor and NMR showed that tumor mass attached on the upper frontal wall of the left atrium. In January 2007, reoperation was performed and dark red, friable tumor mass measuring 1.1×0.8×0.7 cm, attached by fibrous stalk to the middle of the interatrial septum was resected. The location of the second tumor, near the fossa ovalis, was clearly separated from the previously resected tumor. Microscopically tumor had the same texture as the previously diagnosed one. In the medical family history, parents have nevi and "spotty" efflorescence. On the grounds of clinical aspect and family history of recurrent atrial myxoma, facial and backbone nevi, oral cavity pigmentation, vulvar and perianal nevi and hirsutism, the diagnosis of Carney complex was established. Patient is on regular follow-up.
CONCLUSION: Cardiac myxoma in young adults is rare and clinically behave aggressively because it recurres frequently and is associated with embolism. The surgery is treatment of choice and regular follow-up of the patient is recomended.

Soft Tissue, Joint and Bone

P-168
Clinicopathological features of a series of 10 cases of malignant triton tumors diagnosed over 10 years at a teriary cancer hospital, Mumbai, India
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Background: A rhabdomyoblastic differentiation in a malignant peripheral nerve sheath tumor (MPNST) is designated as a malignant triton tumor (MTT). Clinicopathological features and outcomes of 10 such cases, diagnosed over a 10 year period, are presented.
Method: H & E stained microsections were available in all the cases. Immunohistochemical (IHC) markers included vimentin, S100, desmin, myoglobin, CD34, MIC2 and glial fibrillary acidic protein (GFAP). p53 and MIB-1 staining were carried out in all cases.
Results: The average age of occurrence was 30 years, with maximum cases (60%) in the second decade, including males outnumbering the females. 62.5% cases were identified in the setting of neurofibromatosis. Average tumor size was 12.6 cms. On histology, 80% cases were of high grade. Distinct rhabdomyoblastic cells in form of 'strap' cells and pleomorphic forms were noted in 3 cases, each, respectively. The distribution of rhabdomyoblastic cells was focal, especially in close proximity to vessels and nerves in most of the cases that displayed features a MPNST. IHC showed varying S-100 expression, along with desmin and myoglobin positivity in all cases. Two cases showed GFAP positivity, including 1 case that also showed neuroectodermal differentiation. Surgery was performed in form of wide excision in 4 cases; marginal excision in 2, amputation in 2 and intracapsular excision in 2 cases. Adjuvant chemotherapy (CT) and/ or radiotherapy (RT) were offered in 9 cases. On follow-up, out of 7 cases, 3 showed local recurrences, including 1, which, in addition to other 2 cases, showed lung metastasis. One patient succumbed to the disease. This case along with another high grade case displayed diffuse MIB-1 and p53 positivity.

Conclusion: MTT is an uncommon tumor, invariably of high grade and stage and displays an aggressive behavior. High MIB-1 counts and p53 positivity indicate a dismal prognosis. Surgery with clear margins is the treatment mainstay. Adjuvant RT is effective.

P-169

Ossifying Fibromyxoid Tumor of Soft Tissue

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Ossifying Fibromyxoid Tumor (OFMT) is a distinctive and rare soft tissue neoplasm of uncertain lineage, first described by Enzinger in 1989. The purpose of this study is to report four cases and review the literature.
We report four cases of our consultation files, all adult males aged between 32 and 53 years old. The involvement sites were abdominal wall, the supraclavicular area, lower extremity and shoulder.
All lesions were superficial soft tissue tumors with a diameter ranged from 3 to 10 centimeters. Patients complained of a painless sucutaneous mass which had been present from 2 to 15 years. Available X-ray and mamographic studies of the surgical specimen in 3 of the 4 cases revealed a circumscribed mass with irregular calcification inside it, surrounded by an incomplete ring of bone. On cut sections these masses were white, firm and rubbery-textured. Histologically showed encapsulated and lobulated tumors composed of small, uniformly-round cells with eosinophilic cytoplasm, arranged in cords and rows in a myxoid, fibromyxoid or hialinized stroma. Immunohistochemistry showed S100 protein positive in 3 of 4 cases.
Differential diagnosis was made with heterotopic ossification, extraskeletal myxoid chondrosarcoma and extraskeletal osteosarcoma. None of the cases showed atypical features neither increased mitotic activity nor cellularity. The treatment in all the 4 cases was the surgical resection of the tumoral mass without recurrence in six years, three years, 18 months and 6 months, respectivelly.

P-170

Abberant Cdx2 expression in pyloric gland adenoma of the gallbladder

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Introduction: The aim of the study is to survey cdx2 expression in pyloric gland adenoma (PGA) of the gallbladder and whether the cdx2 expression is related to intestinal differentiation, to beta-catenin expression, or to spindle cell metaplasia (SM).
Materials and methods: We reviewed 25 cases of PGAs. With immunostaining, the antibodies (Cdx2, beta-catenin, MUC2, MUC6, HIK1083) were reacted and detected with Envision plus. The immunostaining was scored as follows: 0: negative, 1+: <10% positive cells, 2+: 10-<30%, 3+: 30-<50%, 4+:50-<70%, 5+: 70–100%. The number of positive nuclei for cdx2 and beta-catenin was counted in the well-labeled high power fields (HPFs). The mean labeling indices (LIs; %) were calculated by counting 1000 cells in the three HPFs. Separately, positive cells(%) were calculated in the SM foci.
Results: PGA was ranged in size from 3 to 16 mm. Four cases (16%) showed SM. In the background mucosa, cdx2 was not expressed. Although low score(1 or 2), cdx2 was expressed in 23 cases (92%). However, Muc2 was only positive in the small number of goblet cells in three cases (12%). The cdx2 score was not correlated with that of Muc6, HIK1083 and not strongly with beta-catenin. The LIs of Beta-catenin was correlated with those of cdx2 ($r = 0.705$). In all PGA cases with SM, positive cells(%) in the SM foci were higher than the mean LIs.
Conclusion: Cdx2 expression is specific to PGA with high frequency (92%) despite lacking any expression in the background mucosa, and is not related to goblet cells-type intestinal differentiation. The correlation of mean LIs between cdx2 and beta-catenin was identified, whereas the cdx2 score was generally low and was not strongly correlated with that of beta-catenin. Especially, high expression of cdx2 and beta-catenin in the SM foci raises a possibility that the mutation of beta-catenin genes promotes cdx2 expression.

P-171

Metachronous adenocarcinoma of the large intestine in a male patient with aggressive angiomyxoma

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Aggressive angiomyxoma (AA) is a rear, locally infiltrative, but nonmetastasizing soft-tissue tumor that usually arises in the pelvis or perineum of premenopausal women. Only 44 cases of AA occurring in male patients have been reported in the English literature. Sites frequently involved include

the scrotum, spermatic cord, inguinal region and perineum. Correlation with carcinomas has not been reported.

We report the case of a 56-year-old man who presented with an inguinal hernia. During the operation, a nodular mass was identified attached to the spermatic cord. The hernia was subsequently repaired and the mass was excised. Histological examination showed features compatible with AA. The patient received no further treatment and had no evidence of systemic disease. Three months after the diagnosis of AA was made he presented with anemia and rectal bleeding. Colonoscopy demonstrated a large infiltrative tumor of the rectum, partially obstructing the lumen. Histological examination of the biopsies taken, revealed a moderate differentiated adenocarcinoma of the rectum. Because the tumor was unresectable the patient received systemic chemotherapy and radiotherapy and 10 months later is in good condition without evidence of metastatic disease.

In the present study we present an extremely rare case of AA in a male patient. To our knowledge, this is the first reported case of an AA with a metachronous adenocarcinoma. Correlation or common pathogenetic mechanisms are presently unknown.

P-172

A case of sarcoidosis established by gastrocnemius muscle biopsy.

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Sarcoidosis is a multisystem disorder of unknown cause, characterised by granuloma formation in affected organs. Its diagnosis is established by the combination of clinical, laboratory, radiological and histological findings. Symptomatic muscle involvement is reported to be between 0.5 and 1.4% of known cases of sarcoidosis.

We report the case of a 42-year-old man who was admitted to our hospital due to bilateral painful enlargement of the ankle joints of 20 days duration with coexisting arthralgies especially in the hands, feet and knees as well as myopathic symptoms. On physical examination there was swelling of the ankle joints with rubor and pain during the palpation. There was no peripheral lymphadenopathy or hepatosplenomegaly. His autoantibody profile was negative. Inflammatory markers like C reactive protein and erythrocyte sedimentation rate were raised. His lactate dehydrogenase, creatine kinase as well as transaminases and fibrinogen levels were also elevated. Complete blood count was normal. Vidal-Wright reactions and Mantoux test were negative. A chest x-ray showed bilateral hilar lymphadenopathy. Thereafter, he underwent a 67Ga-Citrate scintiscan which showed 67Ga collection in hilar lymph nodes and in lesser amount in the joints. A gastrocnemius muscle biopsy was performed and histological examination showed the presence of epithelioid non-necrotic granulomas. In accordance with the clinical and histological findings, the diagnosis of sarcoidosis affecting the skeletal muscle was made. The patient received treatment with high dose methylprednisolone followed by oral prednisolone and 7 months later is in good condition.

We report the case of sarcoidosis presenting with polyarthritis and myopathic syndrome confirmed with gastrocnemius muscle biopsy. The impressive sensitivity of muscle biopsy, its safety and ease of performance, along with the extreme rarity of muscle involvement by other granulomatous diseases, may render it as the procedure of choice for the histologic confirmation of sarcoidosis.

P-173

Diagnostic usefulness of synovial biopsy in monoarthritis and oligoarthritis: A retrospective analysis of 146 synovial biopsies over 11 years (1990–2000)

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Background: Synovial biopsies are seldom used for diagnostic purposes. In general, patient interview, clinical examination, routine laboratory testing, radiographic examination and synovial fluid analysis usually suffice to establish a correct diagnosis. Synovial biopsy analysis is only justified in cases with a high degree of suspicion of atypical infectious agents, evaluation for intra-articular tumours and diseases with characteristic histological features.

Aim of study: Our goal is to assess the utility of synovial biopsy in establishing correct diagnosis of oligoarthritis and monoarthritis through a confrontation of histological findings with clinical diagnoses. We will also discuss its limitations and compare our results with those reported in literature.

Materials and Methods: Between January 1990 and December 2000, 146 patients have undergone arthroscopic or surgical synovial biopsies as a diagnostic procedure for monoarthritis and oligoarthritis of unknown etiology. For each case, we proceeded with a confrontation of histological diagnoses with clinical findings after a mean follow-up period of 5 years.

Results: Our study group included 69 men and 77 women aged between 7 and 85 years (mean=41.2 years). One hundred and twenty nine patients (88.4%) had monoarthri-

tis and 17 patients (11.6%) had oligoarthritis. Diagnosis yielded by synovial biopsy was concordant with clinical findings in 84.2% of the cases. Discordant diagnoses were mainly related to rheumatoid arthritis (n=5), osteochondrmatosis (n=1), chondrosarcoma (n=1) and tuberculous arthritis (n=1).

Conclusion: Our results are similar to those reported in literature and show that synovial biopsy is reliable in establishing correct diagnosis of oligoarthritis and monoarthritis.

P-174

Accuracy of fine needle aspiration biopsy in soft tissue tumors diagnosis - Diagnostic meta-analysis

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Context - Accurate diagnosis is relevant for the treatment and prognosis of soft tissue tumors (STT). Open incisional and/or excisional biopsy are still gold-standard in the diagnosis of these neoplasias. The real accuracy of fine needle aspiration biopsy (FNAB) in the diagnosis of soft tissue tumors is still controversial in literature.

Objective - To assess the accuracy of FNAB compared to gold-standard in STT diagnosis.

Methods - Systematic research on MEDLINE, EMBASE and COCHRANE databases (1996–2007) for STT accuracy diagnostic studies was done. Six studies were included, showing 310 cases in the assessment of malignant and benign diagnosis and 190 cases for specific diagnosis. Articles were assessed through STARD checklist criteria and Oxford levels of evidence and grades of recommendation. Only the ones compared to gold-standard were included. Non-mesenchymal tumors and articles that did not allow for the exclusion of these cases despite having good methodological quality were excluded. There was no language restriction. MetaDisc software for statistical analysis was used.

Results - In the malignant and benign diagnosis, scores of 89.8% (95% CI; 84.3–93.8) for sensitivity; 88.8% (95% CI; 82.2–93.6) for specificity; 6.807 (95% CI; 4.262–10.874) for positive likelihood ratio (LR); 0.136 (95% CI; 0.083–0.224) for negative LR and 63.499 (95% CI; 28.818–139.91; p=0.425 and I2=0.0%) for diagnostic odds ratio (DOR) were found. For specific diagnosis, scores of 55.5% (95% CI; 45.7–64.1) for sensitivity; 51.1% (95% CI; 40.3–61.8) for specificity; 1.245(95% CI; 0.963–1.609) for positive LR; 0.803 (95% CI; 0.587–1.099) for negative LR and 1.644 (95% CI; 0.896–3.018; p=0.560 and I2= 0.0%) for DOR were found.

Conclusions - FNAB, based on current literature data, presented high accuracy in malignant and benign diagnosis and low accuracy in the specific diagnosis of STT.

P-175

Prostheses-related sarcomas of the hip and knee

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Background: Prostheses-related sarcomas are unusual butserious complications of orthopaedic surgery. Patients have an average age of 66 years and the mean time between surgery and the diagnosis is 6 years. They are usuallylocated in the hip area and the most common type is malignant fibroushistiocytoma. Prognosis is poor and most patients died in the first year.

The reason of this association is not known.Although some of the prostheses components, like chromium, nickel or cobalt,have been implicated, a casual association cannot be ruled-out.

Case Reports:

Case 1: A 78-year old woman with total hipreplacement twenty years ago. She complaint of pain in the right femur for oneyear, that increased the last week. Radiological examination revealed apathological bone fracture with associated lesion in the surrounding softtissues, which was considered of infectious origin. Examination of thecurettage specimen showed a neoplasm with histopathological immunohistochemicalfeatures of malignant fibrous histiocytoma. The patient alive with livermetastases, four months after surgery.

Case 2: A 65-year old woman with bilateral kneereplacement fifteen and one year ago. She complaint of pain from the moment ofthe last surgery. Radiological studies showed non-specific findings. The prosthesiswas removed and pathological examination revealed a soft tissue neoplasm. Histopathology and immunohistochemistry showed to be a leiomyosarcoma. Supracondylealamputation was performed and the patient is free of disease four months aftersurgery.

Conclusions: We report two unusual cases of sarcomas. Theassociation with prosthetic implants is well documented, but far from clear.Moreover, our case 2 is a leiomyosarcoma, which has been only rarely reportedin this context. The lapse of time from surgery is short, but some reportedcases have demonstrated even shorter intervals.

In patients with prosthetic devices and softtissue lesions is mandatory to exclude a neoplasm.

P-176
CEMENTIFYING FIBROMA- CLINICAL CHARACTERISTICS, HISTOLOGICAL FEATURES AND CORRELATION

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Cementifying fibroma (CF) is a fibro-osseous lesion which produces cementum. The aim of the current study was to further refine its clinical and hystological features and to make clinico-pathological correlations. The authors report 10 cases of cementifying fibroma diagnosed on the Institute of pathology School of Medicine, Belgrade during the 15-years (1995–2005.). CF showed no sex predilection (5 males and 5 fimales). The youngest patient was male 14 years old, and the oldest was female 54 years old (the average years were 27,1). The maxila was the most frequent site (80%), just 1 patient had lesion in the baseos cranii with invasion of sella turcica. All the patients reported history of pain and edema lasting from 3 to 20 months (midle 10,4 months). The other clinical simptoms were: face assimetry, tooth dislocation and bulbus protrusion. The evolution of the tumor was longer and clinical cours indolent in the patients with mandibular localisation (mandibula-12,3 months, maxilla -9,5 months).
Clinical cours and longitude of anamnessis are in the correlation with histological feature of CF. The main histomorphological diagnostic criterium for CF are «psammoma like structures». There number and mineralisation of bone spheruls varyed during the tumor maturation. In old lesions with long anamnessis increase the number of "psammomatoid" bodys. In the opposit, dramatic clinical simptoms were discrabed in the CF with stromal haemorrhage and inflammation. We had one patient with secondary superposition of aneurysmal bone cyst on the prime CF.
Differential diagnosis of cementifying fibroma and other fibro-osseous lesion of cranial bones just by histological evaluation is often difficult and asked for permanent cooperation between clinical doctors and pathologists.
Kee words: cementifying fibroma, psammoma like body

P-177
Three-dimensional collagen-chondroitin sulfate-hydroxyapatite composite scaffolds for bone regeneration

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Background. Osteogenesis is a natural repair mechanism that occurs in the human body but in critical sized bone defects, a transplantation of bone tissue or bone substitute is needed to regain bone integrity. In recent years, scaffolds built from synthetic or natural materials for guiding the proliferation and spread of different cell types were developed. The aim of our study was to characterize new biomaterials composed of collagen type I (COL), chondroitin sulfate (CS) and hydroxyapatite (HA), and to investigate their in vitro effect on a human osteoblast culture.
Methods. Two variants of COL-CS-HA composite scaffolds were prepared by mixing natural polymers with HA in different weight ratios (COL:CS:HA 1:0.5:1 (I); 1:0.5:2 (II)). Their structure and morphology was analyzed by scanning electron microscopy (SEM). Scaffold biodegradability in the presence of collagenase was measured. Osteoblast viability, adhesion and morphology were assessed by MTT test and DAPI-staining of cell nuclei.
Results. SEM micrographs showed a highly porous structure with small white aggregates of HA, non-homogenously scattered in the COL framework. The porosity decreased proportional with the increase of the HA quantity. In vitro biodegradability test demonstrated that the exposure to ultraviolet light increased the resistance of porous composites to collagenase digestion. The MTT test indicated the highest cell viability for variant I. Nuclear staining with DAPI showed that cells retained their normal morphology and adhered, spread and proliferated inside the porous scaffolds.
In **conclusion**, porous COL-CS-HA composites with good physico-chemical and biological properties were developed by HA powder integration in a mixture of COL-CS. Both composite variants were biocompatible with human osteoblasts in vitro. These biomaterials could be used as scaffolds for bone regeneration.
This work was supported by Romanian Programme 4 "Partnership in Strategic Domanis", Project No. 61012.

P-178
Clinicopathologic features of retroperitoneal tumors.

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Introduction: Actuality of investigation of retroperitoneal tumors is connect with considerable difficulties, such as their clinicolaboratorial and morphological diagnosis.
Method: Complex clinicomorphological (histological, immunohistochemical, cytological, electron microscopic) investigation of biopsy and surgical materials of 74 patients

(19 men and 55 woman, aged 22–75), treated in 2001–2007 for retroperitoneal tumors, was performed.

Results: In 6 cases neoplasms affected viscera (kidney, adrenal glands, uterus), in 3 cases cancer metastases (2 – adenocarcinomas, 1 – epidermal cancer) without detection of primary focus were diagnosed, in 3 cases – hematomas with sing of formation, in 1 cases – benign hyperplasia of lymphatic nods. In 61 cases (21%) of non-organic retroperitoneal tumors affections cystic tumors were detected: 6 cases – celomitic cysts, 7 cases – cystic lymphangiomas; in 21% of the cases lypolitic tumors were revealed: 3 – lipoma, 10 – lyposarcoma, in 18% - muscular tumors: 1 – leiomyoma, 10 – leiomyosarcoma (Ki67 - 23%). In 9 patients peripheral nerves tumors were detected: 5 – neurofibroma, 2 – neurinoma, 2 – malignant neurinoma (Ki67 - %). In 13% of the cases fibrous tumors were diagnosed: 7 malignant fibrous histiocytoma (Ki67 - 12%), 1 – fibrosarcoma (Ki67 - 22%). In 3 cases nonchromaffin paraganglioma (Ki67 - %), in 2 – hemangioma, in 1 – venous-cavernous angiodisplasia, in 1 – gastrointestinal stromal tumor (CD117+, CD34+, Ki67 - 12%) were detected.

Conclusion: Final decision regarding the origin of retroperitoneal tumors can be drawn after complex morphological study with obligatory application of immunohistochemical methods.

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A multilocular large gastrointestinal stromal tumors of the small intestine

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BACKGROUND Gastrointestinal stromal tumors (GIST) are the most common mesenchymal neoplasms arising from the gastrointestinal tract and typically occur in adults older than 40 years with a peak incidence in the sixth and seventh decades. Gastro-intestinal stromal tumours are a heterogenous group of mesenchymal tumours mostly arising from the stomach and small intestine which may be benign or malignant. In the present case we reported a patient with multilocular large GIST and discussed the relevant literature on this topic.

METHODS: A 48-year man presented with abdominal pain and progressive distension of abdomen. Preoperative CT evaluation revealed two masses; one superior to bladder with septas and the second 7×5 cm solid mass. Both masses were interrelated and included some calcified areas. Microscopically, the tumour consisted of anaplastic spindle cells with high mitotic activity (90 mitoses per 50 high-power fields). Immunohistochemically, the spindle cells were positive for c KIT and CD34. The final diagnosis was high-grade malignant gastrointestinal stromal tumour of the jejunum.

CONCLUSION Considering the recent advances in the diagnosis and treatment of GIST, this neoplasm should be taken into account in the differential diagnosis of the tumours appearing in the jejunal region.

P-180

A case of synovial sarcoma; a solid mass in the inguinal region

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BACKGROUND Synovial sarcomas in this location have been characterized by slow growth, intense pain in some which is not explained by the microscopic image, intense vascularity and, macroscopically, by precise peripheral limits.The great majority of the tumors known as synovial sarcomas are found in the extremities, near to, but not directly continuous with joint spaces.In the present case we reported a patient with synovial sarcoma presented as right inguinal fine defined mass that was presented as lymphadenopathy.

METHODS: A 21 years old female was presented with restlesness, weight loss and mass in the groin. History revealed inguinal mass that was present for 3–4 monthes and pain. The postoperative diagnosis revealed that the tumor was positive for vimentin and CK 7. The mass was diagnosed as biphasic synovial sarcoma.

CONCLUSION We suggest that the clinical and pathologic data concerning the origin, location and microscopic appearance of these tumors infer that they might better be named synovial sarcoma.

P-181

THE PROGNOSTIC AND PREDICTIVE VALUE OF ANGIOGENESIS IN RHEUMATOID ARTHRITIS

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Background: Angiogenesis is an important process in a number of physiological and pathological processes, such as inflammation, tissue repair and neoplasia. In rheumatoid arthritis (RA) the angiogenesis is a fundamental component of pannus development, which covers and erodes the articular cartilage.

Material and methods: In synovial biopsies, we determined the immunohistochemical expression of the following antibodies, provided by Lab-Vision: CD31, CD34, CD105 (Endoglin) and VEGF. We used the immunoperoxidase method. We measured the positive vessels' area versus total tissues' area.
Results: In RA, high levels of VEGF, FGF1,2, TGF-beta and other angiogenic growth factors are present in the synovial fluid. We observed a high expression of VEGF in the hyperplasic synovial cells, respectively CD105 in neoformed vessels and in the activated endothelial cells.
Conclusions: The angiogenesis may have important diagnostic, prognostic and predictive value in RA. It correlated with the progression of joint destruction and with the activity of the disease. The antiangiogenic therapy could stop the progression of RA. We belive that the synovial biopsy could be an important method in the estimation of the RA evolution.

P-182

Pleomorphic Hyalinizing Angiectatic Tumor of Soft Parts: A case report

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Pleomorphic hyalinizing angiectatic tumor (PHAT) is a rare, recently recognized neoplasm occurring predominantly in the subcutaneous tissue of the lower limbs of adults. We report a case of PHAT in an 71-year-old man who presented with a 4.5×4.0×3.5 cm mass in the deep subcutaneous layer of his left inguinal area. The left inguinal mass was growing slowly in two years. No symptom was detected. Ultrasonographic findings showed a well-defined, slightly lobulated, heterogeneous, echogenic mass in the deep subcutaneous layer. The possibility of malignancy could not be excluded. Excisional biopsy was done. Histologically, the tumor was well circumscribed by a thin fibrous capsule and predominantly composed of fusiform cells with eosinophilic cytoplasm and round-to-oval or pleomorphic nuclei. The tumor had irregular-shaped mono- or multinuclear cells and marked bizarre cells with prominent intranuclear cytoplasmic inclusion. Also prominent angiectatic vesssels showing the endothelial lining with a thick eosinophilic and hyalinotic amorphous material. The positivity of CD68 and CD34 immunohistochemical stains was detected.

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Atypical organizing seroma following treatment for soft tissue sarcomas of the extremities

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Background Postoperative seromas are a well recognized complication in patients who have undergone excision of extremity soft tissue sarcomas. Due to atypical MRI findings, these lesions may be surgically explored to exclude the possibility of sarcoma recurrence or of development of a radiation induced sarcoma.
Method We retrospectively reviewed 6 cases of postoperative seroma occurring in patients treated at our center for soft tissue sarcoma of the extremities, who were biopsied or surgically treated with the clinical suspicion of recurrence or radiation induced sarcoma. The initial diagnosis were leiomyosarcoma in 4 patients, synovial sarcoma in one and myxoid liposarcoma in one. All patients had been surgically treated and 4 had received radiation therapy. Hematoxylin and eosin stained slides were retrieved for each case and immunohistochemical staining were performed on paraffin embedded sections according to standard protocols.
Results All lesions contained fibrin clots partially covered by a single layer of flattened cells, with inconspicuous cytoplasm and oval, sometimes bulging, nucleus, either forming interconnected pseudovascular spaces containing erythrocytes or solid sheets. In two cases, there were focal areas showing a papillary architecture, with fibrin cores lined by flattened cells. Scattered cells, both lining pseudovascular spaces and within the fibrin clots, showed enlarged nucleus with prominent nucleolus, but no real pleomorphism as well as mitotic figures were observed. CD 34 immunostaining was observed in 5 cases, while CD31 and D2–40 were detected in 4 cases. At least one marker was expressed in all cases. MIB-1 nuclear staining was present in all cases (18–35% of cells).
Conclusions We present the histological features of a small series of soft tissue lesions following treatment for sarcoma clinically simulating a recurrence or a radiation induced neoplasm. The primary importance of recognizing these lesions lies in their possible misdiagnosis as angiosarcoma under histologic examination.

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TETRASPANINS EXPRESSION ANALYSIS IN SOFT - TISSUE TUMORS

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Background: Tetraspanins are transmembrane proteins with at least 33 members in humans. These proteins can be associated each other and with a large number of different transmembrane proteins. They are involved in cellular processes like cell adhesion, motility, differentiation and proliferation, and the profile of expression or the role of these proteins in soft tissue tumors (STT) are unknown. The aim of this study was to evaluate expression of tetraspanins CD9, CD37, CD81, CD63 and CD82 by immunohistochemistry in several histological types of STT.
Methods: We analized the protein expression in 352 samples of STT (including fibromatosis, leiomyosarcoma, liposarcoma, synovial sarcoma, fibrosarcoma, pleomorphic sarcoma, schwannomas and others) spotted in duplicated in 3 tissue microarray paraffin blocks. Correspondent normal mesenchymal tissues were also analyzed. We classified the profile of the expression in 4 groups based on intensity of reaction (negative, weak, moderate and strong positive). For statistical analysis, we evaluated some clinical-pathological parameters such as hystological grade and metastasis.
Results: There was no expression of CD37 in normal or tumor tissue. Expression of CD63 was detected mainly in pleomorphic sarcomas (51%) and leiomyosarcomas (40%). CD81 and CD82 were found in almost every tumors tissue types, but in weak intensity. Expression of CD9, mainly moderated, was detected in leiomyosarcomas (28%) and pleomorphic sarcomas (22%). We found significant difference of expression of CD63, CD9 (both with $p<0{,}001$) and CD81 ($p<0{,}036$) between malignant and benign tumors.
Conclusion: all tetraspanins, except CD37, were present in soft tissue tumors. CD63 and CD9 were the most expressed, mainly in pleomorphic sarcomas and leiomyosarcomas. Our results showed that tetraspanins role in soft tissue tumors must be better investigated.

P-185
HHV-8 (LNA-1) POSITIVE MULTIFOCAL INFLAMMATORY MYOFIBROBLASTIC TUMOR

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Background: Inflammatory myofibroblastic tumor (IMT) is a rare soft tissue tumor with intermediate biological potential. It is mostly found in lungs, generally in children and young adults. Proliferation of myofibroblastic spindle cells accompanied by inflammatory infiltrate characterizes its histology. Anaplastic lymphoma kinase (ALK) gene translocations are observed in almost 50% of the cases. Recent detection of EBV and human herpes virus (HHV)-8 in some cases may indicate the possible role of infectious agents in pathogenesis.
Case: An 80-year-old male presented with abdominal pain, fever, and loss of weight. Subsequent to detection of tumoral lesions in his right scrotum and mesentery of ileum, partial ileum resection and right orchiectomy were performed. Grossly, scrotal tumor neighboring epididimis was well-circumscribed and 2 cm in its greatest diameter. The larger $11\times10\times9$ cm mesenteric tumor infiltrating the ileal wall without obvious mucosal destruction exhibited focal cystic areas, necrosis, and hemorrhage. Microscopically, both tumors consisted of spindle-shaped myofibroblastic cells, ganglion-like giant cells, and inflammatory cells of mixed type settled in a myxoid stroma. Tumor cells demonstrated evident pleomorphism, but no mitosis was noted. Spindle cells immunohistochemically expressed vimentin, smooth muscle actin and HHV-8 (LNA-1). No reaction with epithelial membrane antigen, pan-cytokeratin, ALK, HMB45, CD34, factor VIII, desmin, S-100, CD68, CD 117(C-kit) and glial fibrillary acidic protein was observed in tumor cells. Considering the histopathological and immunohistochemical findings, the case was diagnosed as multifocal inflammatory myofibroblastic tumor.
Conclusion: IMT is an uncommon tumor with varying clinic and histopathologic characteristics. The current case is presented with literature review considering its concurrent existence in two separate extra-pulmonary locations and immunohistochemical HHV-8 (LNA-1) reaction of tumor cells.
Key words: Inflammatory myofibroblastic tumor, human herpes virus-8, immunohistochemistry.

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Angiomatoid Malignant Fibrous Histiocytomas

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We studied four cases of angiomatoid malignant histiocytoma. The patient's ages ranged from 20 to 39 years. There

was a marked female predominance with a female-to-male ratio of 3:1. There was a predilection for the extremities (2 out of 4 cases). Three patients had tumors within subcutaneous tissue. The tumors ranged in size from 2 to 8 cm in greatest diameter. They consisted of partially cystic, circumscribed masses surrounded by a thick layer of connective tissue where a cuff of chronic inflammatory cells with formation of lymphoid follicles was present in variable amount. The cystic cavity was filled with blood and had no endothelial lining. The cytology of the tumor was bland with presence of uniform, spindle-shaped or ovoid cells forming clusters or lobules. Hemosiderin pigment was invariably present. Three of these patients had clear surgical margins at the time of the original excision and remained free of disease. Only one patient was clinically diagnosed with hematoma which was incised and drained. The tumor had been growing for 14 months and was excised, and the patient was free of tumor after 80 months. Our study shows that Angiomatoid Malignant Fibrous Histiocytomas is better treated by surgical excision, and appears to follow a low grade malignant course, characterized by local aggressiveness.

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EXTRASKELETAL OSTEOCHONDROMA OF THE CHEST WALL: A CASE REPORT

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Background: Extra skeletal osteochondroma are benign soft tissue tumors occurring in extra-osseous and extra-synovial locations, predominantly composed of adult mature hyaline cartilage, with prominent ossification. The majority of tumours occurs in the region of the fingers and is rare in atypical sites such as chest wall.

Method- Results: We present an unusual case of an extra skeletal osteochondroma of the chest wall in a 31-year-old woman and illustrate the clinical, radiographic and histological features of this entity.

We review the literature and discuss the differential diagnoses.

Conclusion: An integrated clinical-pathologic diagnosis helps to clarify the nature of extra skeletal cartilaginous tumors that can arise at unusual site. Complete local surgical excision is the management of choice.

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Isolated intraosseous gouty tophus in hallux sesamoid. A case report

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INTRODUCTION Gout is a systemic disorder associated with the deposition of sodium urate crystals in the joints and soft tissue. Often, but not invariably, the serum urate levels are elevated as a result of overproduction or underexcretion of urid acid. Intraosseous gotty invasion is a relatively rare condition. We are reporting an unusual case of intraoosseous tophus of a great toe sesamoid, presenting as a persistent pain without prior history of gouty attack or underlying systemic disorders.

CASE REPORT An otherwise healhty 38-year-old man had had left great toe pain intermittently for about one year, after a car accident. On physical examination there was no inflammatory signs and analysis was normal. Radiograph, CT scan and Radionuclide scintigraphy showed a sesamoid fracture and a probable bone necrosis. Sesamoid bone was removed and the tissue obtained showed numerous tophaceousdeposits composed of crystalline material with surrounding histiocytes, giant cells, chronic inflammatory cells, and fibrosis in the destroyed bone. The pathologic diagnosis was intraosseous gout in sesamoid. Serum urate levels were never abnormal in posterior analyses. Three months later the patient had a characteristic attack of acute arthritis and began treatment. He had had no other clinical symptoms until now, six months after the diagnosis.

CONCLUSION Gout is a common disease in adults and tophaceous deposits usually appear after a long time of hyperuricemia; in fact, most of the previous cases of intraosseous gout have been described in long standing hyperuricemic patients. Thus, in our case the final diagnosis was completely unexpected. It is important to be aware of this entity as a source of sesamoid pain that can be cured by medical therapy

P-189

Malignant Psammomatous Melanotic Schwannoma Of The Axilla. A Case Report

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Background: A 46-year-old woman with neurofibromatosis 1, but not Carney's complex, presented with a mass of the right axilla, without history from her breast. A

malignant psammomatous melanotic schwannoma was diagnosed, a rather unusual case, as malignant schwannomas have been reported in only 3,1% of patients with Von Recklinghausen's neurofibromatosis.
Method: A solid, partly cystic mass of ~9,5 cm of the right axilla with orange brown pigmentation was excised. Paraffin-embedded tissue was stained with H-E, PAS, Gomori and Fontana. Immunohistochemistry was performed with the antibodies: S-100, Vimentin, HMB-45, NSE, Collagen IV, Desmin, SMA, HHF-35, EMA and CK8,18,19.
Results: Spindle-shaped and epithelioid neoplastic cells, with large vesicular nuclei and eosinophilic nucleoli, were observed. Multinucleated cells and cells with cytoplasmic vacuoles like mature adipose tissue, were also present. Mitotic activity was increased, with abnormal figures. Numerous laminated calcispherites (psammoma bodies-PAS+) and melanin pigment as brown/black granules (Fontana +) were also observed. Zones of necrosis were present, especially near cystic parts of the tumor. An inter- and pericellular reticulin staining pattern was revealed. Immunohistochemistry: S-100(+), HMB-45(+), Vimentin (+), NSE(+), laminin(+), focally Collagen IV(+). The main and most difficult differential diagnosis is with neurotropic malignant melanoma, depending on clinical course and presence of giant melanosomes ultrastructurally.
Conclusion: Our case, a malignant psammomatous melanotic schwannoma of the axilla (involvement of cervical spinal nerve) is extremely rare, as psammomatous type of melanotic schwannomas usually involves alimentary tract tumors. Most melanotic schwannomas are benign, slowly growing tumors, psammomatous form of which usually presents with Carney's complex, (a dominantly transmitted autosomal disorder), not our case. Tumors may follow a malignant course, usually after local reccurence.

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Utrophin assay in Becker Muscular Dystrophy

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Background: Utrophin(Dystrophin Related Protein) is a protein which show marked homology with Dystrophin.It is normally found in neuromuscular junction and peripheral nerves but is absent in sarcolemma.In Dystrophinopathies, Utrophin is increased in normal position of Dystrophin and could be detected by Immunohistochemistry & Immunoblotting.Methods:10 clinically suspected BMD (Becker Muscular Dystrophy) were selected.Muscle biopsy was performed from Deltoid muscle for allof the cases.Immunostains (Spectrin,D1,D2,D3& Utrophin) were done on frozen muscle sections followed by Western blot analysis of protein extract for each muscle sample.Result: All of the patients showed an on & off pattern of all subtypes of Dystrophins & were confirmed as BMD using Immunoblotting technique.Abnormal expression of Utrophin is also evident in BMD patients (confirmed by both immunostaining & immunoblotting).This pattern is not evident in other subtypes of Muscular Dystrophies.Conclusion:Utrophin assay is an important & complementary tool for the diagnosis & differentiation of muscular Dystrophies.

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SUBSCAPULAR ELASTOFIBROLIPOMA

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BACKGROUND Elastofibrolipoma is a rare entity that was first described by De Nictolis et al. in 1995, and that, at the present, only two cases of which have been reported.
METHOD Tissue samples were fixed in 10% buffered formalin, embedded in paraffin blocks; 0,4-mm-thick histologic sections were taken and stained with hematoxylin and eosin, Verhoeff elastin and Masson trichrome stains.
RESULTS A 72-year-old male patient presented with a recidivant subscapular lipoma (previously studied and diagnosed in other institution). On gross examination, it was a 5× 4×2-cm mass with a capsulated and yellow-brown appearance. The cut surface was solid and lipomatous, showing streaks of white tissue irregularly alternating with areas of yellow tissue. Microscopic examination revealed that the lesion has a thin fibrous capsule. The mass consisted of bands of hypocellular, fibrous connective tissue, which separated numerous lobules of mature adipose tissue. In addition, numerous elongated or globe-like, eosinophlilic deposits, sometimes arranged linearly, were haphazardly distributed throughout the fibrous connective tissue. These deposits were strongly colored with an elastic stain. A diagnosis of benign mesenchymal tumor, i.e. elastofibroma, was made.
CONCLUSION Our findings supports that the elastofibrolipoma is a true benign neoplasm. This rare and recently recognized entity, must be bear in mind in the differential diagnosis of subscapularly located, deep-seated, well-circumscribed masses.

P-192

Intranodal kaposi sarcoma

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75 years old patient presented with dispne, and coughing to the Internal Medicine Department of Taksim's Education and Research Hospital. Multiple mediastinal, bilaterally axillary, inguinal, celiak, vertebral and paraaortic lymphadenomegaly had seen by MRI, CT and USG. Hematologically there was trombocytopenia and normochromic normocyter anemia. Because his PSA was high, prostatic biopsy was planned. And the result of the biopsy was prostatic adenocarsinoma. Besides axillary lymph node was excized and the histopathological result was intranodal Kaposi sarcoma. HIV markers were negative. Before he had the diagnosis of chronic renal failure. Because of prostatic adenocarcinoma and chronic renal failure he was immunologically insufficient, this prepared the situation for intranodal Kaposi sarcoma. We present the case because it is quite rare in literature.

Liver and Pancreas Pathology

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Cellular senescence in pancreatic ductal adenocarcinoma protection

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K-Ras oncogenic activation is a very frequent genetic alteration in human Pancreatic Ductal Adenocarcinoma (PDA). The generation of a mouse model with controlled temporal expression of K-RasG12V under its endogenous promoter in acinar and centroacinar cells has allowed us to study the generation of preneoplastic lesions, PanINs, and their evolution to PDA1. It has been recently described that preneoplastic lesions of the lung developed by mice with widespread expression of K-RasG12V were enriched of cells positive for markers of cellular senescence2. For this reason, we decided to characterize the senescence response during pancreatic neoplasia, since senescence has proved to be a relevant tumor suppressive mechanism *in vivo*3.
Moreover, the characterization of pancreatic neoplastic lesions by oncogenic K-Ras in mice revealed that K-RasG12V expression in adult animals does not result in neoplastic development unless accompanied by chronic pancreatitis. Thus, we decided to explore how continuos low doses of caerulein, a treatment causing mild pancreatitis, can affect the senescence response.
Finally, since a high percentage of human PDA has been firmly linked with chronic pancreatitis, we wondered whether halting the pancreatitis treatment would allow the control of the neoplastic development, if senescence has a role in this control and how effective would the senescence response be in controlling tumor development.

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Inflamatory Myofibroblastic Tumor presenting as a pancreatic mass

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Inflammatory myofibroblastic tumor of the pancreas is extremely rare, an entity previously known as an inflammatory pseudotumor. It has generally been considered a benign pseudosarcomatous inflammatory lesion which may have an infectious aetiology, very rare in the pancreatico-biliary region. We report a case of a 63-year-old man with a 3,5 cm pancreatic mass originally diagnosed as malignancy, which required surgical exploration and complete resection. Histologically the tumor consisted of a smooth muscle actin and CD68-positive spindle cell population and a more abundant mononuclear inflammatory cell population, primarily composed of T-lymphocytes and few macrophages. Genomic hybridization analysis of the tumor showed no chromosomal imbalances, with polyclonal rearrangement of IGH gene (CDRII, CDRIII).
It can be extremely difficult to differentiate inflammatory myofibroblastic tumour from pancreatic malignancies, and most cases require surgical exploration and complete resection to obtain an accurate diagnosis. The relation of the pancreatic IPT to autoimmune pancreatitis is reported.

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Assessment of liver biopsy specimens in chronic viral hepatitis (B,C)and autoimmune hepatitis referred to shahid sadoughi hospital yazd, Iran

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Background:we decided to evaluate liver biopsies in chronic viral hepatitis (B,C) and autoimmune hepatitis based on sex,age,clinical signs and symptoms with staging and grading of the disease. Methods:This descriptive study was conducted through case series method all the patients are positive for Hbs Ag,HCV Ab or autoimmune hepatitis, so liver biopsies were assessted for staging and grading by using scheuer system. Other data accumulated from

hospital folders, data analyzed statistically using SPSS 13 and chi-squqre test.Results:120 patients were included in this study, forty-eight were HbsAg+,fifty- eight HCV +, sixteen had autoimmune hepatitis, and twoof them had both (B,C).there is significant relation between HCVAb with sex (pv:0.000),cirrhosis and age(pv:0.00),cirrhosis and sex (pv:0.00),staging of hepatitis with duration of clinical signs and grading of disease (pv:0.00),frequency of cirrhosis and beginning of clinical signs (pv:0.00)and significant relation between incidence of autoimmune hepatitis and sex. Conclusion:we advised all patients with chronic hepatitis diagnosed based on clinical signs and serological markers undergone liver biopsy and assisted by sceuher or other staging and grading system to prevent and decreased progression into the end stage phase

Keywords:viral hepatitis,autoimmune hepatitis,cirrhosis

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Spectrum of Alpha-Methylacyl-CoA Racemase expression in the hepato-billiary system and pancreas: a wide tissue microarray analysis of normal and neoplastic epithelia

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Background: AMACR is a mytocondrial and peroxisomal enzyme involved in the β-oxidation of dietary fatty acids. It was initially described as a diagnostic marker of prostatic carcinoma, although recent reports indicate its expression in other neoplasms, especially colorectal carcinoma. In order to precisely establish the frequency, significance and possible clinical utility of AMACR expression in the hepatobilliary system, we have performed a wide screening of AMACR expression including normal, preneoplastic and neoplastic tissues using tissue microarray (TMA) technology and immunohistochemistry.

Design: A total of 204 samples of normal (76) and tumoral (128) human tissues from the hepatobilliary and pancreatic system were selected from the surgical pathology archives. Immunohistochemical studies were carried out using anti-AMACR (Policlonal, 1:100, Dako) and the Envision System (Dako). The staining was classified as negative, weak or strong, according to intensity and percentage of positive cells.

Results: Hepatocellular carcinomas (HCC) showed a stronger expression of AMACR when compared to normal, cirrhotic liver ($p=0.015$) and to high grade dysplastic nodules ($p=0.023$), but we found no differences in AMACR expression between single and advanced HCC, and between normal and cirrhotic liver. Well differentiated neoplasms showed increased expression of AMACR when compared to poorly differentiated tumors ($p=0.007$). Normal biliary ducts were frequently negative. Three out of 14 colangiocarcinomas showed strong AMACR expression and 6 stained weakly. Interestingly, HCC showed a statistically significant stronger staining for AMACR compared with cholangiocarcinomas ($p<0.001$). Neoplastic and pre-neoplastic lesions arising in the pancreas only showed a mild AMACR expression, mainly in PANIN lesions.

Conclusions: AMACR is strongly positive in some neoplastic and preneoplastic lesions of the hepatobilliary system and may be a marker of malignant transformation. In that context, it may be a useful marker in daily practise to help the pathologist to find the origin of metastatic lesions to or from the liver.

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HEPATOCELLULAR CARCINOMA WITH AN UNUSUAL NEUROENDOCRINE COMPONENT

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Background: Primary hepatocellular carcinoma is a well known liver condition. It exhibits histologic polymorphism even within a single nodule. The neuroendocrine character has been observed immunohistochemically and ultrastructually in some tumor cells within some hepatocellular carcinoma nodules. Only few cases of coexisting neuroendocrine carcinoma within hepatocellular carcinoma with clear boundaries have been reported.

Method: We describe a case of a tumor of the liver with features both of primary hepatocellular carcinoma and neuroendocrine carcinoma. The clinicopathological features and pathogenesis of this rare association are studied.

Results: A 56-year-old man was referred to the department of surgery for nodular lesions of the liver detected at abdominal ultrasonography at the time of exploration of a persistent fever with lost of weight and anorexia. Serum α-fetoprotein (AFP) was 15 ng/mL. The carcinoembryonic antigen (CEA) was within normal limits. Viral hepatitis serological testing was negative.

Intraoperatively, the liver, the spleen and the peritoneum showed numerous nodules associated with ascite. A liver surgical biopsy was performed. Histologically, the majority of the tumor was a hepatocellular carcinoma that was positive for hepatocellular antigen and alpha-fetoprotein. This component was focally intermingled with large nests of small round cells that were positive for chromogranin and synaptophysin corresponding to a moderately differen-

tiated neuroendocrine carcinoma. The patient died one month later.

Conclusion: The admixture of hepatocellular carcinoma and neuroendocrine tumor with clear boundaries is rare. The origin of neuroendocrine tumors in the liver is controversial. They may represent a differentiation of the malignant liver cells into a neuroendocrine tumor or the two tumors grow independently of each other. More cases need to be documented to obtain a better insight on the pathogenesis of this association.

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AUTOIMMUNE PANCREATITIS, AN UNDERDIAGNOSED ENTITY. DIAGNOSIS IMPORTANCE OF IgG4.

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BACKGROUND: Autoimmune pancreatitis (AIP) seems to be a disease with an heterogeneous appearance, that produces pancreatic nodular areas with duct estenosis, wich can mimic carcinoma. Patients often show elevated serum IgG4 and they usually respond to corticosteroid treatment, so it is important to differentiate AIP from pancreatic ductal adenocarcinoma. Our intention is to establish some key histopathological criteria and evaluate the diagnostic relevance of biopsy specimens.

MATERIAL AND METHODS: Histopathological, clinical and radiological features were recorded from 12 patients from 2000 up to 2006: 10 pancreaticoduodenectomy specimens (Whipple resections) and two biopsy specimens. The specimens displayed chronic pancreatitis and there was no evidence of pseudocysts, calculi, irregular duct dilatations, pancreas divisum and/or duodenal wall inflammation. The severity and the situation of the chronic inflammation as well as the intensity of IgG4- positive plasma cell infiltration were graded. The storiform pattern of the fibrosis and obliterative phlebitis were also determined.

RESULTS: Using previously published criteria the cases were subdivided into AIP "*concluent*" (n=1) showing periductal lymphoplasmacytic infiltrate, obliterative phlebitis, storiform fibrosis and abundant (>10 cells/HPF) IgG4 positive cells; "*suggestive*" (n=3) showing periductal lymphoplasmacytic infiltrate, storiform fibrosis and moderate (5–10 cells/HPF) IgG4 positive cells and "*doubtfu*l" (n=2) showing periductal lymphoplasmacytic infiltrate without IgG4 positive cells or lymphoplasmacytic parenchymatous infiltrate with IgG4 (<5 cells/HPF) positive cells. Ordinary chronic pancreatitis (alcohol or gallstones associated) showed lymphoplasmacytic infiltration and fibrosis without or minimal expression of IgG4.

CONCLUSIONS: 1. Benign biopsies proved to be a very helpful tool to establish the diagnosis prior to Whipple resection. 2. Autoimmune pancreatitis is characterized histologically by a dense periductal lymphoplasmacytic infiltration, with variable number of IgG4-positive plasma cells, fibrosis and venulitis. 3. IgG4-positive plasma cells are a useful immunohistochemical marker for the morphological diagnosis of AIP.

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Solitary fibrous tumor of the liver. A case report.

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Background: Solitary fibrous tumor (SFT) is a rare neoplasm of the adulthood. For many years it has been referred to the serous surfaces of the body, namely pleura, peritoneum and pericardium; recently cases arising in mesenchymal organs such as lung, mediastinum, liver and paranasal sinuses were also reported. Our aim is to present a new case of (SFTs) of liver.

Methods: A 81 year old female presented to our hospital with jaundice. Grossly the lesion was a well-circumscribed, encapsulated, firm, lobulated, gray-white to yellow-white mass. On dissection the tumor had frequent whirling and fasciculation and measured 18×13×8 cm.

Results: Microscopically the tumor showed alternating cellular with relatively acelular areas. The cellular areas were composed of uniform collagen forming spindle cells which were arranged in interlacing fascicles, while the acelular areas were edematous with stretched out tumor cells. The neoplastic cells were usually oval-shaped and contain bland nuclei with scattered chromatin and inconspicuous nucleoli surrounded by an indistinct rim of amphophilic cytoplasm. In some areas the cells are arranged around ectatic vessels in a haemangiopericytoma-like pattern. The tumor characteristically expressed Vimentin, CD-34, bcl-2 and reacted negatively to epithelial markers (CK), desmin, neurofilaments, NSE, S-100, SMA, CD117, CD31 and Factor VIII.

Conclusions: Our differential diagnosis included Peripheral Nerve Sheath Tumors which express NSE and S-100. The number of SFTs of the liver reported to date is too limited and overall experience is limited to confirm the definite prognosis of the tumor. Although most SFTs are benign, some may have malignant histological features and recur locally or metastasize. Malignancy should be considered when there is greater cellularity, cellular pleomorphism and increased mitotic activity. Treatment of choice for solitary fibrous tumors is extensive surgical resection.

P-200

Mutation and overexpression of β-catenin in hepatocellular carcinomas and hepatoblastomas

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Hepatocellular carcinoma is the major primary malignant tumor in the human liver, but the molecular changes for β-catenin has not yet been investigated in hepatocellular carcinoma and hepatoblastoma. We have examined the status of the β-catenin gene in 19 hepatocellular carcinomas and 3 hepatoblastomas by methods of RT-PCR, SSCP, western blot and immunohistochemical stain. In addition, mutation in the β-catenin gene is evaluated for 10 hepatoma cell lines such as SNU 182, 354, 368, 387, 398, 423, 449, 475, HepG2, Hep3B. Three cases out of nineteen hepatocellular carcinomas (16%) and Hep3B cell line had activating somatic mutations in exon 3 of the β-catenin gene. One case with β-catenin gene mutation showed β-catenin gene expression of stronger density by RT-PCR, and β-catenin overexpression by immunohistochemical stain in tumor than non-tumorous tissue, but no evidence of truncated β-catenin protein in western blot. And this case was also poorly differentiated tumor and had numerous mitoses. β-catenin overexpression had been detected mainly in the cell membrane of seventeen hepatocellular carcinomas (89%) and in the nuclei of three hepatoblastomas (100%). Thus β-catenin gene alterations are involved during liver tumorigenesis and suggest that disregulation of wnt-β-catenin pathway is a major event in the development of hepatocellular carcinoma and hepatoblastoma in humans.

P-201

The role of life-long feeding with different dietary lipid sources on the development of non-alcoholic steatohepatitis (nash) in aged rats

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Background. Morphofunctional studies suggest that the liver, compared with other organs, ages fairly well. Nevertheless, age does not spare the liver. Dietary fat has been related with changes in the susceptibility to suffer several liver diseases and with the aging process itself. We investigated the role of feeding rats life-long on different dietary fat sources (virgin-olive, sunflower or fish oils) differing in their fatty acids (mainly n-9 monounsaturated, n-6 polyunsaturated or n-3 polyunsaturated) on the susceptibility to develop NASH with aging.

Method. One-hundred and forty seven male wistar rats were fed life-long on three semysinthetic, normolipemic (4% w/w) and isoenergetic diets differing in the lipid source (virgin-olive, sunflower or fish oils). At the age of 24 months, twelve rats per group were slaughtered and the liver, blood plasma and urine were collected. A portion of the liver was processed for histopathological studies and another portion was used for mitochondrial isolation.

Results. Animals fed on fish oil showed a significantly higher NASH grade than those fed on virgin-olive oil (animals fed on sunflower oil were not different from the rest of groups). These results positively correlated very well with finding on centrolobulillar inflammatory infiltrate. Also a good positive correlation was found between NASH grade and the liver:body weight ratio and pro-inflammatory soluble factors (MCP1, ICAM and IL-6). Concerning oxidative stress findings, animals with the lowest NASH grade reached the highest levels of the mitochondrial antioxidant coenzyme Q, the lowest activity for mitochondrial catalase as well as the lowest concentration of urinary F_2-isoprostanes

Conclusion. Our results indicate that fish but not virgin-olive oil, when fed life-long leads to the development of NASH. That might be related with inflammatory and oxidative-stress processes intrinsic to each dietary fat source.

P-202

Comparative immunohistochemical profile and identification of programmed cell death (apoptosis) in hepatocellular carcinoma and cholangiocarcinoma.

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BACKGROUND: The histological diagnosis of poorly differentiated hepatocellular carcinoma (HCC) can be complicated by difficulty in differentiation from intrahepatic peripheral cholangiocarcinoma (PCC) and metastatic carcinoma, often requiring the use of immunohistochemistry for diagnosis. The reproducible identification of apoptotic cells in malignant tumours of the liver is important in so far as apoptosis occurs in later stages of carcinoma development.

METHOD: Our aim was to analyse the immunohistochemical profile of the 73 primary liver tumors, diagnosed, between 2002–2007. Primary antibodies used are: CEA,

CK 7, CK 8/18, CK 19, CK 20, CD34, Bcl-2, VEGF, Ki 67, PCNA, p53. For detection of apoptosis we used the terminal deoxynucleotidyl transferaze (TdT)-mediated deoxyuridine triphosphate (dUTP) - nick end-labeling TUNEL method, in 24 from our 73 cases.

RESULTS: From the 73 primary liver tumors, 47 were HCC and 26 were PCC. CK 7 was positive in 42,36% from cases with PCC and in 11,11% from cases with HCC. CK 19 stained positively in 82,34% from PCCs and in 30,34% from HCC. On the other hand CK 20 was positive in 17,15% from cases with PCC, and in 10,89% from cases with HCC. 58,23% of PCC stain positively with CEA and 9,78% of HCCs show patchy staining with CEA. In CD 34 reactions we noticed the strongest reaction in sinusoidal endothelial cells around HCCs. The PCNA–labeling index was higher in PCC than in HCC.

CONCLUSIONS: -The percentage of apoptotic cells is significantly higher in PCC than in HCC, suggesting that the degree of apoptotic cell death is severe in PCC, but is relatively mild in HCC.

– The apoptotic cells are consistently negative for PCNA and vice versa, suggesting that apoptotic cells do not show proliferative activity.
– IHC results should be interpreted in the larger context of the case.

P-203

Clinicopathological Characteristics of Turkish Patients with Invasive Pancreatic Ductal Cancers

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Background: Pancreatic ductal adenocarcinoma (PDAC) is the major pancreatic tumor and carries an extremely poor prognosis. The aim of this study was to identify these prognostic factors in patients undergoing primary curative resection.

Method: The study retrospectively analyzed 90 patients with PDAC who were operated with whipple resection. Their clinicopathological parameters revised, documented and statistically analysed for correlations.

Results: Average age of study group was 61,5 year and F/M was 1/1,5. Most prominent location was head of pancreas (44,4%), and the second was periampullary region (40%). Lymph node metastases were found in 54%. The rates of lymph vessel (LVI) and perineural invasion (PNI) were 90% and 82%, respectively. 25% of patients had blood vessel invasion (BVI). Mean of tumor size was 3,4 cm (1–11 cm). Pancreatic intraductal neoplasia (PANIN) was seen 33% of patients. 20% of cases was associated with IPMN. There was significant correlation between tumor location and mean tumor size (2,8 cm for periampullary; 3,8 cm for head; 6,7 cm for corpus; and 6,8 cm for tail of pancreas; PNI was also associated with distal location and lymph node metastasis.

Conclusion: Our preliminary study support that PDAC which is located in tail of pancreas seem to have larger size, lymph node positivity and blood vessel invasion. Tumor location may be considered as poor prognostic factors in pancreatic adenocarcinomas.

P-204

Intraductal papillary neoplasm of the bile duct associated with Clonorchis sinensis infection

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Intraductal papillary neoplasm of bile duct (IPNB) is one of the precursor lesions of cholangiocarcinoma. Only one preexisting condition, hepatolithiasis has been extensively studied in association with IPNB. However, to the best of our knowledge, there was no comprehensive study regarding IPNB association with Clonorchiasis. We performed immunohistochemical studies of mucin proteins (MUC1, MUC2, MUC5AC, CDX2) and cytokeratin (CK7, CK20) pancels of 12 IPNB cases with Clonorchis sinensis infection. Four cases were non-ivasive IPNB (1 borderline and 3 carcinoma in-situ) and 8 contained an invasive carcinoma component (7 tubular carcinoma and 1 colloid carcinoma). Tumors were located in extrahepatic bile duct (EBD) in 7 cases, intrahepatic bile duct (IHD) in 4 cases, and both EBD and IHD in 1 case. Seven cases were positive for MUC1 and 11 for MUC5AC, while only 2 cases were positive for MUC2. The predominant immunolabeling pattern was pancreatobiliary type (MUC1 / MUC5AC /CK7) and it was associated with tubular carcinoma and has higher stage than colloid carcinoma case. Our data revealed different immuno-phenotype with that of IPNB from hepatolithiasis, which was known to be related with an intestinal type. These results suggest that IPNB with Clonorchiasis may have a different tumorigenesis pathway than IPNB with hepatolithiasis.

P-205

ANGIOGENESIS IN HEPATOCELLULAR CARCINOMA AND CIRRHOSIS: IMMUNOHISTOCHEMICAL STUDY WITH ENDOTHELIAL MARKERS

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Background: Hepatocellular carcinoma (HCC) is a highly vascularized tumor and angiogenesis has been studied in this neoplasia. During the hepatic carcinogenesis, structural and functional changes characterized by sinusoid-like vessels, take place in the vascular architecture. Studies have demonstrated that in the HCC the capillary vessels express CD34. The CD105 is a glycoprotein associated with endothelium cell proliferation and is identified as a marker of neoangiogenesis, but its expression is controversial in HCC.

Aims: To evaluate the expression and the distribution of CD105 and CD34 proteins in HCC and surrounding cirrhotic regenerative nodules (RN).

Methods: Tissue samples of tumors and cirrhotic regenerative nodules were obtained from 16 explanted livers. Thirty-four RN and 25 HCC were studied by immunohistochemistry with human CD34 and CD105 monoclonal antibodies. Immunoexpression was semiquantitatively evaluated in sinusoidal area as follows: group 1 (negative); group II (positive to 30%); group III (positive >30%).

Results: The HCC showed CD34 and CD105 expression in 24/25 (96%) and 22/25 (88%), respectively. However in cirrhotic parenchyma the CD34 value changes 24/34 (70%) of the RN while the CD105 was similar 30/34 (88%). An important observation was the semiquantitative evaluation of the immunoexpression. CD 105 was higher in RN than in HCC. It was observed in more than 30% of the sinusoidal area in 21/34 (61.7%) of the RN, and only in 5/25 (20%) in the HCC. Conversely, the HCC showed high CD34 expression in 21/25 (84%), a difference statistically significant when compared with RN 2/34 (5.8%). CD105 showed diffuse positive immunostaining in all HCC and RN. CD34 expression was also diffuse in all the HCC, whereas only 2/34 (5.8%) of the RN were positive.

Conclusion: The higher expression of CD105 in cirrhosis when compared to the HCC suggests that may not be a good endothelial marker of the hepatic carcinogenesis as opposed to CD34.

P-206

Pancreatic tuberculosis in non immunocompromised patients: report of three cases.

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Introduction Isolated pancreatic tuberculosis is rare and can mimic pancreatic carcinoma.

Cases We report three cases of pancreatic tuberculosis.

34 years old and 50 years old women presented with obstructive jaundice. For the first patient, abdominal CT scan showed suggested tuberculosis and resection was avoided. The second underwent whipple procedure.

Third case was 48 years old alcoholic man who presented with recent history of painful mass of left hypochondre. Cystic tumor of pancreas tail was suggested in CT scan. A bloc resection of tumor pancreas tail and spleen was performed.

The three patients had antitubercular therapy after histological confirmation of pancreatic tuberculosis. Follow-up was respectively 3 years, 5 months and 2 years free recurrence.

Conclusion Tuberculosis origin of an isolated pancreatic mass may be suspected in young, especially in endemic areas.

Electron Microscopy

P-207

Ultrastructural alterations of large intestine before and after endovascular hemostasis in treatment of acute intestinal hemorrhages in experiment

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Background: The aim of this study was to prove at ultrastructural level the possibility of carrying out the roentgen-endovascular hemostasis at acute intestinal hemorrhages without risk of occurrence of irreversible changes in wall of mucosa of large intestine.

Method: The material for morphological research were the sites of large intestine on which different methods of roentgen-endovascular hemostasis were spent. Extracture of tissue pieces of large intestine for electron microscopic research spent at animals from three groups in different terms after endovascular interventions.

Results: The analysis of ultrastructural alterations after roentgen-endovascular hemostasis by means of microspirals has shown, that ultrastructural architectonics of column

epitheliocytes, goblet exocrinocytes vascular capillaries and smooth myocytes of the muscular layer of the large intestine of rats endured basically compensative-adaptable reorganisation. Alterations of organellas were in limits of physiological indemnification. Nevertheless, there were observed moderately expressed dystrophic disturbances, leading of which were mitochondrial dysfunction, structurally expressed in swelling of mitochondrions, loss of electron density of the matrix, disorganization of cristas and partial focal lysis of external membranes. These alterations testify to disturbance of oxidation-reduction reactions, that in their turn involves also disturbance of synthetic and reparative intracellular processes. It is necessary to notice, that the described alterations, according to depth and expression degree, are reversible.
Conclusion: Having been carried out electron microscopic researches of large intestine after roentgen-endovascular hemostasis by mechanical damage of an intima artery by guide wire or spiral, and also simultaneous introduction of chemodrugs with vasospastic and haemostatic drugs have shown convertibility of the ultrastructures alterations of mucosa of large intestine. The convertibility of ultrastructural alterations that was revealed during the experiment allowed to recommend the use of given methods of roentgen-endovascular hemostasis in clinic for treatment of patients with intestine hemorrhages.
Conclusion: Having been carried out electron microscopic researches of large intestine after roentgen-endovascular hemostasis by mechanical damage of an intima artery by guide wire or spiral, and also simultaneous introduction of chemodrugs with vasospastic and haemostatic drugs have shown convertibility of the ultrastructures alterations of mucosa of large intestine. The convertibility of ultrastructural alterations that was revealed during the experiment allowed to recommend the use of given methods of roentgen-endovascular hemostasis in clinic for treatment of patients with intestine hemorrhages.

P-208

Solitary fibrous tumour. An ultraestructural and immunohistochemical study of 11 cases

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BACKGROUND Solitary fibrous tumour (SFT) is a rare ubiquitous mesenchymal neoplasm of probable fibroblastic type with a prominent haemangiopericytoma-like vascular pattern. Since their initial description as arising from the pleura, SFTs have been reported in a variety of extraserosal sites. It is now commonly accepted that this neoplasm is derived from mesenchymal cells but histogenesis is not well known. In addition, because of the variability of morphological patterns, SFT may be easily misdiagnosed, specially when appears in an unusual location. We have performed the analysis of 11 cases correlating clinicopathological, immunohistochemical and ultrastructural features in order to understand the biology and help in diagnosis of this entity.
METHODS We gathered data on eleven patients diagnosed between 1986 and 2006. Histopathological evaluation was performed on hematoxylin and eosin previously stained slides. Tissue-arrays were constructed assembling two cylinders from representative areas, to perform inmunohistochemical tests. For electron-microscopic examination tissue was processed with Karnovsky reactive and embedded in epoxy resin.
RESULTS Eigth of the lesions arose in thoracic cavity and three cases were extrathoracic. One case died within the next 5 months following surgery and other was diagnosed during autopsy. Three cases recurred but there were not metastatic disease. Histopathological study showed varying degrees of cell density and mitotic activity which correlated with clinical behaviour. Inmunohistochemically all tumours stained positively for Vimentin, CD99 and CD34 and only 7 cases for bcl-2. Ultraestructural appearance of the eleven tumors showed myofibroblastic differentiation in all cases with focal smooth muscle features in three of them. In addition nine cases showed perivascular undifferentiated cells.
CONCLUSION SFT is an uncommon neoplasm with different histological patterns and clinical behaviour. We have hypothetised that the perivascular undifferentiated cells found might correspond to a quiescent stage of adult stem mesenchymal cell and may be implied in pathogenesis of SFTs.

Breast Pathology

P-209

Quickscore assessment in Bcl- 2 and Bax protein expression in invasive ductal carcinoma of breast and relation with other molecular Markers.

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Objective: Different score such as H- score, multiplicative and additive quickscore were used for ER status by Immunohistochemistry (IHC) methods.
This study investigates the incidence of Bcl- 2 and Bax expression with IHC method and quick score assessment, in invasive ductal carcinoma(IDC) of breast relative to their

prognostic factor and other biological markers including ER and PR status.

Materials and Methods 92 cases of formalin fixed paraffin embedded tissue of IDC of breast were selected and immunostained using primary antibody to Bcl- 2, Bax, Ki- 67, ER and PR quantified with H- score, multiplicative and additive quickscore.

Results: There were high significant positive correlation between ER, PR with Bcl- 2 and significant negative correlation between Ki- 67 with Bcl- 2.

There were high significant correlation between H- score ER and PR and multi and additive quick score ER and PR with multi and additive quick score Bcl- 2.

Conclusion: Bcl- 2 is associated with good prognostic factor in IDC of breast. Bax expression with both quickscores does not necessarily correlate with quickscore and H- score of ER and PR. We can use quickscore in evaluation of ER and PR status instead of H- score.

Keyword: Bax, Bcl- 2, Invasive ductal carcinoma, Ki- 67, Oestrogen receptor, Progestron receptor.

P-210

PRIMARY BREAST AMYLOIDOSIS PRESENTING AS MICROCALCIFICATIONS

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Primary breast amyloidosis presenting solely as microcalcifications is extremely rare. We report a case of a 73 year old woman with persistent radiologically suspicious microcalcifications without palpable mass. The diagnosis of amyloidosis was established histologically by the presence of an amorphous eosinophilic material which was positive for Congo red and was dichroic under polarized light. Paraffin immunohistochemistry showed the presence of kappa light chains (AL-type amyloidosis. The amyloid deposits were associated with microcalcifications. A complete workup was performed to exclude other localisations and was negative. The primary beast amyloidosis presenting solely as microcalcifications is discussed and a brief review of the litterature is presented.

P-211

Interpretation of estrogen and progesterone receptor immunohistochemistry may be biased by laboratory technique and observer expertise

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Background: Estrogen (ER) and progesterone receptor (PR) status plays an importantrole in treatment of breast carcinoma patients. ER and PR immunohistochemistry (IHC) is the current standard forassessment. However, the subjectivenature of IHC interpretation may lead to inaccurate receptor status andpossibly inappropriate therapy.

Method: 100 consecutive breast carcinoma cases were studied. Interpretation was compared between a single-pathologist with expertise in IHC (EP) and ten general pathologists (GP) withvarying IHC experience. Percentage of nuclear immunoreactivity wassemi-quantitated: positive, negative, and focal positive. Focal positive tumors showed < 5%immunoreactivity and/ or weak staining.

Results: There was 91% agreement for both ER and PR.21 and 27 cases were negative for ER and PR, respectively for both observers.There were 18 discrepancies, ER: 4 cases were negative by GP but positive byEP. Three focal positive cases by GP were positive by EP. There were 6 caseswith discordance between positive and negative for the GP and EP. For PR, 96% concordance between positive andnegative. Three cases were negative by GP but positive by the EP and 1 case wasnegative by GP and positive by the EP. Five focal positive cases by GP werepositive by EP.

Conclusions: Concordance between GP and EP was 94% and 96%, respectivelyfor ER and PR. For ER and PR similar rates of discordance between negative byGP and positive by EP were observed (4 and 3%) as well as focal positive by GPand positive by EP (3 and 5%). Subjective interpretation and technicalvariations may limit accurate quantitation in an inherently qualitative test.Discrepant rates of 4–6% may have a clinically significant impact on patienttreatment and therefore survival.

P-212

Infiltrating micropapillary carcinoma of the breast and sentinel lymph node biopsy.

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BACKGROUND: To evaluate the indications of sentinel lymph node (SLN) biopsy in patients with infiltrating micropapillary carcinoma (IMPC) of the breast and the results of this procedure when it has been performed.

METHODS: During the last ten years in our Center SLN biopsy has been performed in 494 patients. During the

same period 52 IMPC have been diagnosed. This is a retrospective and descriptive study of the technique used for nodal staging in these patients, and the reason why SLN biopsy was not performed. The results of SLN biopsy in those patients with IMPC were compared with the results of this technique in patients with other histologial types of breast cancer.
RESULTS: SLN biopsy could be done in only 15 (29%) patients with IMPC and in 10 (67%) cases in was involved. Five had metastases, three micrometastasis and two isolated tumor cells. Only one patient showed more lymph nodes involved in the axilla. In those patients in whom the SLN biopsy could not be done, positive fine needle aspiration under ulltrasonographic guidance and multifocal breast involvement were the main contraindications. In 1036 breast cancer of non IMPC diagnosed in the same period, SLN biopsy could be done in 479 (46%), being involved in 146 (31%) cases.
CONCLUSION: SLN biopsy is infrequently indicated in those patients with IMC and, when performed, the percentage of lymph node metastasis is high but mostly limited to SLN.

P-213

Sarcoma of the female breast in Zaria

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Background:Breast cacinoma,an epithelial tumour is the second commonest tumour among women in zaria,and is a leading cause of cancer-related death in women in developing countries.However,tumours of the mesenchymal origin also occur in the breast.
Materials and Method: A seven year analysis of consecutive women with breast sarcoma from January 2000 to December 2006.tissue biopsies were processed in paraffin waxand histology sections were stained with haematoxylin and Eosin,reticulinand periodic acid Schiff.
Results:9 females were studied.Their ages ranged fron20 years to 60 years with a median age of 32 years. Clinically, these women presented with progressively increasing breast lumpsof varying sizesassociated with pain. One patient presented with a recurrent lump associated with skin ulcer which ruptured spontaneously.the duration of symptoms was from 1 year to 3 years.Examination revealed well circumscribed breast lumps and axillary lymph nodes enlargement. Clinical diagonosis included fibroadenoma and advanced carcinoma.Three women had exicisional biopsy,while swix had mastectomywith axillary clearance.Histologic diagonosis of the sarcoma subtypes included stromal sarcom-7, osteogenic sarcom-1 and Angiosarcoma-1.Microscopically, they were cellular lesions composed of spindle cells having hyperchromatic to vesicular nuclei and moderate amount of cytoplasmarranged in whorls,fascicles and herringbone pattern.They showedvarying degree of myxoid degenerationand necrosis.Variable number of mitotic figures were seen. All sampled nodes showed reactive hyperplasia only.
Conclusion:Breast carcinoma,though uncommon,affects younger women compared to carcinoma in our setting. Tumour spread is haematogenous hence the sparing of lymph nodes.Additional diagonostic methods are helpful in differentiating the various subtypes.This is important in patient care and management.

P-214

A STANDARD, HIGH SENSITIVITY, IHC PROTOCOL AND SCORING METHOD WITH THE AID OF IMAGE ANALYSIS FOR THE DETECTION OF HUMAN ESTROGEN RECEPTOR ALPHA

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Background: Immunohistochemical (IHC) assays of differing sensitivity and scoring systems with varying positive/negative cut-off values for estrogen receptor (ER) are major causes of inconsistent results between laboratories. The ER/PR pharmDx™ kit has been developed and 510(k) cleared, and provides a standard protocol for staining and scoring ER and PR with a defined cut-off. Here we describe the development of the ER cocktail antibody, automated IHC protocol, and a prototype image analysis algorithm aimed at improving staining and scoring consistency. **Method:** The epitopes for mouse anti-ER clones 1D5, ER-2–123, and 6F11 were characterized by SPOT analysis and a synthetic array. Clones 1D5 and ER-2–123 were tested separately or in combination in a highly sensitive IHC assay. Staining with the optimized ER cocktail antibody was evaluated on two automated platforms, Autostainer Plus and Autostainer Plus Link. Image analysis was performed using a prototype ER pharmDx™ algorithm on ACIS III. **Results:** Anti-ER antibody clones 1D5 and ER-2–123 demonstrated reactivity with discrete epitopes on ER; ER-2–123 and 6F11 reacted with the same epitope. IHC results using a cocktail of anti-ER clones, 1D5 and ER-2–123, revealed a synergistically increased sensitivity compared to results with individual clones. ER staining on two automated platforms showed equivalent and reproducible results. Image analysis using a prototype ER pharmDx™ algorithm showed promising results as an aid to consistent scoring. **Conclusion:** The sensitivity and quality of immunohistochemical staining of ER is dependent on selection of ER antibody and IHC protocol. Two complementary ER antibody clones were

combined to increase assay sensitivity and are provided as part of a standard protocol in the ER/PR pharmDx™ kit. The kit protocol includes a validated scoring system with defined cut-off, which combines staining intensity and proportion scores. Automated staining improved workflow and staining reproducibility. A prototype ER pharmDx™ image analysis algorithm facilitated consistent scoring.

P-215

Phyllodes tumour of the breast with features of malignant triton tumour

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Background: Malignant phyllodes tumour of the breast represents a rare biphasic neoplasm with epithelial and stromal components. Approximately 10–20% of all breast phyllodes tumours are malignant, most commonly with an homogeneous sarcomatous differentiation. Anyway, multiple heterologous lineages of differentiation are occasionally found in these neoplasms. Here we report a case of malignant phyllodes tumour showing features of rhabdoid and schwannian differentiation. Clinical history and Methods: The patient, a 80-year-old lady, presented with a 9 cm mass in outer superior quandrant of the right breast. At quadrantectomy, the lesion was greyish in colour, had poorly defined borders and a microcystic appearance. Tissue were fixed in 10% buffered formalin, paraffin embedded and stained with haematoxylin-eosin. Immunohistochemistry was performed with Ventana automated stainer. Results: Histologically, the tumour was constituted by a hypercellular stromal proliferation. Stromal cells had a marked nuclear atypia and numerous mitoses (up to 10 /10 HPF). Only scattered residual mammary glands were present. In most part of the lesion, neoplastic cells were round shaped and had abundant eosinophilic cytoplasm and round vesicular nuclei with prominent nucleoli. These elements merged with areas of spindle cells with elongated nuclei and wavy cytoplasm. By immunohistochemistry, the rounded cells were strongly and diffusely positive with rhabdomyoblastic markers (myogenin, desmin, striated actin) and focally positive with nervous markers (Neu-N and S100 protein). An opposite phenotype was found in the spindle cell population, as they were diffusely positive with Neu-N and S100 protein and only focally expressed rhabdomyoblastic markers. Conclusions: These histological and immunohistochemical findings are consistent with malignant triton tumour, a rare soft tissue neoplasm characterized by divergent differentiation towards skeletal muscle and Schwann cells. To the best of our knowledge, this is the first documented case of phyllodes tumour with features of malignant triton tumour.

P-216

PROGNOSTIC SIGNIFICANCE OF IMMUNOHISTOCHEMICAL EXPRESSION OF CATHEPSIN D, CYCLIN D1, AND THYMIDINE KINASE 1 IN INVASIVE DUCTAL BREAST CARCINOMAS WITH PREDOMINANT INTRADUCTAL COMPONENT

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Background: Breast cancer is the most common malignant neoplasm in women and, in the light of recent studies, new therapeutic strategies keep on developing. We present an immunohistochemical study that was performed in order to evaluate the expression of cathepsin D (CD), thymidine kinase 1 (TK1), and cyclin D1 (CycD1) in invasive ductal breast carcinomas with predominant intraductal component and to determine their prognostic value.
Method: The examination of slides stained with Haematoxylin-Eosin of 70 patients were performed retrospectively, and clinical and prognostic data were reached. The histopathological grading of invasive tumour component was done by using the Nottingham modification of Bloom-Richardson grading system, and staging by using the system proposed by American Joint Committee on Cancer. Pearson's chi-square, Likelihood ratio, Fisher's exact tests, Kaplan-Meier survival analysis were used as statistical tests and the significance of observed differences was set at $p<0.05$.
Results: Intraductal component showed higher degree of staining for CD than invasive tumour component. A correlation was found between the CD expression and the reactive stromal cells and also the increasing degrees of invasive tumour. The negativity or weak expression for CD, TK1, and CycD1 in non-tumoural areas was found to be significant. CycD1 expression is found to be lower in advanced and/or high grade carcinomas and is found to be related to better survival rates in patients with invasive ductal carcinoma.
Conclusion: The finding of significantly higher expressions of CD, TK1, and CycD1 in the tumour component compared to the non-tumoural areas, raised the possibility of usage of all three antibodies in determining the malignant character of breast lesions. CD expression in intraductal component or in the reactive stromal cells may be used as an indicator of invasive potential of the tumoural lesion. CycD1 expression may be related to a better prognosis.

P-217

A study of twenty-eight 9-gauge vacuum assisted breast biopsies for microcalcifications: Columnar Cell Lesions, a frequently detected entity in patients with microcalcifications.

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BACKGROUND: Columnar cell Lesions are a group of proliferative lesions of the terminal duct lobular units (TDLUs) characterized by dilatation of TDLUs lined by columnar epithelial cells with prominent apical snouts and intraluminal secretions. There is currently no internationally accepted classification or terminology for this group of lesions. CCL are considered as a candidate precursor in the progression to low grade ductal carcinoma in situ (low grade DCIS) and low grade invasive carcinoma. METHOD: We have revised twenty-eight 9-gauge vacuum assisted breast biopsies for microcalcifications performed between July and November 2007, and looked for CCL. We performed immunostaining for CK5 and ER in cases where CCL were suspected histologically. RESULTS: CCL were detected in 22 (78,6%) of 28 cases, showing strong overexpression of ER and CK5 negativity. None of these lesions expressed CK5. 6 (85,7%) of 7 cases of any grade DCIS and 5 (83,3%) of 6 cases of any grade infiltrating ductal carcinoma were associated to CCL. 7 (63,6%) of 11 cases diagnosed as non-malignant presented CCL. 2 (50%) of 4 cases diagnosed as Columnar Cell Hyperplasia displayed Flat Epithelial Atypia (FEA). FEA was present in 15 (61,2%) of 22 CCL. CONCLUSIONS: CCL are frequently detected in patients with microcalcifications, and they are often associated with DCIS and invasive ductal carcinomas of any grade. FEA was the most common form of CCL detected. CCL can be frequently underdiagnosed. Immunostaining for CK5 and ER is a useful diagnostic aid and it can discriminate between CCL and its mimics, like apocrine metaplasia. All the CCL detected in this revision showed a pure epithelial (luminal, glandular) immunohistochemical phenotype.

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Risk of additional axillary-node metastases in women with breast cancer and positive sentinel lymph nodes.

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Background: The involvement of sentinel lymph nodes (SLN) is classified as macrometastases (M) (>2 mm), micrometastases (mic) (2 to >0.2 mm), and isolated tumor cells (ITC) (<0.2 mm). Although the risk of additional axillary metastases is significant in patients with **M**, the risk in patients with **mic** and **ITC** is less known, and the indication for completion of axillary dissection is controversial.

Design: 640 patients with breast and SLN biopsy were studied. Patients and primary tumor characteristics included: age, tumor size, histologic type, grade, lymphatic invasion, number of positive SLN, number of patients with additional axillary lymph nodes (AALN), number with positive AALN and size of metastases in AALN.

Results: 28% (n=181) of the patients had positive SLN. According to the size of the metastases, the cases were classified as **M**, 42.5%(n=77); **mic,**36.4% (n=66); and **ITC,** 20.9% (n=38). The number of SLN studied per patient was 1 to 5, and the number of positive SLN 1 to 3. **AALN** were obtained in 159 patients, 98.7% (n=76) with **M,** 93.9% (n=62) with mic, and in 55.2% (n=21) with **ITC.** Of the patients with **M** in the SLN, 34.2% (=26), had positive AALN, 25 were **M** and 1 was **mic**.Of the patients with mic in the SLN, 16.2% (=10), had positive AALN, all were **M.** Of the patients with **ITC** in the SLN, 9.5% (=2) had positive AALN, one **M** and one **mic**.

Conclusions: The risk of AALN metastases is related to the size of the metastases in the SLN, and most AALN metastases are **M**. Completion of axillary dissection is indicated in patients with **M** in the SLN. In patients with **mic** and **ITC** in the SLN, assessment of predictive factors of metastases in AALN is recommended, because there is a risk of macrometastases.

P-219

Methods of differential diagnostics of the locally extended thymomas at myasthenia

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Background: There were studied the diagnostic importance of specific immunophysiological markers associated with various types of local extended thymomas at myasthenia.

Methods: There were defined the phenotype and the density of leukocytic antigens HLA-DR of II class, the expression of differenciative markers CD3, CD4, CD8,

$\tilde{N}$D16, $\tilde{N}$D19, contents of serumal cytotoxic factors, concentration of cytokines and presence organ-specific antibodies to the tissue of liver, lungs, heart.

Results: The immunocytochemical methods of research have found the following types of thymomas: lymphoepithelial thymoma (LEÒ) – 59%, epithelial (EÒ) – 24%, lymphoid (LÒ) – 9% and granulomatous thymoma (GÒ) – 8%. The locally extended thymomas more often associated with the phenotype of antigens HLA-DR1, HLA-DR3 and HLA-DR7. Patients with LÒ have phenotype HLA-DR1 and HLA-DR5, and patients with LET have HLA-DR3 and HLA-DR7, patients with EÒ have phenotype HLA-DR1 and HLA-DR3. Phenotype HLA-DR5 and HLA-DR52 is associated with GÒ. In patients with LT $CD4^+$ T-helpers, $CD16^+$ are significantly increased and level $CD8^+$ is decreased. The increase of $CD19^+$ and $CD16^+$ in 2 times in comparison with referential values has been found at patients with LET. There was found the significant increase of $CD2^+$ at LÒ and GÒ in 1,5 times. Subpopulation of $CD3^+$ at EÒ and LEÒ is significantly decreased. Subpopulation of $CD4^+$ at LET and EÒ is considerably decreased. Cytotoxic $CD8^+$ cells in these groups of patients were also below the control parameters in 2 times. At GÒ the level of $CD3^+$, $CD4^+$ and $CD8^+$ does not differ from control value.

Conclusion: The alterations of immunophysiological reactions at various types of thymomdependent myasthenias allows to carry out differential diagnostics of thymomas, to make a choice of treatment tactics, including excisions of the thymus and to prognosticate the current of disease.

P-220

Immunoexpresion of HER family in Invasive Ductal Carcinomas of the Breast

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Aim: To investigate the frequency of expression of erbB/Her family of growth factor receptors in invasive ductal carcinomas of the breast.

Material Method: 59 invasive carcinomas ductal carcinomas of the breast has been studied for EGFR, c-erbB-2, c-erbB-3 and c-erbB-4 by streptavidin- biotin horseradish method and only for c-erbB-2 by FISH technique. The frequency of expression of the antibodies, their coexpressions, correlation of immunohistochemistry results and FISH for c-erbB-2 and correlations with the histopathological prognostic parameters as tumor stage, grade, lymph node status, hormonal status, age, and patients' 3 years clinical follow-up, have been investigated.

Results: Of the 59 tumours 44 (75%) were ER+, 37 (63%) PR+, 4 (7%) EGFR+, 6 (10%) c-erbB-2, 6 (12%) c-erbB-3, 14 (24%) c-erbB-4 by immunohistochemistry. Seven of eleven c-erbB-2 ++ (equivocal) cases were positive by FISH. Thus, c-erbB-2 amplification increased to 22%. Statistically significant correlations between tumor stage and lymph node status and between ER and PR status were found ($p<0.05$). There has been a statistically significant correlation with ER negativity and EGFR overexpression ($p<0.05$); a borderline positive correlation with EGFR and c-erbB-2 has also been noted ($p=0.064$). C-erbB-2 overexpression demonstrated significant correlation with the tumour high grade, PR negativity, and c-erbB-3 immunoexpression. C-erbB-3 expression and c-erbB-4 expression were found to be statistically correlated ($p<0.05$). Also significant association with c-erbB-4 expression and the patients good clinical out come ($p<0.05$) were found.

Conclusion: The results of our study suggests a subgroup of invasive ductal carcinomas of the breast which are ER-negative, EGFR positive or PR negative, c-erbB-2 positive. These groups correlate with high tumor grade, high stage, and lymph node metastasis.

P-221

CYCLİN D1 AND P21 ASSESMENT AT TAOXİFEN RESİSTANT BREAST CANCER

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Introduction: Cyclin D1 is an important nuclear protein which is necessary for progression of cells through the G1 to S phases of the cell cycle. p21 activated kinase plays inhibitor role at cell cycle. Quantifying of their proliferative potential may help in prediction of biological behavior of tumors with comparable histology.

The aim of this study was to explore the usefullness of Cyclin D1 and p21 expression as proliferative and predictive markers at tamoxifen resistant invasive ductal breast carcinomas and compare these two markers with classical prognostic parameters (tumor size, histological and nuclear grade, perineural and vascular invasion, estrogen and progesteron reseptor status, cerb-B2 positivity, axillar lymph node status).

Materials and methods: We investigated 66 cases of invasive ductal breast carcinomas of which diagnosed between 2002–2007 in our hospital. Paraffine-embedded archival tissue was used with monoclonal antibodies against Cyclin D1 and p21.

Conclusions: In this study, there was no statistically significant relationship with tamoxifen resistance and Cyclin D1 and p21 expression but we found association between estrogen and progesteron receptor positivity with Cyclin D1 expression.

P-222
HIGH AGREEMENT BETWEEN FISH AND CISH BASED TESTING FOR HER2 AND TOP2A GENE AMPLIFICATION

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Background. We have developed a prototype CISH visualization kit, which used on top of FISH gene probes converts the fluorescence signals into stainings that can be evaluated by bright field microscopy. The Dako CISH kit is a dual color assay that detects both the *HER2* or *TOP2A* gene and CEN-17 on the same slide. The dual color technique makes it easier to distinguish true gene amplifications or deletions from chromosomal aneuploidy compared to single color CISH assays. The aim of the present study was to investigate the concordance between CISH and FISH technologies for detection of *HER2* or *TOP2A* gene amplifications/deletions
Method. The study was divided into two parts, one comparison for *HER2* gene detection and one for *TOP2A* FISH gene detection, including 24 and 20 FFPE breast cancer specimens, respectively. The CISH and FISH analyses were performed on serial sections. Evaluation was done blinded.
Results. For both the *HER2* and *TOP2A* status, the concordance was 100% and the corresponding Kappa values were 1,0. The correlation of the gene copy number to CEN-17 ratios were similarly high, with a correlation coefficient (r) for both *HER2* and *TOP2A* above 0,95.
Conclusion. The results from the study showed a complete agreement between CISH and FISH technologies, and a larger study is in planning to confirm the findings in the present study.
The data indicates that the technology used for the Dako prototype CISH assay holds significant promise as a future method.

P-223
Cell membrane and cytoplasmic immunoreactivity of MIB-1 in invasive breast carcinoma

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Background The monoclonal antibody MIB-1 recognizes the nuclear Ki67 antigen and is used to assess proliferative activity of tumors. In the last years a peculiar cell membrane or cytoplasmic MIB-1 staining has been occasionally reported in a few tumors, including breast carcinoma. We investigated clinicopathologic features of invasive breast carcinomas with non-nuclear MIB-1 immunoreactivity.
Method 295 invasive breast carcinomas stained with MIB-1 from May 2002 to September 2007 were reviewed. They were 236 usual ductal carcinomas, 35 lobular carcinomas and 24 special types. Staining method was LSAB2 (185 cases) or Envision (110) (Dako). MIB-1 antibody was diluted 1:25 and 1:75 respectively. Non-nuclear immunostaining was evaluated as + (isolated cells), ++ (focal, minority of cells) or +++ (diffuse and strong staining). In positive cases GCDFP-15, CD10, alcian blue and PAS-diastase techniques were done.
Results Fourteen cases (4.7%) showed non-nuclear reactivity (3 +, 4 ++, 7 +++): in lateral cell membrane (6 cases), luminal membrane (6) and cytoplasm (4), both with LSAB2 (9 cases, 4.8%) as with Envision (5 cases, 4.5%). Staining did not correlate with GCDFP-15 or CD10 reactivity. Positive cases were 8 usual ductal carcinomas, one pleomorphic lobular carcinoma and 5 other types, staged pN0 (6 cases), pN1(4), pN2 (1), pN3 (1) or pNx (2). Bloom-Richardson grade was low (5 cases), intermediate (5) or high (4). Estrogen receptors were positive in 8 cases and progesterone receptors in 7 tumors. PAS-diastase and alcian blue were positive.
Conclusion Cell membrane or cytoplasmic MIB-1 immunoreactivity is a rare feature of invasive breast carcinomas (4.7%). It does not depend on the visualization technique and is related to a cross-reactivity of this antibody with other epitope, probably a glycoprotein. It occurs in different types of breast carcinoma and no special clinicopathologic features have been identified, although larger series need to be investigated.

P-224
Myxoid Liposarcoma of the Breast in Female-case raport

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Breast liposarcoma is very rare malignant breast tumor and it have an incidence of 0,3% of all breast sarcomas.
A 66- year old woman presented with a tumorous mass of the left breast. The excided part of breast measured 6×5×3,5 cm. The serial sections revealed well-circumscribed nodular mass yellowish, glistened and lobular. Tumor mass measured 3,2×3×3 cm. Histological examination showed proliferation of spindle shaped cells and signet-ring lipoblasts in myxoid matrix as well as delicate branching capillaries were noted.Cytoplasm of tumor cells were positive staining for Sudan III dye. Ultrastructuraly numer-

ous fatty vacuoles of variable size were found. Phylodes tumor was excluded becauseof abscense of epithelial component.
Aditional mastectomy was done and adjuvant therapy has not been given.

P-225
Nuclear comparative morphometric study between DCIS and normal resting mammary gland tissue

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Background. DCIS is the malignant epithelial cells proliferation that affect only ducts, including lobular, without basement membrane interruption. Benign or malignant cells phenotype is concerned by nuclear appearance. Morphometric analysis could provide quantitative information about nuclear profile in several lesions. In this study we proposed to assess nuclear morphometric features of mammary epithelial cells in DCIS compared to normal resting mammary tissue.
Methode. The study comprise breast tissue from 20 women histopathologically confirmed with DCIS. Normal resting breast tissue were obtained from another 20 women surgically treated for fibroadenoma, tumor condition that associate low risk of cancer development. Nuclear morphometry was performed on H&E slides, using a light microscope Nikon E600 with a Nikon camera linked to a computer and Lucia Net Software. For each case 300 ductal epithelial nuclei were randomly selected at a magnification 400×. We assess: Nuclear Area (μm^2), Nuclear Perimeter (μm), Maxim Diameter (μm), Minim Diameter (μm), Elongation and theirs standard deviations (dst). t-Test and ANOVA were used to compared morphometric parameters between selected groups ($p<0,05$).
Results. Following microscopical exam of normal resting breast tissue, 10 cases were included in follicular phase of menstrual cycle and another 10 in luteal phase. Morphometric assessment showed insignificant size diferences between ductal epithelial nuclei in follicular and luteal phases. The means values were: Nuclear Area 23,63± 3,63 μm^2; Perimeter 17,12±1,34 μm; Maxim Diameter 6,45±0,61 μm; Minim Diameter 4,66±0,56 μm; Elongation 1,41±0,24. Microscopical features of DCIS selected for morphometric assessment were: comedo, cribriforme, solide and papillary. The means values for ductal epithelial nuclei in DCIS were: Nuclear Area 83,29±15,25 μm^2; Perimeter 32,46±2,97 μm; Maxim Diameter 12,2± 1,33 μm; Minim Diameter 8,57±1,17 μm; Elongation 1,45±0,25. Applying t-Test for all evaluated morphometric parameters we obtained $p<0,05$.
Conclusions. Morphometric assessment showed that in DCIS ductal epithelial nuclei presents pronounced polimorphism and high size levels that in normal resting mammary tissue.

P-226
Expressıon of PTEN and Survıvın Correlates wıth Apoptosıs ın In Sıtu and Invasıve Breast Cancer

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Background: During epithelial homeostasis proliferation, migration, differantiation, and apoptosis (programmed cell death) occur. Disturbance of these processes leads to tumorigenesis. Survivin is a recently identified inhibitor of apoptosis and PTEN plays an important role in arrangement of signals required for cell growing and apoptosis. In case of absence or mutations of PTEN, tumor cells can protect themselves against apoptosis by the way of lipid signal transduction.
Methods: In this study, the relationship of PTEN and survivin expression with apoptosis was investigated by the means of immunohistochemical method in 39 patients with invasive breast cancer (IBC) and ductal carcinoma in situ (DCIS) within the same breast tissue.
Results: A decrease or loss in PTEN expression was observed in an important portion in DCIS(46.7%) and IBC (71.8%). In cases with invasive ductal carcinomas, strong staining with survivin was observed in 33.3% of cases, while there was weak staining in 61.6% and no staining in 5.1% of cases. All of the DCIS, tumor cells were positive with survivin but staining was weak. Apoptotic index was higher in IBC than in DCIS, but there was no significant differences. No relationship was found between apoptosis and PTEN or survivin expression.
Conclusion: Our results suggested that a decrease in PTEN expression and increase in apoptosis be important in progression of IBC. Further studies with large case series are needed to clarify importance of PTEN and survivin in progress of IBC and their relationship with apoptosis.

P-227
Presence of basement membrane material around the tubules of tubulolobular carcinoma

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Published guidelines recommend both myoepithelial and basement membrane stains (the latter including collagen IV

and laminin) in the differential diagnosis of tubular or well differentiated ductal carcinomas versus microglandular adenosis or complex sclerosing lesions. Differentiating these lesions in core needle biopsy specimens may be difficult.

Two core needle biopsy samples are presented, both of them containing small acinar (tubular) proliferations arranged in an infiltrating pattern. A basement membrane like staining with collagen IV was seen in both cases, whereas the laminin immunostain was obviously negative. (A positive EMA and a negative S100 stain were also features against the diagnosis of microglandular adenosis.) The tumours were classified as tubulolbular carcinomas after excision.

The collagen IV staining around invasive glands of tubulolobular carcinomas (and probably other well differentiated tubule forming carcinomas of the breast) partially challenges the recommendation of using this immunostain in the differential diagnosis of benign versus malignant lesions of the breast. Knowledge of this phenomenon (the possible presence of a basement membrane like staining with collagen IV around the glands of a malignant lesion) may help in classifying some lesions as B5 (diagnostic of malignancy) instead of B4 (suspicious for malignancy) on core needle biopsy specimens.

P-228

E-cadherin expression in invasive breast cancer correlates with grade of the tumor

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Background E-cadherin is a calcium-dependent transmembrane adhesion protein, with molecular weight of 120 kDa, belonging to the type I classical cadherins family, and is localized in adherens junctions. Data from the literature support the tumor supressor activity of this protein, based on the lost of expression and function during tumorigenesis and tumour progression in different types of cancer. The aim of this study is to evaluate the expression of E-cadherin in invasive breast cancer and its correlation with tumor grade and the number of tumor metastases.

Material and methods There were investigated 22 cases of primary invasive breast carcinoma. E-cadherin expression was assessed immunohistochemically, with the monoclonal antibody (incubation with the primary anti-E–cadherin antibody clone NCH-38 was done for 30 minutes, dilution 1:75) and LSAB+ working system.. The expression of the immunostaining was considered *normal* if over 70% of the tumour cells were positive, and *aberrant* if less than 70% were positive.

Results Normal glandular structures showed an intense positive immunostaining, with membrane pattern, both in luminal and basal cells. From the invasive breast carcinomas, 45.5% (n=10) have presented normal reaction, whereas 54.5% (n=12) have presented aberrant reaction. The percent of cases with aberrant expression of E-cadherin decreases along with growth of the tumour grade. Thus, 100% of the tumors with G1 grade showed aberrant expression, which decreases to approximately 44.5% for G2 and to 37.5% in G3 tumors. The tumors with less than 2 cm in diameter had aberrant expression in 83.3% of the cases, and the percent decreased to 56.25% in the case of the tumors with over 2 cm in dimensions.

Conclusions E-cadherin immunohistochemical expression decreases in ductal invasive breast cancers as compared with normal mammary tissue. The percent of the cases with aberrant expression of E-cadherin decreases with the increase of the tumor grade.

P-229

A survey in EBV genome frequency in breast cancer of iranian population

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Background: Breast cancer is the frequently diagnosed malignancy of women & is the second cause of death from cancer in women. As the etiology & progression of breast cancer remain incompletely understood, novel mechanism of disease pathogenesis is needed to be considered. Recent interest has focused on Epstein-Barr virus (EBV) an oncogenic herpes virus. Investigation of this association could not only broaden understanding of breast cancer etiology but also have implications regarding early detection, treatment and prevention. Studies of an association of this virus with breast cancer have had notably inconsistent results, marked by varying EBV presence from nil to 50% of tumors.

Objective: To assess EBV presence in breast cancer of Iranian series & investigation of this frequency with some tumoral indexes or some factors relevant to the patients.

Method: To evaluation of the presence of LMP genome of EBV by PCR on paraffin embedded blocks of 100 Iranian females with breast cancer.

To differentiate EBV in tumor cells from EBV in background immunocytes, on positive cases, we did microdissection & PCR, and then we investigated this finding with different factors.

Results: Expression of LMP genome was observed primarily in 8 of 100 cases & it was positive in 6 cases after microdissection.
No significant association was observed EBV expression & some tumoral index such as morphology, histological grade, lymph node involvement, skin & perineural & vascular invasion, tumoral necrosis, & some index included ER,PR,P53,Her-2 markers($p<0.005$).
It seems this is due to low frequency of this genome in the cases of breast cancer in Iran.

P-230

Vimentin, p-cadherin and basal type cytokeratins expression in invasive breast carcinoma.

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Background: Basal-type breast carcinomas defined by the CK5/6, CK14 and CK17 expression and ER and HER2 negativity are related with poorer outcome. There were suggestions that these tumours more often present vimentin and p-cadherin expression and these antigens characterise high grade and ER-negative tumours.
Method: We investigated the expression of vimentin, p-cadherin, cytokeratins: 5/6, 14, 17 and ER, PGR, HER2, Cyclin E and Ki67 in 193 invasive ductal breast cancer by immunohistochemistry, to find out the relationship among vimentin and p-cadherin expression and basal cell phenotype. We check also association of analysed antigens with clinical and morphological factors.
Results: Forty one (21,2%) cases expressed vimentin, whereas 111 (57,5%) tumours were p-cadherin-positive. Expression of CK5/6 showed 66 (34,2%) tumours, 48 (24,9%) were CK17-positive and 48 (24,9%) CK-14 positive. Expression of vimentin showed 32 (45,7%) cases from the group of "CK5/6 or CK14 or CK17" - positive tumours and 9 (7,4%) remaining tumours ($p<0,001$). P-cadherin was positive in 73,2% and 48,4% ($p<0,001$) cases accordingly.
Conclusions: P-cadherin and vimentin expression are more often present in the group of basal cytokeratin positive tumours, but can not be regarded as sensitive markers of basal-type tumours.

P-231

Metaplastic breast carcinoma. a clinico-pathologic review of 24 cases

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INTRODUCTION Metaplastic breast carcinoma (MBC) is characterized by an admixture of adenocarcinoma and metaplastic elements, wich can be homologous or heterologous. MBCs account for 1–3.7% of breast carcinomas. Our aim was to analyse the clinico-pathological features of a series of MBCs.
MATERIAL AND METHODS MBCs diagnosed between 1996 and 2007 were reviewed. Patient's age, size, tumor type, border appearance, presence of necrosis, osteoclast-like cells and lymph node status were assessed. Immunostains for oestrogen (ER) and progesterone receptors (PR), HER2, p53, p63, CK5–6, EGFR, vimentin, smooth muscle actin (SMA), bcl-2 and CD10 were performed.
RESULTS Twenty-four cases were found. The age ranged from 31 to 94 years (mean 47.8). By ultrasonography, the most part of tumours were lobulated. MBC was diagnosed or suggested by cytology and/or biopsy in 10 cases. 41.6% were grossly expansive. The mean size was 50.1 mm. Lymph node metastasis were seen in 41.6% of cases. 33% of patients presented recurrence between 2 and 37 months after surgery.
13 of 24 cases (54.1%) were ductal adenocarcinoma with squamous metaplasia, 12,5% pure squamous carcinoma, 12.5% with spindle differentiation, and 20.8% had condroid elements. Osteoclast-like giant cells were seen in 33.3% of cases.
62.5% of cases were positive for p53, 79.2% for p63, 50% for bcl-2, 95.8% for CK5–6, 41.7% for EGFR, 87.5% for vimentin, 16.7% for SMA and 54.2% for CD10.
21 of 24 tumours (87.5%) were negative for ER, PR, and HER2. Of these, 33% expressed EGFR, 95.8% CK5–6, 76.1% p63 and 61.9% expressed CD10.
CONCLUSIONS Most of MBC have lobulated, expansive margins.
The most frequent component is squamous metaplasia.
A high percentage are triple negative tumors. Most of them express basal and myoepithelial markers.
Recurrence is observed in 33% of cases in a shorter interval than conventional ductal adenocarcinoma.

P-232

Radiation impact on breast carcinoma after chernobyl accident

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Background: The accident at the Chernobyl nuclear power plant in 1996 caused radionuclides release over large areas. Radioactive Caesium-137 has a half-life of 30 years and Strontium-90 has a half-life of 50 years. In the atmosphere were also spread vapors of Iodine-131 and Plutonium-239. These radionuclides are incriminated in increase numbers of breast, thyroid, urinary cancer and leukemia.
Method: Statistical study of breast carcinoma cases new diagnosed between 1986- 2006, in Morphopathology Department of County Hospital, Constanta, following a few parameters: sex, age, tumor stage, microscopic features.
Results: From our study resulted an increasing number of new diagnosed breast cancer between 1986 - 2006 (144 cases in 1986 to 204 cases in 2006), this increase being due to life conditions change, new diagnose methods and to latency period needed for genetic mutations provoked by post-Chernobyl emission of radiations to accumulate. The study showed anticipation phenomena with a lot of new cases diagnosed between 30–50 years (20 cases in 1986 to 56 cases in 2006). Anticipation phenomena observed may be due to radiation level but further studies are needed. For this period the number of breast cancer diagnosed in first stages increased (64 cases in 1986 to 160 cases in 2006). Number of breast cancer with mixed histological features increased between 1986 -2006 (16 cases in 1986 to 100 cases in 2006). Predominance of breast cancer in female patients maintains in this period but from 1986 to 2006 had been diagnosticated cases of breast cancer in man patients.
Conclusions:

- Breast cancer is continuously increasing because radionuclides half-life.
- In breast cancer cases evolution it is observed an anticipating phenomena.
- Radiations determined an increasing in number of mixed histological features of breast cancer specimens.
- Cases of breast cancer in man are more frequent.

P-233

BREAST CANCER STEM CELLS RESIST APOPTOSIS THROUGH THE REGULATION OF BCL2

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Background: cancer stem cells arise from their normal stem cell counterparts that undergo accumulation of genetic changes until the cells acquire a malignant phenotype. Breast epithelial stem cells are the primary targets in the etiology of breast cancer. The capacity of breast tumour development has been shown to be restricted to CD44+/CD24$^{-/low}$ epithelial tumour cells. Identification of breast cancer stem cell is important because of its implication on development of new therapeutic strategies. Stem cells can resist apoptosis through some mechanism such as the regulation of Bcl2.
Methods: The present study included 150 primary operable breast cancer patients with a median age of 46 years, in order to investigate the prevalence of cancer stem cells. Iimmunohistochemistry was applied to identify the population of CD44+/CD24$^{-/low}$ cells in the paraffin embedded tissues. The prevalence of cancer stem cells are then correlated with level of expression of anti-apoptotic protein Bcl2, Estrogen Receptor (ER), Progesterone Receptor (PR), also with prognostic factors including tumour size, lymph node stage, differentiation and metastasis.
Results and conclusion: Univariate analysis showed a significant correlation between higher level of expression of Bcl-2 and smaller tumour size (P=0.001), and lower grade of breast tumours (p=0.007). There was a strong correlation between the presence of Bcl-2 and the presence of ER and PR (p=0.042). Moreover, a positive correlation was found between CD44 and Bcl2 expression; i.e. CD44+ cells express higher levels of Bcl2, therefore can resist apoptosis. Anticancer therapy including chemotherapy normally target non-tumorigenic cells in tumour, while cancer stem cells are still survive and leading to tumour recurrence. Therefore, targeting this population of cells which are more resistance to apoptosis in combination with current treatments will be more effective in breast cancer patients.

P-234

Comparison of diagnostic accuracy of Triple Test Score with pathologic findings of breast masses

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Introduction:palpable masses of breast are common and usually benign.Combination of physical examination, imaging and Fine Needle Aspiration (FNA) for diagnosis of malignant or benign breast masses are useful.
The Aim: comparison of accurate diagnosis in combination of physical examination, imaging, FNA(Triple Test Score) with pathologic findings that obtained from biopsy or mastectomy of breast masses
Material and Method: 100 women with different ages with breast mass that physical examinations were performed, devided in to 3groups:

Scor = 1 for benign cases, Score=2 for doubtful cases,and Score =3 for malignant.Then these patients were sent to imaging and were given scores on basis of benign, doubtful and malignant. Finally FNA were done and prepared smears,and were stained by Papa and Geimsa.Then pathologic results of biopsy and mastectomy are compared with findings of TTS.

Result: 100 patients were studied by physical examination, imaging and FNA. In this survey,63cases were 3–4 points in TTSscoring, and all of them were benign,and 32 cases which were TTS> or =6, in the final diagnosis were malignant.Therefor, TTS had 100% speciality and 100% sensitivity in diagnosis of breast masses.

Coclusion: the TTS was 100% accurate in the diagnosis of palpable breast masses when all three elements were concomitant.

Key words: Fine Needle Aspiration, Imaging, Palpable masses.

P-235
HER-2/NEU IN BREAST CANCER. IMMUNOHISTOCHEMICAL COMPARISON USING MONO AND POLYCLONAL ANTIBODIES. HYBRIDIZATION IN SITU CHROMOGENICA (CISH) AMPLIFICATION.

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Breast cancer is placed as the fourth most common cancer pathology in our region, with a standard rate of 20.4 per 1000 000 women. There are prognostic and predictive indicators in breast cancer. An available molecular marker is Proto oncogen Her-2/neu. The aim of the present work was to determine by means of immune histochemistry (IHC) with monoclonal and polyclonal antibodies the over expression of the protein. Genes of patients 2+ and 3+ were amplified using in situ chromogenic hybridization CISH).

Methodology: 246 patients diagnosed with breast cancer were evaluated with IHC and CISH panels. Data obtained were related to age, histological type, histological grade and lymph node metastasis.

Results: 96.3% of the results were infiltrated ductal carcinoma; from this group 80.9% were NTH II. Age prevalence was 40 – 49 years old, Of the cases studied. 67 patients (27.2%) expressed the protein by means of polyclonal antibodies (AO485), 70 cases (28.5%) used monoclonal antibodies (CB11). Amplification by CISH was 24% and 23.58%, respectively. Polyclonal antibodies sensitivity was 98.33%, with a specificity of 50% and monoclonal antibodies sensitivity was 96.97%, with a specificity of 25%. The group of patients submitted to MRM (38) with node metastasis, 28 amplified the gene (73.68%). Sensitivity and specificity of CISH 3+ were 100%.

Conclusions: Expression of Her-2 protein by IHQ for both monoclonal and polyclonal antibodies is a high sensitive technique, being less specific to monoclonal antibodies. Based in our results, we recommend CISH as a genetic expression validation test in 2+ cases.

P-236
DEDIFFERENTIATED ADENOID CYSTIC CARCINOMA OF THE BREAST WITH AGGRESSIVE BEHAVIOUR.

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The present paper describes a case of adenoid cystic carcinoma (AdCC) of the breast with dedifferentiated features and aggressive behaviour. A 63-year-old woman presented a 4 cm mass of the right breast. On histology, the tumour showed an in situ and an invasive component, both with typical features of AdCC intermingled with areas of solid, poorly differentiated carcinoma. The poorly differentiated areas loose markers of myoepithelial differentiation, while were diffusely positive for low weight cytokeratin. At the genetic point of view, the Neighbor Joining tree derived from mtDNA D-loop sequence analysis showed that areas of typical AdCC and dedifferentiated were similar. Furthermore both components revealed some genetic alterations in common identified by aCGH (amplifications in 5q, 6p22, 8q24, 19p13.2, 21q22.3). Additionally dedifferentiated areas showed three peculiar deletions in 2q21.1, 6q25, Xp11.22 and six peculiar amplifications in 6p21.33, 9q34.3, 11p15.5, 11q13.1, 14q32.3, 16q13.

One axillary node metastasis was evident at presentation. Presently, six years after surgery the patient is alive with bone and lung metastasis.

AdCC of the breast even if presenting solid areas is usually associated to good prognosis. The present case differs from those previously reported as showed an overgrowth of a dedifferentiated epithelial component similarly to dedifferentiated AdCC of salivary glands. These features should be better known also in breast as associated to more aggressive behaviour.

P-237

Malignant transformation of breast adenomyoepithelioma

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Background: Breast adenomyoepitheliomas are rare tumours composed by a double cell population. They are usually considered benign, although they can recur locally.
Case report: We present the case of a 54 year-old female whose radiological study reveals two polilobated cystic nodules in upper outer quadrant and central region of right breast. These two nodules are resected.
Results: Histological study of the two lumpectomy specimens shows a double component, epithelial and myoepithelial. Thus, they are diagnosed of adenomyoepitheliomas with stromal overgrowth. Besides, resection margins are affected, so the patient is reoperated. In the specimen of re-excision of margins malignancy is found, in the way of myoepithelial carcinoma growing from adenomyoepithelioma. Then, mastectomy is performed and multiple adenomyoepitheliomas are seen all over breast parenchyma. Seven months after surgery the patient presents two subcutaneous nodules in the surgical area, which consist in two foci of myoepithelial carcinoma infiltrating major pectoralis muscle. Six months after treatment, there is no recurrence.
Comments: Myoepithelial carcinoma growing from adenomyoepithelioma is rareness. Histological features that indicate malignancy (atipia, necrosis, high mitotic index and infiltration) are more frequent in recurrences. Although myoepitheliomas are considered benign, there is a rank of behaviour that includes local recurrence, malignization and rarely distant metastases.

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Correlation between pathological features of breast tumors and stem cell content as measured by sphere formation ability

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Background Breast cancer stem cells have been defined as $CD44^+CD24^-Lin^-$ cells, however only about 20% of this population has the ability to recontitute the original tumor. Therefore, a further definition of breast cancer stem cells is required for its use in the clinic as prognostic criteria. By using the unique ability of stem cells to grow as floating spheres in culture we aim to identify and quantify the presence of stem cells in tumor sprecimens and correlate with clinical, phenotypic and immunohistochemical features of the tumor.
Methods We analyzed 60 breast carcinomas diagnosed at Instituto Oncologico in 2007, for which clinico-pathological data was collected. These data includes age, treatment (when applicable), size, grade, histological type, estrogen and progesterone receptors, Her2 and Ki67 expression. A fragment homogeneus for tumor tissue content was used to isolate tumor cells by enzimatic digestion and single cells cultured on non-adherent conditions to allow for sphere formation. Spheres were counted after 10–14 days in culture, time when they were disaggregated to demonstrate presence of cancer stem cells.
Results & Conclusion We have used the number of spheres formed as representative of the content of stem cells in the original tumor (SFU, sphere forming units). While only 30% of the tumors were capable of forming spheres in vitro, irrespective of the number of cells isolated, we found association between the ability to form spheres and several clinico-pathological features of the tumor, such as Her2. This association suggests that the content of stem cells may be of prognostic value. Furthemore, the identification of tumors with highest stem cell content will make possible the identification of specific stem cells markers for use in breast cancer prognosis by directing the search to those tumors.

P-239

Microvessel density in breast cancer patients with polymorphism in the endostatin gene

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Introduction: Angiogenesis is an important step in breast cancer (BC) development and progression. In addition to producing proangiogenesis cytokines, neoplastic cells also play a role in the generation of endogenous antiangiogenic proteins, including endostatin (ES). ES is a terminal fragment of collagen XVIII, the product of the *COL18A1* gene. The *COL18A1* gene polymorphism (D104N) in homozygosis is associated with an increased risk for sporadic breast cancer (SBC). Microvessel density (MVD) is the most commonly used technique to quantify intratumoral angiogenesis in BC. **Aims:** To test whether D104N polymorphism altered MVD in BC patients. **Patients and Methods:** Genomic DNA was obtained from peripheral blood samples from 84 untreated female patients with SBC and was analyzed using the polymerase chain reaction followed by restriction endonuclease digestion with *Mse*I, to evaluate the presence of D104N polymorphism. The

MVD of formalin-fixed paraffin-embedded tissues of all patients was determined in CD34-immunostained sections using the Imagelab software for morphoanalysis. Statistical significance of the median intratumoral MVD differences between groups was calculated by Kruskal-Wallis and Wilcoxon tests. **Results:** The median intratumoral MVD was different when we compared the patients with wild-type (DD), heterozygous (DN) and homozygous (NN) genotypes (19.00, 12.83 and 28.66, respectively; $P=0.02$). Surprisingly, there were no significant differences between the median intratumoral MVD in DD plus DN, in comparison with NN patients (18.50 *vs* 28.66; $P=0.08$). **Conclusion:** It was not possible to conclusively determine any association between D104N polymorphism in *COL18A1* gene with the degree of angiogenesis in BC. (Supported by FAPESP and CNPq).

P-240

Thymoma mimicking a primary breast cancer

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Background. Most tumours of the breast originate in mammary tissue. Metastases to the breast mimicking a primary breast cancer are uncommon and direct invasion from a tumour located to the thorax is rare. We report a case of a thymoma which was diagnosed on a core biopsy of a breast mass.

Case report. A 83-year old woman presented with a palpable polycyclic 3 cm large mass in the UEQ of the left breast. Her medical history was significant for an asymptomatic mass of the anterior mediastinum, incidentally detected on radiographic imaging 12 years ago, but never histologically documented. Histological analysis of breast core biopsy showed a mixture of small ovoid or spindle-shaped cells with clear cytoplasm, bland cytologic features and trabecular or hemangiopericytoma-like growth pattern and small round basophilic cells. The clear cells were immunoreactive for pan cytokeratin, CK7, CK5/6, and p63, with a very low Ki-67 index. The basophilic cells expressed CD3, CD1a, TdT and had a Ki-67 index of 100%. The breast tumour was diagnosed as an extension of a mediastinal type AB thymoma (WHO classification). TDM confirmed the presence of a large intrathoracic mass occupying most of the left pleural space.

Discussion. Without knowledge of the past history of the patient, a solid variant of lobular carcinoma, a myofibroblastoma or a myoepithelial tumor would have been considered. Stage III thymomas may invade surrounding organs such as lung, pleura or pericardium. Rare cases of thymoma with endobronchial growth or transdiaphragmatic extension to the liver have also been described, but to our knolwledge direct invasion of the breast mimicking primary breast cancer has never been reported.

Conclusion. In case of histologic features unusual for a primary breast tumor, the possibility of metastasis to the breast, but also of direct extension of an advanced thymoma should be considered.

P-241

P53 and c-myc in advanced breast cancer. a tma immunohistochemical evaluation.

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Background - *c-myc* and p53 genes were frequently deregulated in invasive ductal carcinomas of the breast (IDC), but *c-myc* role in mammary carcinogenesis remains controversial.

Method - To determine if the concomitant expression of the two oncogenes might have prognostic value, stage, a retrospective series of 40 samples of lymph nodes with metastasis; hormone receptor status and survival of 80 consecutive IDC patients and clinical data were analyzed in the light of p53 and *c-myc* expression assessed by immunohistochemistry in a TMA block.

Results - Positive *c-myc* and p53 staining was detected in neoplastic epithelial cells respectively in 89% and 40% of the cases. Respective lymph nodes expressed *c-myc* in 38/40 cases and p53 in 12/40. Concomitant p53 and *c-myc* positive immunolabelling in lymph nodes emerged in 12 out of the 80 cases. Clinicopathological parameters and overall-survival of patients were not associated with the immunoreactivity of p53 or *c-myc* considered separately or grouped in subsets.

Conclusion - p53 and *c-myc* expression in tumor or compromised lymph nodes were not capable to predict outcome ou correlate to prognostic variables in advanced breast cancer.

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Cytokeratin 5 as a prognostic marker in invasive ductal carcinoma

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Background - Cytokeratin (CK) 5 is a marker of basaloid carcinoma in breast cancer. CK expression is considered a primordial cell feature and related to worse prognosis. To evaluate this hypothesis we assessed CK5 immunohistochemical expression in 80 advanced invasive ductal carcinoma (IDC) and correlated to classical clinicopathological prognostic variables.
Method - In a retrospective series of 80 T2-T4 NxMx IDC samples of invasive tumor and 40 compromised lymph nodes were selected to construct a Tissue Micro Array. Immunohistochemical essay with CK5, Estrogen receptor (ER), Progesterone receptor (PR), c-erbB2, MIB-1 and p53 were performed in an ABC streptoavidin method. More that 10% of stained cells were considered positive. Chi-square correlations with T-Test were performed with SPSS statistical software.
Results - CK5 staining were positive in 14 cases, negative in 63 and missing in 3. Positive cases correlated to CK expression at the lymph node (p=0,003); histological grade (p=0,03); lymph node status (p=0,01) and p53 status (p= 0,02). Proliferation, hormone receptors and c-erbB2 status did not correlate to CK5 expression.
Conclusion - We found that CK5 reflects important prognostic variables and has a potential value as a predictive marker in advanced IDC.

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Immunohistochemical assessment of axilar positive lymph nodes in breast cancer patients.

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Background - Lymph node (LN) metastasis is considered a worse prognosis feature in invasive breast cancer (IBC). However, little is know about the biological behavior of metastatic neoplastic cells compared to primaty tumor source. Objetive to evaluate the immunohistochemical profile of LN metastasis of advanced invasive ductal carcinoma (IDC) compared to primary tumor by Immunohistochemical method.
Method - In a retrospective series of 80 T2-T4 NxMx IDC samples of invasive tumor and 40 compromised lymph nodes were selected to construct a Tissue Micro Array. Immunohistochemical essay with CK5, Estrogen receptor (ER), Progesterone receptor (PR), c-erbB2, MIB-1, *c-myc*, EGFR, c-KIT and p53 were performed in an ABC streptoavidin method. More that 10% of stained cells were considered positive. Chi-square correlations with T-test were performed in SPSS statistical software.
Results - c-KIT was negative in IDC and LN samples. *c-myc* was positive in 89% of IDC and 80% of LN samples. CK5 positive cases correlated to CK5 expressionl at the lymph node (p=0,003); p53, c-erbB2 and EGFR IDC expression did reach a positive correlation to LN.
Conclusion - We conclude LN metastatic cancer does not necessarily develop the same primary IDC profile, with may reflect the polyclonal feature of neplastic cells. Luminal and basaloid cells managed to maintain the same similar markers as ER and CK5 in IDC and LN.

P-244

C-erbB-2 amplification in Turkish patients with invasive ductal carcinomas of the breast

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Aim: The aims were two-fold: 1) To determine the frequency of the immunoexpression and amplification of c-erbB-2 inTurkish patients with invasive ductal carcinoma of the breast, and 2) to search the correlation of the estrogen receptor (ER), progesterone receptor (PR), tumor histologic grade, stage andamplification of the c-erbB-2 in these cases.
Material - Method: Onehundred thirty three invasive ductal carcinomas of the breast have been studied for c-erbB-2, by streptavidin - biotin horseradish peroxidase method and FISH technique. The correlations with the histopathological prognostic parameters as tumor stage, grade, lymph node status, hormonal status, and c-erbB-2 amplification have been studied.
Results: Of the 133 tumours 14% (19/133) were 3+, 9% (12/133) 2+ for c-erbB-2 by immunohistochemistry. Eight of12 c-erbB-2 equivocal (2+) cases demonstrated amplification by FISH. Overall, c-erbB-2amplification found to be 20,3% of the cases. 33% (9/27) of the c-erb-B2amplified cases were grade 3, 67% (18/27) were grade 2. While 67% (18/27) ofthe cases were stage II, 26% (7/27) of the cases were stage I. 7,4% (2/27) ofthe cases were stage III. ER /PR negativity was found to be 63% (17/27) of the c-erbB-2 amplified cases.
Conclusion: In the recent study the frequency of the amplificationhas found to be 20,3% for c-erbB-2 in Turkish patients with invasive ductal carcinomas ofthe breast. c-erbB-2 amplification apppears to be correlated with ER / PR negativity.

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CORRELATION OF CD24, CD44 AND GALECTIN-1 EXPRESSIONS WITH TUMOR DIFFERENTIATION AND POTENTIAL LYMPH NODE METASTASIS IN INFILTRATING DUCTAL CARCINOMA OF BREAST

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Background: Breast cancer is one of the most common cancers in women and infiltrating ductal carcinoma constitutes the most common type. Axillary lymph node metastasis is found in 40–50% of the cases. Many immunohistochemical markers that may reflect the nature of the tumors have been used to establish early diagnostic methods, new targets for therapeutic strategies, and predictive parameters. In this study, the correlation of CD24, CD44 and Gal-1 expressions in infiltrating ductal carcinoma with tumor differentiation and potential lymph node metastasis as well as clinicopathologic parameters such as age, localization, tumor size, lymphovascular invasion was investigated.
Method: The study group consisted of 40 cases of infiltrating ductal carcinoma diagnosed in the Pathology Department of Zonguldak Karaelmas University School of Medicine between 2001 and 2007. Slides from each case were reevaluated in terms of grade, lymhovascular invasion and lymph node metastases and immunohistochemistry for CD24, CD44 and Gal-1 were performed.
Results: CD44 had no statistically significant correlations. There were significant correlations between tumor grade and size ($p=0.030$) as well as between lymphovascular invasion and lymph node metastasis ($p=0.011$). CD24 immunoreactivity correlated with lymph node metastases ($p=0.012$) and grade ($p=0.05$). Statistically significant associations were also observed between Gal-1 immunoreactivity and the number of metastatic lymph nodes ($p=0.05$); between Gal-1 expression in primary tumor cells and in metastatic tumor cells ($p=0.004$); and between Gal-1 expression in stromal cells in the primary tumor and in stromal cells in the metastatic foci ($p=0.003$).
Conclusion: The current study suggests that CD44 has no predictive value in infiltrating ductal carcinoma of breast. On the other hand, CD24 expression seems closely related with tumor differentiation and metastatic potential of tumors. Importantly, increased Gal-1 expression in human breast carcinoma tissues underlies the potential key role of Gal-1 particularly in tumor-stroma interaction.
Key words: CD24, CD44, Galectin-1, Breast carcinoma, Immunohistochemistry

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SENTINAL LYMPH NODE BIOPSY IN BREAST CARCINOMA

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Aim: Sentinal lymph node (SLN) procedure enables selective targeting of the first draining lymph node where the initial metastases will form. A negative SLN predicts the absence tumour metastases of the other regional lymph node with high accuracy. The aim of the study was to search the reliability of intraoperative frozen section investigation of the SLN to detect metastases.
Material and Method: Ninety three SLNs were detected by gamma probe detection of nanocolloid and visual accumulation of patent blue in breast carcinomas. The identified SLNs were immediately investigated by frozen section and imprint cytological investigation. Diagnosis were confirmed on paraffin material, and in case of negative frozen sections and hematoxylin and eosin and immunochemistry were performed.
Results: While 66 SLNs (70,96%) were negative for metastasis, 17 (18,27%) of the cases contained metastesis by the frozen section procedure. Ten cases (10,75%) were false negative as they were found to be metastatic LNs on the paraffin sections. While the sensitivity of the frozen section procedure was found to be 62,9%, spesivity of the procedure was 100%. Mong the SLN positive cases 37% (10–27) were T1N1 and 51,8% (14–27) were T2N1. 77,7% (21–27) of the positive SLNs were estrogen and progesterone positive.
Conclusions: Intraoperative frozen section analysis is a reliable procedure by which a high percentage of SLN metastases can be detected in breast cancer patients without false negative results. In up to 10% of the cases the final paraffin sections will reveal micrometastases that were not detected by the frozen section and in these patients axillary lymph node dissection will have to be performed in a second session.

P-247
The relation of COX-2 expression with other prognostic parameters in invasive ductal carcinomas

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BACKGROUND: Cyclooxygenase (COX) is an enzyme catalysing the conversion of arachidonic acid to prostaglandins. The inducible form cox-2 is known to be essential in promotion of carcinogenesis, invasion and angiogenesis. In studies, cox 2 overexpression is observed in about 40% of human breast cancers. This overexpression is reported to be related with especially c erb B2 overexpression. The aim of this study is to investigate the relation between clinicopathological parameters such as histological grade, metastasis, ER, PR, c erb B2 and cox 2 expression in invasive ductal carcinomas.
MATERIAL&METHODS: Cox 2 expression is examined immunohistochemically by using peroxidase antiperoxidase method in slides prepared from paraffine blocks of 62 invasive ductal carcinomas patients. Data on other clinicopathological parameters, c erb B2 expression and hormonal status (ER, PR) are obtained from patient files.
RESULTS: Cox 2 expression was present in 47 of 62 cases (75.81%). There was a negative correlation between Cox 2 expression and estrogen receptor positivity and a statistically significant result was obtained ($p<0.008$). However, a statistically significance was not found between histological grade, progesteron receptor positivity and c erb B2 expression ($p>0.005$).
CONCLUSION: Regarding the statistically significant relation between cox 2 expression and estrogen receptor, cox 2 expression in breast carcinomas should be considered as a poor prognostic parameter.

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Analysis of the Pathologic Response to Neoadjuvant Chemotherapy (NC) in Patients with Locally Advanced Breast Cancer (LABC), according to Hormonal Receptors (ER/PR) and ERBB2 Status.

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PURPOSE: Gene expression analysis identifies several breast cancer subtypes. We examined the relationship of NC response to outcome among these breast cancer subtypes, defined by immunohistochemical profile (IP) as surrogate of molecular classification. EXPERIMENTAL DESIGN: We used IP (ERBB2+ and ER/PR- for HER2+ type; ERBB2-and ER and/or PR+ for luminal type; ERBB2- and ER/PR- for basal-like type; and ERBB2+ and ER and/or PR+ for HER2+ plus hormone receptor (HR)+type) to subclassify a prospectively maintained series of patients treated with neoadjuvant anthracycline-based (doxorubicin plus cyclophosphamide) chemotherapy. We analyzed each subtype for pahtologic response to NC (evaluated by Miller&Payne system, G1-G5 from no response to complete pathologic response) and examined the relationship of pathologic response to overall (OS) and disease-free survival (DFS). RESULTS: Of the 117 patients tested, 23 (20%) were basal-like; 55 (47%) were luminal; 20 (17%) were HER2+; and 19 (16%) were HER2+/HR+. Pathologic complete response (G5) occurred in 50% of HER2+, 39.1% of basal-like, 31.6% of HER2+/HR+ group and in 7.3% of luminal type (p

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Tumors initiated by constitutive Cdk2 activation contain an invasive basal component

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Background: The basal-like subtype of breast cancer is associated with invasiveness, high recurrence rates, and poor prognosis. Aside from inactivation of the *BRCA1* tumor suppressor gene, little is known concerning the mechanisms that cause basal breast cancer or the mechanisms responsible for its invasiveness.
Method: Cell lines were isolated from MMTV-CyclinD1-Cdk2 transgenic tumors. These cell lines were examined for the expression of luminal and myoepithelial differentiation markers by immunoblot. The cell lines were injected into the mammary fat pads of wild type syngeneic FvB mice to form tumors. The mechanisms responsible for the invasiveness of the tumors and lack of cell-cell cohesion were investigated by immunohistochemical analysis (IHC) of the tumors and immunoflourescence (IF) analysis of cells grown *in vitro*.
Results: Heterogeneous MMTV-Cyclin D1-Cdk2 (MMTV-D1K2) transgenic mouse mammary tumors contain regions of spindle-shaped cells expressing both luminal and myoepithelial markers. Cell lines cultured from these tumors exhibit the same luminal/myoepithelial "mixed-lineage" phenotype that is associated with human basal

breast cancer, and express a number of myoepithelial markers including Cytokeratin 14 (CK14), P-Cadherin, -Smooth Muscle Actin, and Nestin. The cell lines form highly invasive tumors when injected into mouse mammary glands. Invasion is associated with E-Cadherin localization to the cytoplasm, or loss of E-Cadherin expression. E-Cadherin localization to the cytoplasm correlates with lack of colony formation and β-Catenin and $p120^{ctn}$ localization to the cytoplasm. The MMTV-D1K2 cancer cell lines also express the proinvasive proteins Nestin and Zyxin.

Conclusion: Together the results suggest that constitutive Cdk2 activation may contribute to the formation of basal breast cancers, provide explanations for the invasive behavior of basal breast cancers, and indicate that it may be possible to suppress tumor invasiveness by restoring cell-cell adhesive functions pharmacologically.

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TOP2A - EGFR correlations in triple-negative (TN) breast cancer

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Background. The basal type breast cancer as identified by gene profiling expresses immunophenotypically, basal cytokeratins and EGFR. It is negative for ER, PGR, and Her2/neu proteins, and has an agressive biologic behavior. Topoisomerase II alpha (TOP2A) is involved in the anthracyclins responsivity. EGFR is a basal type marker and a molecular therapeutic target. We studied the EGFR expression in TOP2A positive TN and nonTN invasive ductal carcinomas (IDC), as a possible associated therapeutic target.

Method. We analyzed retrospectively 102 consecutive IDC positive for TOP2A (files of "Victor Babes" Institute), tested also for ER, PGR (Novocastra), cerbB2, Ki67, p53 (Dako), and for EGFR (Dako). The TN tumors were selected. The IHC bistadial indirect technique was performed using producer's development kit.

Results. From 102 breast IDC (G2 and G3) (age range: 28–83 years), 18 TN cases were founded (only 4 cases aged under 45 years). Top2A was positive in: 1 to 50% of tumoral cells in TN and 1 to 30% in nonTN tumors. The EGFR positivity was localized in tumoral cells (4/14 TN and 16/81 nonTN tumors tested), but also in vascular endothelium (3/14 TN and 18/81 nonTN tumors tested). The EGFR positivity in tumoral cells was associated with absence of cerbB2 in all 4 cases of TN tumors and with a high percentage (>20%) of Ki67 and p53. The EGFR positivity in nonTN tumors was associated in 13 cases with cerbB2 negativity, but only in 2 cases, with a high percentage of Ki67 and p53.

Conclusion. In TOP2A positive selected cases, the patients' age is higher than ussually for TN tumors. The associated TOP2A/EGFR positive tumoral cells appear in cerbB2 negative cases, but with a high percentage of positivity for Ki67 and p53, known as poor prognosis markers. The association between TOP2A and EGFR could represent a combined therapeutic target.

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Insulin-like Growth Factor 1-Receptor (IGF1R) activity and mRNA expression levels in early breast carcinoma with local recurrence

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Background. The IGF1R regulates cell survival signals and apoptosis mainly through the PI3K/Akt signaling cascade. Whether the receptor activity or mRNA levels have an impact on clinical outcome in patients with early invasive breast carcinoma (IBC) undergoing breast-conserving surgery (BCS) and radiation therapy (RT) is not well known.

Method. Our series of 197 eligible BC patients in stage I-II treated with BCS and RT, included 33 (16.8%) who developed a local recurrence (LR) and 16 (8.1%) with distant metastasis. Median follow-up was 100 months. Clinical and pathological features were assessed. Immunohistochemistry (IHC) for the phosphorylation of IGF1R beta-subunit (active form), Estrogen and Progesterone receptors (ER/PR) were performed. In addition, in tissue samples from 85 primary BC (42 without recurrence, 31 with LR and 12 with distant metastasis) and 31 LR, we evaluated the IGF1R mRNA expression by quantitative real-time PCR (qRT-PCR). The relationship between pathologic, IHC and qRT-PCR results were studied and correlated with the outcome.

Results. LR was significantly associated with high grade, PR-negative, lower expression of p-IGF1R (all $p>0.05$) and as a trend with extensive *in situ* component (EIC) ($p=0.06$). However, there were no differences regarding the mRNA levels ($p>0.05$; Kruskall-Wallis test). For local disease free survival, histologic grade, EIC, PR and p-IGF1R emerged by multivariate analyses as significant breast relapse predictors (all $p\leq0.05$; Cox regression analysis).

Conclusion. Patients with early BC treated with BCS and RT, those with tumors showing lower p-IGF1R and PR-negative expression, high histologic grade and EIC developed more frequently LR. Therefore, the IGF1R active form content is a favourable prognostic indicator.

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CGH Analysis of Basal-Like Type of Breast Cancer. Comparison with Chromosomal Imbalance Pattern in HER2+ Type.

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PURPOSE: The most consistent immunophenotype seen in the basal-like tumours of the breast, identified by gene-expression profile, is negativity for hormone receptors and HER2 (triple-negative type), and positivity for vimentin, EGFR, cytokeratin 8/18, and cytokeratin 5/6, with variable expression of myoepithelial markers (SMA, p63, CD10). We have investigated whether this group of breast cancers has a distinguishing pattern of genetic alterations, defined by CGH. EXPERIMENTAL DESIGN: CGH performed on frozen material from 12 cases of breast cancer triple-negative type. Immunophenotypic profile evaluation with cytokeratin 5/6, cytokeratin 17, EGFR and p63. Comparison of the results with the chromosomal imbalance pattern observed in a series of 10 cases of HER2+ breast cancer (previously reported). RESULTS: Of the 12 cases, 10 (80%) showed positivity for EGFR and for cytokeratin 5/6 and/or cytokeratin 17. CGH found gains at 1q (91.6%), 8q (75%), 3q, 7q (66.6%) and 1p, 6q, 11q, 12p, 2q (50%); and losses in 17p (75%), 19p (66.6%), 1p (58.3%) and 8p, 16p, 17q (41.6%). CGH in HER2+ group showed gains in 17q (90%), 1q, 8q (80%), 16p, 19q, 20q (60%) and 7q (50%); and losses in 3p, 5q, 11q, 14q (50%), 4q, 9p, 13q (40%) and 6q, 8p, Xq (30%). CONCLUSIONS: 1) Chromosomal imbalance pattern of basal-like group showed an increased prevalence for losses at 16p, 17q (alterations associated with pure myoepithelial carcinomas) and 19p, opposite to those of HER2+ group, with gains in these regions and at 20q and 7q; 2) Gains at 6q and 11q are present in up to 50% of basal-like group, while HER2+ group showed losses at these regions; 3) Gains at 1q and 8q indicate shorter disease-free and overall survival times, and are common to both groups; 4) The different CGH profiles demonstrate the inherent differences in the molecular evolution of these tumour groups.

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Pigmented mammary Paget's disease simulating malignant melanoma

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Paget's disease of the nipple represents a cutaneous manifestation of an underlying breast malignancy. Pigmented mammary Paget's disease represents a rare variant of this entity that clinically and histopathologically simulates a melanoma. We describe a case of pigmented lesion involving the nipple with an underlying intraductal and infiltrating breast carcinoma.

A 57-year-old woman presented with a pigmented lesion in the right-breast nipple. On physical examination, nipple demonstrated a heterogeneous, dark, pigmented lesion with irregular borders. There was no evidence of erythema or excoriation, neither palpable breast mass. A skin-punch-biopsy showed an intraepidermal neoplasm characterized by cells with enlarged and pleomorphic nuclei with pale cytoplasm, some of which contained melanin pigment. The cells were arranged in a pagetoid pattern and involved all levels of the epidermis. There was infiltration of the dermis by cells forming nests and cords. Immunohistochemical studies demonstrated that the tumor cells were positive for keratins(Cam 5.2) and negative for HMB-45 and S-100 protein. Both oestrogen and progesterone receptors and HER-2 were positive, supporting the diagnosis of mammary Paget's disease. Mammographic and ultrasound studies revealed no abnormalities. A lumpectomy with excision of nipple-areola complex with sentinel lymph node evaluation was performed. Grossly the only pathological change was the nipple pigmentation. Histologically there was a 2 mm. grade 2 infiltrating ductal carcinoma and two lactiferous ducts with intraductal carcinoma. The epidermal lesion simulated a malignant melanoma. The lymph node was negative.

Comment: Pigmented mammary Paget's disease should be differentiated from melanoma clinical and histopathologically. The differentiation is based on histopathologic and immunohistochemical criteria.

Local production of melanocytic chemotactic factor by neoplastic cells when they reach the dermoepidermal junction has been postulated as the cause of the hyperpigmentation. Another possibility is the phagocytosis of melanin from melanocytes to the intraepidermal neoplastic cells of the breast carcinoma.

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Value of phenotype in invasive breast carcinoma evaluated by needle core biopsy

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Background: Phenotype of invasive breast carcinoma (IBC) based on immunohistochemical findings, shows prognostic and therapeutic implications. The aim of this study is to evaluate the value of the phenotype from NCB in relation with the surgical specimen (SS).

Methods: 183 cases of IBC with NCB and SS, without neoadjuvant chemotherapy, were evaluated. Immunohistochemical expression of estradiol and progesterone receptors, Her-2 and Ki-67 were obtained in FNB and compared with the same variables in SS. The classification is based on immunophenotype: a)luminal A (ER/PR+/Her-2 -/Ki-67<20%) b) luminal B (ER/PR+/Her-2 -/Ki-67≥20%) c) Her-2+ (Herceptest 3+/2+ confirmed by FISH) d) triple-negative (ER/PR -/Her-2 -). Concordance and kappa value of NCB and SS were assessed.

Results: ER: sensibility 89%, specificity 87%, kappa= 0.712 (p=0.000). PR: sensibility 85%, specificity 76%, kappa=0.601 (p=0.000). Ki-67: sensibility 63%, specificity 79%, kappa=0.423 (p=0.000). Phenotypes in NCB and in SS: luminal A 59% and 56%, luminal B 25% and 12%, Her-2 12% and 17%, triple-negative 15% and 15%, respectively. Luminal A: sensibility 82%, specificity 80%, kappa=0.615 (p=0.000). Luminal B: sensibility 39%, specificity 91%, kappa=0.283 (p=0.000). Her-2: sensibility 96%, specificity 95%, kappa=0.784 (p=0.000). Triple-negative: sensibility 89%, specificity 99%, kappa=0.893 (p=0.000). Kappa global: 0.615 (p=0.000)

Conclusions: Estradiol and progesterone receptors, Her-2, Ki-67 and phenotype obtained in FNB showed a good level of concordance with SS and can be used for diagnostic purposes.

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C-kit and egfr are mostly negative in advanced breast cancer. a tma study

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Background - Receptor tyrosine kinases (RTKs) constitute attractive targets, as quite often their abnormal signaling has been associated with tumor development and growth. Abnormally elevated EGFR activity is associated with the most common human solid tumors and has been detected at all stages of tumor progression. The proto-oncogene C-KIT encodes a transmembrane tyrosine growth factor receptor, plays an important role in the development of certain neoplasms. Several investigators report conflicting results concerning its expression especially in malignant breast lesions.

Method - In retrospective series of 80 T2-T4 NxMx Invasive Ductal Carcinomas (IDC) samples were selected to construct a Tissue Micro Array (epithelium and stroma). Immunohistochemical essay with C-KIT, EGFR and CK5, performed in an ABC streptoavidin method. More that 10% of stained cells were considered positive.

Results - Immunohistochemically stained TMA samples for EGFR (epithelium) were positive in 16 cases, negative in 62 cases and missing in 2 cases. EGFR (stroma) were negative in 80 cases. C-KIT (epithelium) was positive in 9 cases, negative in 63 cases and missing in 8 cases. C-KIT (stroma) were positive in 10 cases, negative in 69 cases and missing in 1 case. CK5 positive/C-KIT negative were expression in 13/80 cases.

Conclusion - We conclude that C-KIT and EGFR mostly negative in advanced breast cancer. These results suggests that tumors expressing EGFR result from multiple genetic changes leading to the expression of additional receptor and nonreceptor TKs that play roles in tumor growth. C-KIT is consistently expressed in normal epithelium cells of the terminal duct lobular unit (TDLU) and poorly expressed in IDC. Correlation with CK5 positive suggests that C-KIT negative invasive breast carcinomas are of basal type.

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HISTOMORPHOLOGICAL CHANGES IN BREAST CANCER WIHT NEOADJUVANT PROTOCOLS. Preliminary observations of pathological responses.

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INTRODUCTION AND OBJECTIVE: Preoperative chemotherapy (QT) is used in cases of locally avanced breast cancer. Tumor size remains as the best parameter for evaluating residual tumor. This study was conceived as an initial step aimed at evaluating the pathological response of breast cancer with neoadjuvant protocols.

MATERIALS AND METHODS: We analyzed retrospectively 20 patients with breast cancer treated with neoadjuvant protocols.In each case the presence of residual tumor was evaluated macroscopically. Sections stained with hematoxylin and eosin were used to confirm the presence of tumor, to determine it histologic type as well as the presence of lymph node metastasis. We also used inmunohistochemical analysis to evaluate hormone receptor and Her 2/neu protein expression.
RESULTS: A minimal pathological response was observed in 50% of cases with macroscopically evident nodes (size between 20×15 mm and 7×7 mm). In the remaining cases the pathological study showed only an area of fibrosis. Seven cases had lymph node metastases. Most of the residual tumors were negative for hormone receptors and did not show Her 2/neu protein overexpression.
CONCLUSIONS: This preliminary study indicate that neoadjuvant protocols were apparently most effective in a 50% of cases whereas the remaining 50% showed only a minimal response.Poorly differentiated carcinomas were most sensitive to QT when compared with moderately differentiated infiltrating ductal carcinomas NOS (this histological type usually associated minimal response). We found no significant differences between the minimal and maximal response related with the presence or absence of axilary lymph node metastases.
According to the literature, most of the cases studied had low antigenicity for hormone receptors and the over-expression of Her 2/neu.

Neuropathology

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FEATURES OF CENTRAL NERVOUS SYSTEM TUMORS FROM UNIVERSITY HOSPITAL OF SANTANDER, BUCARAMANGA COLOMBIA

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Objective. It identified the pathologic features of patients served by tumor lesions of the central nervous system in the Hospital Universitario de Santander, between 1998 to 2006. **Materials and Methods.** A retrospective observational study was made, with a population that corresponds to the patients of the Hospital Universitario de Santander. There were interrogated different sociodemographic, clinical and pathological findings. **Results.** We studied 213 patients with neoplastic lesions of the Central Nervous system (CNS), with age between 1 to 89 years old, with average of 38.385±20.947 years old. The male:female ratio was 1:1. There were identificated 4 CNS cysts, all of them were epidermoid cysts (1.87%). The glial tumors contribute with 51.7% of the lesions, with 93 astrocytics tumors (43.66%), 7 oligodendroglial tumors (3.3%), 8 ependymal tumors (3.75%), 7 gliomas of the mixed type (3.28%), 1 neuro-epithelial tumor of uncertain origin (0.47%) and 1 neuronal-glial tumor. Nonglial tumors contribute with 39.9% of the tumors, 12 were embryonal tumors (5.63%), 3 choroid plexos tumors (1.4%), 2 pineal tumors (0.94%), 31 meníngeals tumors (14.55%), 1 germinal (0.47%), 20 tumors of cellar region (9.39% and 16 mesenquimals tumors (7.51%). At least, 8 metastátics tumors were find (3.75%), three of them with indentificated primary lesion, 1 of ovaric tumor, 1 parotid tumor and 1 breast tumor. **Conclusions.** The central nervous system neoplasms are rare, but their behavior in many regions of the world is known,however is not available studies in Colombia, in this study it was demonstrated that these neoplams have a particular behavior in our population.

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The Correlation between MDM2 Protein and Histologic Grading of Cerebral Astrocytomas

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Background: $MDM_2$is a protein factor that plays an important role in inhibition of $P_{53}$and P_{73} related apoptosis. The aim of this study was to investigatethe correlation between MDM_2 expression and histologic grading ofcerebral astrocytomas in order to reduce misdiagnosis of this tumor incontroversial cases.
Methods: In this study,104 cases of astrocytic tumors including 4 different grades were selectedrandomly. We prepared 4 μm sections of paraffin blocks of tumors. Then theslides were stained by anti MDM_2 antibody.
Findings: The intensityof nuclear staining for MDM_2 marker had a statistically significantcorrelation with histologic grading. There was no significant correlationbetween percentage of nuclear staining for MDM_2 and histologic-grading. The positivity of MDM_2 marker, as well as the multiplicationof intensity and percentage of nuclear staining in S.Score had a significantcorrelation with histologic grading. The S.Score correlation with grading ofastrocytic tumors was analyzed by ROC Cure analysis method and showed that atthe cut off point of 6, this score can be indicative for differentiation of Glioblastom multiform from other grades with specificity of 95.3%.

Conclusion: Our resultsconfirm the role of the MDM_2 protein as a oncogenic factor inprogression of astrosytic tumors. Evaluation the MDM_2 expression inastrocytic cerebral tumors can be specifically indicative for distinction ofglioblastom multiform from other grades of astrocytic tumors.

Key Words: Cerebralastrocytic tumors, MDM_2 protein factor, histologic grading.

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CRIPTOCOCOMA OF THE CENTRAL NERVUOS SYSTEM IN A PATIENT IMMUNOCOMPETENT

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Background. The criptococosis of the Central Nervous System is an important cause of morbidity and mortality in inmunocomprometidos patients; nevertheless, its presentation in inmunocompetentes patients extremely rare, is caused different a clinical phantom, since it is pronounced in this group of people like granulomatosas masses denominated criptococomas. **Objective.** A intracerebral case of criptococoma in a taken care of inmunocompetente woman in the University Hospital de Santander will be described and a discussion of the most excellent aspects of this pathology will be made. **Clinical case.** Patient of feminine sex of 24 years of age, that enters to present/display generalized clónica tonic convulsion, with deviation of the glance upwards, lost sialorrea and of knowledge, without data that suggest inmunosupresión. The Computerized Axial Tomografía of skull demonstrates mass of possible inflammatory origin versus. Neoplásico. Craneotomía with excisional biopsy is taken to surgery practicing. By means of pathological analysis the diagnosis becomes of cerebral Criptococoma and antimicótico handling begins, with favorable evolution, at the moment the patient is in good state of health without evidence of active disease. **Conclusions.** the presence of masses of criptococico origin of the central nervous system, had to a chronic granulomatosa reaction, rarely is reported, these appear in almost all the cases in competent patients inmuno, in these patients is important to discard all the causes of cellular immune commitment. Also I diagnose of these injuries must be considered before the presence of intracraneales masses that show inflammatory characteristics.

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Cell counting in brain tumors using semi-automatic system

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Background Recognition of the cells with Ki-67 staining isimportant for medical diagnosis of brain tumor. Usually it is done manually byvisual inspection of the viewing field of the histological slide. This paperpresents the automatic system for cell counting. Only the viewing field of themicroscope and camera is selected manually, the rest is processed automaticallyby the system.

Method The tissue of the brain tumor is processed using Ki-67staining. The viewing field of the image, selected by the expert, is processedautomatically in order to count and recognize the immunopositive andimmunonegative cells. This is done by the developed automatic system. Thesystem uses thresholding operation, filtering, watershed algorithm and otherstandard morphological operations for extracting and counting tumor cells. Nextthe artificial neural network (Support Vector Machine) is used for recognitionof immunopositive and immunonegative cells.

Results The developed system was applied for cell counting inmeningiomas and oligodendroglioma at assessment of the cell density. 24patients with the meningothelial meningioma and 26 patients with theoligodendroglioma of grade II according to WHO classification were evaluated. We have found that the density of cells within the viewing microscope field ischanging significantly. For meningioma the mean number of cells was 652 with std=141. Most images (95%) contained 390–879 cells. For oligodendroglioma themean value of cells was 448 with std=107. Mostimages (95%) contained 285–669 cells. Even for the same patient standarddeviation was significant: in meningioma 12.3% of mean, in oligodendroglioma12.8% of mean. The results of counting have been compared to the human expertscore. The average discrepancy between these results at Ki-67 index for 50patients (over 500 images) was 0.18% at the investigated levels from 0.6% to7.8%.

Conclusions The semi-automatic system checked on over 50 patientsdelivered the sufficiently accurate results and we hope it may be useful forsupporting the medical diagnosis of brain tumors.

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Subependymomas: A clinicopathological study of 7 Tunisian cases

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Background: Subependymomas are rare slow-growing, benign tumours which commonly involve the fourth or lateral ventricle. They are designated as grade I neoplasms in the latest WHO classification and are histologically characterized by clusters of ependymal cells arranged against a gliofibrillary matrix with frequent microcystic change. Most subependymomas are incidental findings discovered at autopsy; however, they may grow large enough to be symptomatic.
Aim of study: This study retrospectively reviews the clinical, radiological and pathologic features of a series of 7 subependymomas.
Patients and Methods: Between 1997 and 2007, 7 cases of subependymomas were diagnosed at the pathology department of La Rabta hospital. Medical records and microscopic slides were available in all cases and were retrospectively reviewed.
Results: Our study group included 6 men and one woman aged between 24 and 47 years (mean=38.5 years). The most common clinical presentations included headache (n=7), nausea/vomiting (n=6) and seizures (n=1). CT scan was performed in all cases. Six tumours were intraventricular and one was intraparenchymal. Six patients underwent gross total resection whereas one patient had subtotal resection. Histologically, the tumours were characterized by clusters of ependymal cells arranged against a fibrillary background. Focal cystic degeneration was seen in 6 tumours. Mitotic figures, vascular endothelial proliferation and necrosis were not seen in any of these tumours.
Conclusion: Compared with other ependymal tumours, subependymomas have the lowest rate of cell proliferation as evidenced by MIB-1 immunostaining. Following surgical resection, long-term prognosis is excellent.

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Hydatid disease of the central nervous system: a clinicopathological study of 20 Tunisian cases

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Background: Hydatid disease of the nervous system (echinococcosis) is rare and represents the development of vesicles or cysts by the larval stage of the tapeworm *Echinococcus granulosus* in the cerebrum. Cerebral hydatidosis accounts for 0.2% of all intracranial tumours. Primary hydatid cyst of the brain represents approximately 1–5% of all echinococcosis cases, showing evidence of infestation during childhood. Such cysts are much rarer in adults and constitute only 0.7% of all echinococcosis cases.
Patients and Methods: Between January 2000 and October 2007, 20 patients were operated for cerebral or spinal hydatid cysts at the neurosurgery department of La Rabta hospital. Medical records and microscopic slides were available for review in all cases and were retrospectively analyzed.
Results: Our study group comprised 8 male and 12 female patients aged between 2 and 65 years. CT scan was performed in all cases and demonstrated a round or oval well-defined cystic mass located in cerebral parenchyma (n=15), posterior fossa (n=1) and spinal cord (n=4). All patients were operated and total removal of the cyst without rupture was achieved in all cases. The size of the cysts varied between 2.5 and 8 cm in diameter. pathological examination was consistent with hydatid cyst in all cases. All patients were followed up for at least 5 years after surgery. There were no recurrences and their postoperative recovery was uneventful.

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The status of MGMT methylation and TP53 mutations in patients with glioblastoma.

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Background MGMT (O-6-methylguanine-DNA-methyltransferase) is a DNA-repair enzyme that specifically removes promutagenic alkyl groups from the O-6 position of guanine and protects cells from cytotoxic and mutagenic effects. Epigenetic silencing of O(6)-methylguanine-DNA methyltransferase(MGMT) by promoter methylation can leads to lack of DNA-repair, increased sensitivity to alkylating chemotherapeutic agents and a higher susceptibility to TP53 transition mutations, especially G:C @@@ A:T transitions. The aim of our study was to assess the correlation of promoter methylation of the MGMT gene with TP53 mutations and the clinical characteristics of glioblastomas.
Methods: Tumor samples were obtained from 32 patients with primary glioblastoma (15 males and 17 females, aged

from 40 to 75 years). The methylation-specific PCR (MSP) was performed in a two-step approach.The PCR products were separated on 3% agarose gel containing ethidium bromide and documented using the Gel Doc1000 Bio Rad Image System. For each PCR reaction, methylated and unmethylated DNA were included as positive and negative control.

For statistical analysis Fisher exact test and Cox regression analysis were use.

Results: Methylation of the MGMT promoter was detectedin 72% (23 of 32) of tumors. 31% (10 of 32) of all tumors presented TP53 mutations. 90% of TP53 mutations were detected in tumors that also presented MGMT methylation. We further correlated the results with survival.

Conclusions:

1) Methylation of the MGMTpromoter gene is a frequent molecular event in glioblastoma but there was no statistical correlation between TP53 mutations MGMT methylation (Fisher exact test p=0.223)
2) No corralation between met MGMT and patients survival (HR 0,74, 95%CI, from 0,32 to 1,69, p=0,45
3) Coexistence of MGMTmethylation and TP53 mutations in patients with glioblastoma was not associated with the better prognosis.

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Primary meningeal malignant melanoma

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BACKGROUND: Primarydiffuse meningeal melanomatosis arising in the leptomeninges is a highlyunusual lesion with only few cases reported. The correct and precociousdiagnosis of the disease is a medical challenge and most commonly this is madeonly after autopsy. It is an aggressive neoplasm with poor prognosis.

CASE REPORT: We report a case of a 41-year-old woman withheadache and vision impairment, most severe in the right eye, starting inOctober 2006. Physical examination shows meningism and bilateral paralysis ofthe VIth cranial nerve. A ventriculoperitoneal CSF shunt is created. The MRimages demonstrate subarachnoid infiltration with abnormal contrast enhancementin T1-weighted images. The CSF is negative for malignant cells thoughhypoglucorrarquia is present. A biopsy is performed in October 2007. Onmacroscopic examination, leptomeninges are black-tanned and diffuselythickened. Microscopic examination of brain specimen reveals diffuse neoplasticmeningeal infiltration spreading along the Virchow-Robin spaces. Thetumour cells were fusocellular with vesicular nuclei, coarse cromatin,prominent nucleoli and dusty cytoplasmic melanin pigment. Mitotic activity islow. Parenchymal infiltrating tumour nodules can be seen. Theinmunohistochemical staining reveals diffuse and strong positivity of tumourcells for S-100, HMB45, Melan-A, BCl-2 and p53 but lack EMA reactivity.Ultrastructural images show abundant well-developed melanosomes.

CONCLUSION: Primarydiffuse meningeal melanomatosis is a very rare neoplasia originated fromleptomeningeal melanocytes. The base of the brain is the most common locationand the tumour spreads along the Virchow-Robin spaces reaching the parenchyma.In our case, radiological findings and clinical history of the patient werehighly suspicious and a biopsy was performed. Meningioma and PigmentedSchwannoma were ruled out based on the histopathological findings and theabsence of staining with EMA. Histological, inmunohistochemical,ultrastructural and clinical data (no other primary malignant melanoma wasfound elsewhere) allowed the diagnosis of primary malignant melanoma of theCNS.

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Meningioangiomatosis non Associated with Neurofibromatosis Type 2: Report of Two Cases

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Background: Meningioangiomatosis (MA) is an uncommon condition of disputed nature, which commonly affects children and young adults. Although most cases are sporadic, the association with familial type 2 neurofibromatosis (NF) is well known. The sporadic type usually presents with seizures, whereas that associated with NF is often asymptomatic. Occasionally, it is associated with a neoplasm, most commonly a meningioma. Surgical treatment is recommended and, in most cases, it is gratifying.

Case Report:

Case 1: A 44-year-old woman presented with a history of generalized seizures refractory to different therapeutic

regimens. T2-weighed MRI images demonstrated a heterogeneous lesion on the left temporal lobe. The lesion was removed and histopathological examination revealed a plaque-like proliferation of meningothelial and fibroblasts-like cells, in a richly vascularised stroma, involving the meninges and Virchow-Robin spaces.
Case 2: A 2-year-old child presented with headache and seizures. Neuroradiological studies showed a lesion on the left temporal lobe, with isointense and hypointense areas in MRI. Histopathologic examination showed a lesion characterized by proliferation of small blood vessels accompanied by spindle cells that arrayed in fascicular or storiform patterns and trapping islands of gliotic cortical tissue. Immunohistochemistry showed strong and diffuse staining for vimentin and EMA, while GFAP was negative.
None of the cases was associated to type 2 NF.
Discussion: MA is a benign lesion of unclear pathogenesis but without malignant potential. Two different etiological theories have been proposed. The first holds that the lesion arises from an initial angiomatous component, while the other suggests that MA represents a variant of meningioma. It is important to distinguish MA from other possible cortical lesions since it is a surgically curable cause of seizures. The main pathological differential diagnosis is with meningeal or glial neoplasms.

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Nonsmall cell bronchial carcinoma with paraneoplastic meningoencephalitis

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Background: Paraneoplastic encephalitis is a multifocal inflammation of central nervous system, associated with malignant tumors (small cell bronchial carcinoma in 80% of cases). Immunologicaly anti-Hu, anti-Yo and P/Q antibodies are found.
Patient and Methods: D.G.S., 49 year aged man suddenly develop a loss of memory, desorientation and neuropsychic disorders (dysarthria, aphasia, general weakness, dizziness and diplopia). It was found to be afebril, with normal vesicular respiration, normal heard rhythm and variantional blood presure. Neurological examination was normal except the presence of partial aphasia. Computed tomography of the brain showed no focal changes. Cerebrospinal fluid: Protein-140,4 mg%, Ly-48%, Mo-48%, CMV-320 Ulml (+), VZV-400 Ulml (+), HIV (-), Wass. (-), HSV (-). In MRI on T1 - weighted images and T2 - small lesions (ovoid and linear) in corona radiata (bilaterally), corpus callosum, left putamen and left cerebral peduncule were found. After two months the patient dead with quadripiramidal syndrome, bronchopneumonia (with pleural and pericardial effusion) and trombosis of v.saph.magna.
Results: At autopsy a bronchial adenocarcinoma (0,5 sm. in diameter) was found. Systemic examination of the brain showed the presence of multiple punctiform, linear (oval) inflamatory necrosis with neuronal decomposition, neuronophagy and reactive astroglyosis, associated with scanty perivascular lymphoid infiltrations and lymphocytar meningistis. The changes were localized in the striatum, occipital region (paraventricular), hippocampus, mesencephalon (cerebral peduncle), near to substancia nigra, pons and cerebellum, associated with considerable loss of Purkinje cells.
Conclusion: The course of the disease with leading neurological syndroms and characteristic changes in central nervous system associated with nonsmall cell bronchial carcinoma turn to the diagnosis paraneoplastic meningoencephalitis.

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Apoptosis in Glioblastoma Xenograft: Immunohistochemical expression of caspase 3, 8, 9 and Bax

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Background: Development of new therapies for glioblastoma requires animal models that mimic the biological characteristics of human brain tumors. On the other hand, potential antitumoral effects of a new therapeutic strategy are often established by evaluation of tumor cells apoptosis. Caspases are key mediators in the regulation and execution of apoptosis. Caspase-8 mediates the extrinsic pathway of apoptosis triggered by signaling through receptors while caspase-9 is activated during the intrinsic pathway downstream of mitochondria. Caspase-3 is an effector caspase that initiates degradation of the cell in the final stages of apoptosis. Bax is a pro-apoptotic member of the Bcl-2 family that play key roles in the regulation of intrinsic apoptotic signalling. In the present study we investigated the immunohistochemical distribution of caspase 3, 8, 9 and Bax in intracranial glioblastoma xenograft. **Method:** Orthotopic xenografts were establish by injecting 5×10^5 tumor cells from glioblastoma multiforme cell line U87 into the right striatum of Balb/c nude mice. Tumor growth was monitored *in vivo* by serially sectioning the xenograft

brains at 1, 7, 21, and 28 days postinjection. Immunohistochemistry were performed for detection of the expressions of caspase 3, 8, 9 and Bax. Nuclear morphology of tumor cells was assessed in fluorescence microscopy after 4′-6-Diamidino-2-phenylindole (DAPI) staining. **Results:** Immunohistochemistry showed that some glioblastoma cells with apoptotic morphology were positive for caspase 3, caspase 9 and Bax and negative for caspase 8. The percentage of caspase 3, caspase 9 and Bax positive cells varied in individual mice. However, the expression of caspase 9 was significantly higher than that of caspase 3 and Bax. **Conclusion:** Taken together, these results suggest that glioblastoma cells in vivo have the molecular components associated with intrinsic apoptotic pathway.

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P-268

Intraventricular dysembryoplastic neuroepithelial tumor

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Background: Dysembryoplastic neuroepithelial tumor (DNT) is a glial-neuronal tumor characterized by usually supratentorial cortical location. Cases of DNTs in and around lateral ventricles have been reported very rarely. Here we report another extracortical case of DNT with typical morphology in lateral ventricle.

Case Presentation: A 21-year-old male patient was presented with recent onset of seizure in January 2007. As a result of single episode of seizure, he sustained a head injury with occipital linear fracture. Magnetic resonance imaging (MRI) of the brain demonstrated 2×1.5 cm hypointense mass lesion in the frontal horn of right ventricle on T1-weighted images without contrast enhancement after gadolinium injection. Right ventricle was asymmetric and enlarged compared to left ventricle due to obstructed foramen of Monro.

Materials and Methods: After routine histopathological processing and staining, immunohistochemistry was performed using primary antibodies against glial fibrillary acidic protein (GFAP; MAb, 1:100, Neomarkers), S-100 protein (MAb, 1:100, Neomarkers), synapthophysin (PAb, 1:100, Neomarkers), Ki-67 (MAb, 1:80, Neomarkers).

Results Histopathology: Histologically, the lesion was hypocellular and composed of oligodendrocyte-like cells (OLC) with small uniform round nuclei in an abundant basophilic pale mucinous matrix. Some of these cells were situated around delicate ramifying capillaries. Mature neuronal cells ("floating neurons") were observed in mucinous matrix of cystic areas. Immunohistochemically, those cells were positive for synaptophysin. However, OLCs were not immunoreactive for synaptophysin. Scattered glial fibrillary acid protein-positive reactive astrocytes were also seen. Ki-67 proliferation index was less than 1%. We considered that the lesion was consistent with simple form of DNT.

Conclusion: The present case shows the importance of correct histopathologic diagnosis in this unusual presentation of DNT to avoid overtreatment (chemotherapy or radiotherapy), because of favorable outcome after surgical resection of these benign lesions. Derivation from a secondary germinal layer was considered for the unusual presentation of ectopic DNTs.

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PROGESTRONE RECEPTOR STATUS AND PROLIFERATIVE MARKER KI67, AS A GUIDE IN GRADIND OF MENINGIOMA

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Background: Meningiomas are slow-growing neoplasms which recur locally.Their morphologic grading is simple but do not always correlate with patient outcome.The aim of present study is to evaluate the status of progestrone receptor(PR) and Ki67 in various grades of meningioma in a group of iranian patients.

Methods:78 cases of meningioma were selected from the file of a university hospital.All archival H&E stained sections were reviewed and regraded according to WHO 2000 criteria.Immunohistochemical analysis for PR and Ki67 was performed on formalin-fixed,paraffin-embedded material.The PR status considered positive if >10% tumor cell's nuclei were strongly immunoreactive,or if > than 50% of nuclei were stained with medium intensity..The Ki67 labeling index (LI) is defined as the percentage of positively immuno-reactive tumor cell nuclei in the area with the strongest immunostaining.

Results:PR were positive in 61/63(96.8%)of grade I tumors,2/10(20%) of grade II,and 0/5(0%) of grade III tumors.Ki67 LI was %2.98±2.27 in grade I tumors,%9.30± 5.79 in grade II tumors and %34.00±5.47 in grade III tumors.For both markers,differences between grade I,II and III tumors were significant($P<0.001$).There was a reverse relationship between mean of Ki67 LI and PR status,with increasing grade of tumor.

Conclusion: Evaluation of PR status and Ki67 LI together with conventional histologic evaluation can help in providing more information about the biologic behaviour of meningiomas,especially for those that histological grading is not straightforward.

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Establishment and characterization of a new human cell line derived from a recurrent glioblastoma

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Background. Glioblastomas are the most frequent and aggressive malignant brain tumors in adults which may occur de novo, especially in the frontal and temporal lobes of the brain or may result from progression of low-grade astrocytomas. Despite advances in surgical techniques, radiotherapy and chemotherapy the median survival rate for pacients with glioblastoma is only 1 year. Consequently, gliomas cell lines are useful as models for drug screening and cellular and molecular studies of these brain tumors. The aim of our study was the establishment and characterization of a human glioblastoma cell line derived from a recurrent tumor.

Method. Cell culture was carried out by enzymatic digestion from a tumor specimen removed from a 58-year old woman diagnosed with glioblastoma of the left temporal lobe. Cells were subcultured from more than 100 passages in DMEM with 10% FBS and antibiotics. Morphology, growth kinetics, specific markers expression and chromosome characteristics of this cell line were studied.

Results. Morphologic examination using light microscopy showed a polymorphic culture with spindle cells, star-shaped cells with large round nucleus and giant cells with one or more nuclei. Cell line retained immunopositivity for vimentin but was negative for GFAP. Other specific markers such as S-100 protein, PDGF, p53 protein, NSE, nestin and CD 133 have been also examined. Metaphases analysis of tumor cells revealed hyperdiploid cells with a very complex karyotype. The chromosome number varied between 55 and 72.

Conclusion. Our results have revealed that the new cell line have presented a polymorphic aspect, hyperdiploid cells and was stained negative for GFAP although the cell culture was immunopositive for this marker at first passages. This cell line may be a useful tool for understanding the biology of gliomas and for testing new drugs in the treatment of this malignant primary brain tumor.

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GLIOBLASTOMA MULTIFORME WITH EXTENSIVE NEURONAL DIFFERENTIATION: CASE REPORT

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Background: Glioblastoma multiforme (GBM) is the most aggressive, and the most frequent primary tumor of the brain in adults. The presence of poorly differentiated areas displaying small cell architecture and often immunoreaction for neuronal markers lacking glial fibrillary acidic protein (GFAP) expression is an uncommon phenomenon in GBM. The bases of this type of differentiation and its prognostic significance have remained vague.

Case: A 61-year-old woman with a 1-month history of headache and drowsiness presented with a sudden onset of left facial paresis. Magnetic resonance imaging revealed an enhanced mass lesion located in the right fronto-temporal lobe, exhibiting solid and cystic components and extending into corpus callosum and contralateral hemisphere, suggesting a diagnosis of GBM. Microscopically, the neoplasm was composed of two evidently perceptible areas. The predominant component was characterized by neoplastic cells with pleomorphic, atypical nuclei, and abundant eosinophilic cytoplasm lying on a fibrillary network. Numerous mitoses, blood vessels with endothelial cell proliferation and large areas of necrosis were also noted. Immunohistochemically these neoplastic cells as well as the fibrillary network were positive for GFAP. The second component dispersed throughout the tumor was composed of numerous small and densely packed cells with slightly elongated, hyperchromatic nuclei and scant cytoplasm. These primitive-looking neoplastic cells were immunohistochemically negative for GFAP and chromogranin but expressed synaptophysin, neurofilament protein and NeuN. Considering the age of the patient and high proportion of glial component, it was concluded that the current case represents a GBM with poorly differentiated small cell component expressing neuronal markers.

Conclusion: The significance of neuronal cells in an otherwise ordinary astrocytoma requires further investigations. Therefore, each case must be reported in detail to constitute a sufficient data.

Key words: Glioblastoma multiforme, immunohistochemistry

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99mTc-tetrofosmin brain spect in the assessment of meningiomas. Correlation with histological grade and proliferation index

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Background Meningiomas are common brain tumors. Although benign meningiomas (WHO grade I) have a

favorable clinical course, atypical and anaplastic tumors (WHO grade II and III, respectively) are associated with high recurrence rate and aggressive biological behavior. The expression of the cell proliferation marker Ki67/MIB1 has been used as a useful adjunct for predicting recurrence and tumor behavior. SPECT (single photon emission computed tomography) by various radiotracers can metabolically characterize intracranial tumors. Therefore, in the present study we investigated whether ^{99m}Tc-Tetrofosmin (TF) uptake in meningiomas correlates with histological grade and tumor cell proliferation.

Method Fifteen patients (4 males, 11 females; mean age 62.3 years) with suspected meningioma on structural brain imaging (CT/MRI) were prospectively enrolled.

Brain SPECT by TF had been performed within a week prior to surgical tumor excision. TF uptake was assessed visually and semiquantitatively, by calculating the lesion-to-normal (L/N) uptake ratio. In the excised tumor specimens, the immunohistochemical expression of the Ki67/MIB1 protein was evaluated. Pearson's correlation analysis was performed between the level of tracer uptake (as expressed by the L/N ratio) and Ki-67/MIB1 expression and tumor grade. Statistical significance was set at $p<0.05$.

Results Intensity of TF uptake in the region corresponding to the CT/MRI findings ranged from faint to profound. On histological examination, twelve cases were diagnosed as benign meningiomas and three cases as anaplastic meningiomas. The expression of the Ki67/MIB1 protein ranged from 0.5% to 20%. A significant positive correlation between TF uptake and tumor grade ($r=0.834$, $p<0.001$) as well as between TF uptake and Ki67/MIB1 expression ($r=0.702$, $p=0.004$) was observed.

Conclusion The findings of this pilot study suggest that TF brain SPECT may be useful in differentiating benign from malignant meningiomas and may be a potential indicator of tumor proliferation activity.

P-273

Elevated expression of Cav-1 in brain tumors and its correlation with angiogenic markers – VEGF, bFGF

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Background Caveolins, structural proteins of caveolae, are involvedin many cell functions, including endocytosis, signal transduction and cellproliferation. Their role is explored in tumor growth regulation.

We analysed the potential role of Cav-1 expression inbrain tumor progression and the association between its level of expression andthe one of VEGF and bFGF.

Methods Specimens from 20 glial tumors of various histologictypes and grades were immunohistochemical assayed for caveolin-1, VEGF and bFGFreactivity. Level of Cav-1 in selected samples was determined by Western Blot.

Results In tumoral tissue, both tumor cells and blood vesselsexpressed Cav-1 with a higher percentage compared to peritumoral areas, whereCav-1 expression was confined only to endothelial cells.Western Blot analysisconfirmed overexpression of Cav-1 in tumoral tissue lysates.

Immunoreactivity for VEGF and bFGF was expressed in both endothelial and tumor cells,with increased levels following tumor invasiveness.

Cav-1 expression in tumoral areas had a good correlation-with angiogenic markers. Significantly higher Cav-1 expression was encounteredin cases with increased VEGF and bFGF.

Conclusion Cav-1 was overexpressed in studied brain tumors, alongwith VEGF and bFGF, with a higher score in highly aggressive ones.

Future studies about interrelation between caveolins andangiogenic markers will be of majorinterest as possible therapy targets.

P-274

Correlations between clinical and morphological aspects in hypertensive patiens dead with severe primary intracerebral haemorrhage

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Aims. The study is a retrospective integrated assesment of clinical, imagistic and morphological parameters in severe intracerebral hemorrhages (ICH) complicated with intra-

ventricular extension (IVE) and/or subarachnoid effusion (SE).

Method. The material consisted of patient's medical records (clinical charts, CT scans, autopsy protocols and histopathology records) from 106 cases with ICH associated with IVE and/or SE hospitalised in the Emergency County Hospital of Craiova. The evaluated parameters were clinical (season relation, age, sex, arterial blood presure - HT, motor deficit - MD, Glasgow score at admission - GS) and morphological (ICH sites, size, perilesional edema - pE, microhemorrhages - mH and mass effect - ME).

Results. 65% of the cases showed only IVE and 22% had both complications. Both IVE and SE were more frequent in cold seasons, but IVE in autumn whereas SE in winter. Sex distribution was almost equal, with slight male predominence in VE and slight female predominence in SE. IVE and SE were more frequent in ICH before 60 years, with no predilection for a certain age group. Around 70% of the patients had IIIrd and IVth stage HT at admission, 85% had MD and almost 50% Glasgow scores lower than 6. In half of all cases IVE involved at least one of the lateral ventricles. VE was more frequent in lobar ICH while SE was more frequent in non-lobar ones. In lobar sites involving more than one lobe, arround 85% of hematomas had huge dimensions, half of the CT investigated cases showing ME. CT and HP examinations often revealed multiple mH both near and distant to ICH, and pE.

Conclusion. ICH associated with IVE and/or SE and one or more clinical and morphological poor outcome predictors (HT, MD, low GS, pE, mH and ME) result in patient's death, despite any sustained therapeutical intervention.

P-275

Central neurocytoma: a clinical-pathological study of eight cases

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Background: Central Neurocytomas (CN) are uncommon tumours of the central nervous system, with neuronal differentation, generally found in young adults, in the lateral or third ventricles. Typically, they have a favourable prognosis after surgical total resection, but in some cases the clinical course is more agressive.

Material and methods: Our series are about eight patients (M:F=3:5: men age, 28.6 years) diagnosed between 1995–2007. All are intraventricular tumours showed typical radiological, histological and inmunohistochemical features. Most patients received craniotomy with complete removal of the tumour, except one patient who underwent partial resection.The clinical, pathological and surgical data of these patients were reviewed and analyzed.

Results: Seven tumours showed typical features of CN. Anaplastic features (mitosis, vascular proliferation and necrosis) were observed in one case. Inmunochemistry showed in all reactivity for neuronal markers and negativity for GFAP. Two tumours called atypical neurocytoma presented high proliferative activity (MIB-1 labelling index >5%), one with anaplastic features. Except one case that succumbed two month afteer surgery for another tumour, the remaining seven were alive and apparently asymptomatic during follow-up period wihch ranged from 5 to 80 months (mean 38,7).

Conclusions: CN is an infrequent neoplasm with low incidence, (0.3% in t. series). The most frequent presentation is like intraventricular masses in young adults. Definitive diagnostic require neuronal markers and/or molecular studies (LOH 1p/19q), especially in extraventricular forms. Typically, CN are associated with favourable outcome, althought cases called atypical have more agressive clinical course with local recurrences.

P-276

Metastatic hemangiopericytoma after long free disease interval; presentation of two cases

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Background Hemangiopericytomas (HPC) are rare spindle cell tumors constituting 2,5% of soft tissues neoplasms but only a 0,4% of central nervous system (CNS) tumors. The majority of the tumors are superficially located and attached to the dura with no evidence of infiltration of the CNS parenchyma. HPC are frequently higly aggressive and they have a tendency to recur and metastasize outside de CNS.

Method We describe the cytological and histologycal finfings in a 57-year-old man and a 56-year-old woman who previously presented meningeal HPC and at this moment develop metastatic disease. After a free disease interval of fourteen and nine years respectively they present metastases in liver and pancreas.

Results Metastatic specimens showed a monotonous tumour composed of randomly oriented plump cells with ill-defiened cytoplasm and prominent highly-branched "stag-horn-like" vascular channels. The neoplastic cells were positive for CD·34 and CD99 and negative for CD·31 and keratin, supporting a diagnosis of metastases from the patint's cerebral tumor.

Conclusions Meningeal HPC is a diagnostic challenge due to its propensity to metastasize peripherically despite treatment. Surgical excision is the most common therapy recommende and the use of postoperative radiotherapy is debatable. Recurrences are frequent and metastases may be multiple, the liver is the most common location followed by bone, skin and soft tissues.

P-277

MGMT protein expression and MGMT promoter methylation status in a series of 63 glioblastomas

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Background: DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT) removes alkyl groups from DNA and thus prevents cell death induced by alkylating agents. Almost 30% of glial neoplasms show *MGMT* inactivation, mostly caused by promoter methylation. This phenomenon has been correlated with sensitivity to methylating agents. Additionally, some studies suggest a direct relationship between chemotherapy response and MGMT protein immunohistochemical (IHC) detection. Nonetheless, few data are available concerning the relation between IHC MGMT protein expression and *MGMT* promoter methylation status.
Method: We have studied both MGMT protein expression by IHC and MGMT promoter methylation status by methylation-specific PCR (MSP) in a series of 63 glioblastomas. Two tissue microarrays were constructed for IHC assessment. A total of 100–600 cells were counted in every case and results were expressed as percentage of MGMT-positive cells.
Results: In our series, the percentage of IHC MGMT-positive cells ranged from 5% to 93.5%. On the other hand, *MGMT* promoter methylation was detected in 39 cases. A relation between protein expression and gene promoter methylation status was observed (ROC 0.70 [0.56–0.84]; p=0.007). Placing the cut-off value of MGMT-positive cells at 12%, IHC was a sensitive tool (92.3%) in predicting non-methylation status, although with a low specificity (25%). Using the 12% cut-off value, 53 cases (84.1%) were IHC-positive and 10 were IHC-negative.
Conclusion: In our hands, although MGMT IHC detection could predict non-methylation status with a high sensitivity, its specificity was so low as to need confirmation by *MGMT* promoter methylation status determination. For this reason, we think that implementation of MGMT IHC is not justified for routine clinical management of patients.

P-278

Identification of novel candidate genes associated with recurrent meningiomas by analysis of microarrays

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Background and Aim: Meningiomas (MNGs) account for about 24–30% of primary intracranial tumours, with an annual incidence rate of up to 13 per 100000 individuals. Most of these tumours are benign (WHO grade I) and can be cured by surgical resection. Although generally considered benign, recurrences occur between 10% and 25% of the cases undergoing gross total resection of the tumor and represent the major factor influencing patient's outcome. Molecular mechanisms associated to recurrence are still unclear. The aim of this study was to carry out gene expression profiling in primary and recurrent MNGs in order to identify potential predictive target genes and/or molecular markers of recurrence.
Experimental design: Forty-six tumours (17 WHO grade I, 17 grade II, 7 recurrent MNGs grades I and II) and 5 non-neoplastic meninges were analysed by using oligonucleotide microarrays. Expression profiling results from primary tumors were compared to those of recurrent tumours. Statistical analyses were performed by using GEPAS (CIPF, Valencia, Spain) and GSEA (Broad Institute, Cambridge, Massachussetts).
Results: Unsupervised hierarchical clustering of the samples showed two clusters of tumours, one of them containing all of the recurrences. In addition, using a discriminant approach analysis we identified 899 genes with differential expression between primary and recurrent tumours (FDR<0.15). These genes were related to the Wnt, MAPK, regulation of actin cytoskeleton, insulin, and VEGF signaling pathways.
Conclusions: We have identified gene expression profiles and candidate markers associated to recurrent meningioma.

Additional studies on these candidate genes are warranted to assess its utility as diagnostic and/or prognostic markers.

P-279

Gene expression profiling in diffuse gliomas

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Diffuse gliomas are the most common primarybrain tumours in adults. They are currently classified histologically inastrocytoma (AST), oligodendroglioma (OLIG), and mixed oligoastrocytoma (MOA). However,some gliomas show ambiguous morphology and can be difficult to classify. High-throughput analyses of gene expression has shown to be helpful inidentifying new key genes, networks and pathways which may be clinicallyrelevant. We investigated thepattern of gene expression in 37 gliomas of different types (9 AST, 19 OLIG and9 MOA) and grades (WHO grades II and III) by using oligonucleotidemicroarrays.

Functional analyses by using GEPAS (CIPF, Valencia, Spain)and GSEA (Broad Institute, Cambridge, MA, USA) comparing tumours of WHO grade IIand III revealed a set of genes significantly up-regulated in grade III thatwere involved in cell cycle, cell proliferation and metabolism processes. Moreover, comparing the gene expression profiles between AST, OLIG and MOA, thelatter group showed a gene expression profile more similar to AST than to OLIG.We also found significant up-regulation of ANXA1 in ASTs and MOAs while thisgene was downregulated in OLIGs. Immunohistochemical validation studies in anindependent series of 66 diffuse gliomas confirmed ANXA1 overexpression in 41% of ASTs, but only in 18% of MOA and 5% of MOAs.

Our findings support the molecular relevance of the astrocytic component inthe gliomas with a mixed morphology, and disclose a set of candidate genes ofpotential diagnostic interest.

P-280

Immunohistochemical study for O6-methylguanin-DNA methyltransferase (MGMT) in gliomas: A tissue microarray study of 244 cases.

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Background and aim Hypermethylation of the MGMT promoter has been recently found associated to longer survival and improved response to chemotherapy in patients with glioblastoma (GBM) who receive alkylating agents. The benefit derived from alkylating drugs has been linked to reduced expression and activity of the DNA repair gene MGMT. When the DNA lesions produced by the drug are not repaired by MGMT, cell death is induced. Epigenetic silencing of MGMT occurs between 41% and 47,7% of GBMs and in 39% of anaplastic astrocytomas (AIII). Low expression of MGMT by immunohistochemistry (IHC) has been reported in 32% of GBM, 42% of AA, and 55% of anaplastic gliomas (all types). However, most studies have shown a lack of correlation between MGMT status and MGMT protein expression. Knowing the DNA repair enzyme status of the tumour is becoming a crucial parameter for prognosis and optimizing treatment decisions in glioma patients, however, molecular analyses are not a routine clinical practice in most institutions and a standardized immunohistochemical method for this analysis is not available yet. The present study examines expression of MGMT in a large series of gliomas of adult patients.

Material and methods Tissue microarrays including 128 astrocytomas (A; 98 GBM, 11 grade III, 19 grade II, 13 grade 1), 16 oligodendrogliomas (O; 8 grade III, 8 grade II), 15 oligoastrocytomas (OA; 9 grade III, 6 grade II), 8 ependymomas (E; grade II), 2 subependymomas (SE), and 4 normal brain tissues (NB) were used for the immunohistochemical study. Based on immunostain results tumours in each category were stratified into low expressors and high expressors (strong positivity in >5% of tumour cells).

Results Most positive cases showed a heterogeneous pattern of immunostaining. Low expression was observed in 47% GBM, 54% A III, 53% A II, 38% A I, 62% O III; 37% O II: 53% OA, 37% E, and 0% SE. Low-grade tumours as a group were more expressors than high grade

gliomas. Only 4/98 GBM were strongly positive. All 8 anaplastic Oligodendrogliomas were negative.
Conclusion We conclude that MGMT is differentially expressed in gliomas and that there is an inverse correlation to tumor grade, especially in oligodendrogliomas. Further studies are necessary to determine the precise clinical utility of MGMT expression studies in the different glioma types and grades.

Poster displayed on Tuesday, May 20
Head and Neck Pathology

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EVALUATION OF FINE NEEDLE ASPIRATION BIOPSY WITH HISTOPATHOLOGIC REFERENCE TESTING IN THE DIAGNOSIS OF CANCER OF THE PAROTID GLAND IN A COLOMBIAN POPULATION
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Background. Fine Needle Aspiration Biopsy (FNAB) is used commonly in the study of neoplastic lesions of the parotid gland, however, there exists controversy in its accuracy. **Objective.** To evaluate the performance of the FNAB in the diagnosis of the parotid gland carcinoma. **Materials.** 46 patients with masses in the parotid area from 7 health centers of Bucaramanga, Colombia were selected. **Results.** The mean age of the patients was of 51.78± 16.32 years old, the 58,7% were female. The FNA had a Sensitivity of 53,8%, a Specificity of 90,9%, a PPV of 70%, a NPV of 83,3%, a LR+ of 5,92 a LR- of 0,5 and kappa of 0.48 in the identification of parotid gland carcinoma, in a population with a prevalence of the disease of 28.26%. **Conclusions.** The FNAB had a moderate performance in the diagnosis of the parotid gland carcinoma, which agrees with other previous studies. Its low sensitivity and LR- indicates its limitations as a screening test; in addition its low Kappa shows little correlation in its diagnosis. Therefore, it's advisable to improve the criteria used in its interpretation, as well as to make emphasis on the development of new technologies that allow a better, valid and precise diagnosis of this pathology, and that like the FNAB, offers its service at low cost and with ease of implementation.

P-282
VEGF Biosynthesized in Adenoid Cystic Carcinoma; its role in tumor architectural histogenesis and its impact on tumor behavior
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Background Adenoid Cystic Carcinoma (ACC), the most common malignant tumor of salivary glands, is characterized by its variable histogenitic architecture. Duct-like structures together with few ductal elements constitute the prototypical patterns that typifies the tumor. Vascular endothelial growth factor (VEGF) is a potent tumorigenic growth factor, which is upregulated by hypoxic conditions for tumor cells. It is a must for tumor cells to feasibly gain their nutrients and get rid of their metabolites. How ACC tumor cells can achieve this objective though their poor vascularity, still an open question.
Aim of the work To characterize epigenetic and protein expression related to different ACC histopathology patterns.
Methods To achieve this purpose we will demonstrate the immunohistochemical distribution of VEGF and VEGF receptors (VEGFR) mainly VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1) in different ACC components. Also, show how VEGF and VEGFR architecture-related expression can work to induce the characteristic tumor histogenesis, and how this can influence tumor survival and proliferation. RNA expression of different VEGF spliced variants will be demonstrated in ACC cells in culture,by competitive RT-PCR.
Results Our results immunoprofiled ACC components show strong VEGF/ Flk-1 co-localization in tumor duct-like structures indicates that VEGF functions in formation and maintenance of ductal structures when it is recognized by Flk-1. Enhanced tumor cell proliferative and anti-apoptotic signals surrounding duct-like structures demonstrated by Ki-67, Bcl-2, and Survivin compared to other tumor components.
Conclusion Our evidences suggest an active role for duct-like structures to enhance proliferation and survival.

P-283
OCULAR JUVENILE PILOCYTIC ASTROCYTOMA IN A 7 YEAR OLD NIGERIAN GIRL A RARE FINDING.
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INTRODUCTION: Most optic nerve gliomas are juvenile pilocytic astrocytomas (JPA). Most occur in individuals

between the ages of 2–6 years,and are frequently associated with von Recklinghausen neurofibromatosis type1 (NF1). JPAare uncommon clinical entities rarely seen in our environment.
DESIGN /CASE REPORT. We present a case of JPA seen recently in a 7 year old girl who presented at the eye clinic of the University of Nigeria Teaching hospital with an 18 month history of painless protrusion of the right eye ball, associated marked diminution of vision. Externally an 8 mm non-pulsatile, non-axial proptosis, resistant to retropulsion was noticed on the clinical presentation. Skull x-ray and orbital ultrasound done were indeterminate, while computerized axial tomography of the head yielded a diagnosis of optic nerve glioma. Exision biopsy following exanteration showed a histological diagnosis of optic nerve JPA.
Macroscopically, the tumor is a well circumscribed and envelops the right optic nerve. Cut surface showed a predominantly solid lesion. Microscopically, the lesion demonstrates well-differentiated pilocytes with hairlike glial processes associated with microcysts that contain mucopolysaccharide material. Capillary formationa was present.
DISCUSSION; This case represent the first time juvenile optic nerve astrocytoma is being reported internationally from our environment. Aetiologic factors are unknown. Our patient had no feature of NF1. Transformation to a malignant high-grade tumor is rare. Patients with optic pilocytic astrocytomas associated with NF1 usually have better outcomes than those of other patients with juvenile pilocytic astrocytomas because the tumor is more likely to be confined to the optic nerve. Bilateral optic gliomas are more common in patients with NF1.Patient is currently being reevaluated as to the next line of management.

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PRIMARY CARCINOMAS OF THE UVULA. CLINICO-PATHOLOGICAL STUDY OF 17 CASES

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Background: We analyse clinicopathological features of a series of carcinomas of the uvula (CU) collected in two hospitals.
Design: All the CUs resected in both hospitals during 13 years period were included. Clinical and pathological data have been collected in all cases [age and sex, adjuvant therapy, follow-up, local recurrences, loco-regional extension, second primary tumours (SPT), tumour diameter (TD), growth pattern (GP), histological type (HT) and grade (G), predominant pattern of invasion (PPOI), worst pattern of invasion (WPOI), tumour-associated tissue eosinophilia (TATE), Ki-67 index, pT and pN status].
Results: All patients except one were males. Average age was 61.3 years. In 7 cases surgical resection was followed by radiotherapy and/or chemotherapy. Average follow-up was 27.8 months. In one case local recurrence was detected. Nine cases had SPTs, all of them located in UADT. Three- and 4-year overall survival was 62.7% and 50.2% respectively. Survival associated to SPT did not show significant differences in comparison with the rest. Ten cases showed exophytic GP, 4 were ulcerated-infiltrating and 3 were flat. Average TD was 15.2 mm. HT was as follows: squamous cell carcinoma in 16 cases (mostly G2), and one lymphoepithelioma-like undifferentiated carcinoma. PPOI was pushing in 8 cases, infiltrating in 7, and nested in 2. WPOI was infiltrating in 9 cases. TATE was intense in 3 cases, mild to moderate in 11 and it was absent in 3. Ki-67 index oscillated between 40% and 80%. Staging was: pT1 88.2%; pT2 11.8%; pN0 94.8%; and pN1 5.7%.
Conclusions: Most CUs are diagnosed in males, over the sixth decade, correspond to squamous cell carcinomas diagnosed in early pathological Stages. The prognosis of these patients is worsened by the high incidence of SPTs.

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CARCINOMAS OF THE PYRIFORM SINUS. CLINICOPATHOLOGICAL STUDY OF 40 CASES

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Background: Carcinomas of the pyriform sinus (PS) are rare and aggressive laryngeal tumours. This study reviews the clinicopathological features and clinical outcome of 40 cases.
Design: We diagnosed 40 carcinomas of the PS during the last 9 years. Age, sex, smoking and alcohol abuse, location (L), size, histological type, node status (N), extraganglionar extension (EE), distant metastases (M), local recurrence (LR), second primary tumors (SPT), stage and survival-rates (SR) were evaluated.
Results: Average age was 60.9 years with males predominance (39M/1F). Smoking and alcohol abuse was confirmed in 62.5% of cases. L was as follows: left PS 50%; right PS 45%; bilateral 5%. Surgery+radiotherapy was the

mostly applicated treatment (35%). Squamous cell carcinoma was the most frecuent type (87.5%), followed by basaloid-squamous cell carcinomas (10%), and 2.5% corresponded to carcinoma not otherwise specified. Average TS was 30.8 mm. Node metastases were positive in 67.5% cases, 44.4% of them showed EE. Distant metastases were found in 17.5% cases. LR occurred in 20% of cases and 7 cases had a SPT diagnosis. Most tumors were in advanced stage at diagnosis (I: 2.5%; II: 5%; III: 27.5%; IV: 47.5%) and 17.5% cases were not staged. Follow-up study showed high mortality (16) but most patients are still alive (23). One case was lost on the follow-up. Three- and 5-year overall survival was 41.7% and 34.7% respectively. Three-year survival comparing N and M categories did not show significant differences [(N+ 41.6% vs. N− 59%) (M+, 38% vs. M− 49.3%)].

Conclusions: Most carcinomas arising in PS are conventional squamous cell carcinomas and behave aggressively. Advanced pathological Stage at diagnosis, strong tendency to metastasize, and high incidence of SPT are findings frequently observed in these tumours.

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THYROGLOSSAL DUCT CARCINOMA: CONTRIBUTION OF FOUR NEW CASES FROM COLOMBIA

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Justification. The thyroglossal duct carcinoma is an uncommon disease, which has only an aproximate of 200 reported cases worldwide, 5 of this reported in Colombia. Objective. To describe the features of the thyroglossal duct carcinoma cases diagnosed at the *Hospital Universitario de Santander* (HUS), between 2002 - 2006. **Design.** Descriptive study. **Results.** During the four years of this study, were atended four cases of thyroglossal duct carcinoma at the *HUS*, all of them in women, with ages between 35 and 45 years. All the patients consulted for neck mass, 2 of them previous and the other 2 low to the hyoid bone, with a size between 1.5 and 6 centimeters, from 6 months to 6 years of evolution, and all of them with a rubbery consistence. In this patients the malignancy was discovered ante-surgically in 3 cases, by cytology of a sample obtained with fine needle aspiration. The Sistrunk's procedure was practiced to all the patients, finding thyroid papillary carcinoma in the pahologic study, 2 of them of classic variety and the other 2 of follicular variety. At present, the patients are asymptomatic without relapse signs. **Conclusions.** The thyroglossal duct carcinoma is an uncommon disease in our Hospital, despite that the thyroglossal duct cyst was the most frequent congenital anomaly in the neck, which matches with other series reports. In our experience the antesurgical diagnosis was made on the 75% of the cases, different from the commonly informed. We found thyroid papillary carcinoma in all the cases, data that matches with all the other consulted series and the treatment used was Sistrunk's procedure, which eradicated the disease.

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Morpho-functional peculiarities of the gum tissues at the jaw fractures area due to plasma enriched with platelets application

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Background: Posttraumatic inflammation is a common complication of the fractures of the lower jaw. The aim of present investigation was to study clinical and morphological effect of plasma enriched with platelets on the course of the gum tissues inflammation in the lower jaw fractures area.Methods: 53 volunteers enrolled while receiving dental care in cases of uncomplicated lower jaw fractures. Thirty gums biopsies were taken from patients who got the reposition and fixing of jaws fragments by the teeth fixating devices without plasma application (group of comparison) and 23- from the patients who additionally routine methods had the applications of plasma, enriched with platelets at the area of the jaw fracture. Histological slides stained with H&E and Van Gison, and then studied microscopically and stereologically. The results showed the positive effect of the application of thrombocytes membrane developed from thrombocytes gel at the area of the lawyer jaw fracture under the mucosal and periosteum layers. Plasma rich with platelets filled the alveolus of the tooth (or teeth) extracted from the line of the fracture. Clinical, macroscopical and radiological control under the patient's treatment confirmed the acceleration of the bone repair. The formation and maturation of a bone callous at the zone of the lower bone fracture stimulated by platelets growth factors. The histological evaluation clearly demonstrated a reduction of the inflammation. The number of the vessels and the intensity hyperemia was smaller. Cell infiltrates and areas of connecting tissue dystrophy revealed occasionally. Conclusion: The results showed the positive effect of the plasma application at the area of posttraumatic inflammation due to the stimulation of repair processes within bones and jaw

soft tissues by the plasma growth factors. These processes accompanied with the reduction of morphological and clinical signs of inflammation at the area of jaw fracture.

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Orbital metastasis as primary clinical manifestation of thyroid carcinoma: case report and literature review

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Introduction: Papillary thyroid carcinoma (PTC) is regarded as curable malignance with favorable prognosis. However, a minority of patients may present, or subsequently develop, locoregional and distant metastases that may adversely affect survival. Metastatic thyroid carcinoma (TC) rarely involves the orbit. They constitute 5% to 6.5% of total orbital neoplasms. We report the clinical and pathological features of metastases in orbit from a primary TC. Method and Results: Patient female, 66 years, was admitted with history of unilateral ptosis on right eye for one year. She was submitted a surgery in right orbit with biopsy, which diagnosis of metastases with PTC. Thyroid was palpable during the swallow with a nodule on right lobe. The orbit computed tomographic scan confirmed expansive solid injury projected to upperside of right associated to frontal bone injury with superficial intracranial invasion. Percutaneous US-guided biopsy of right thyroid reveled two solid and hypoecogenic nodes with follicular cells proliferation. The patient was submitted total thyroidectomy. The microscopy showed epithelium heterotype formed by cells in ground-glass and nuclear crank distribution mostly solid and slicly papillary area. The patient involved with spinal toracolombar pain. Began radiotherapy in right orbit. The bone scintillography showed radio contrast hipercaptation in right orbit and T8 to T10. The orbit immunohistochemistry reveld HBME 1, CK-19 and GAL-3 positives. Currently, patient is taking chemistry with Clodronate with palliative care finality. Conclusion: The orbit and globe are not in the list of common sites to which systemic carcinoma metastases. Rarely, the presence of distant metastasis may be the only initial manifestation of TC without clinically apparent disease in thyroid region. The related case shows the importance of immunohistochemistry to strengthen the malignant character of metastatic tumor. This was demonstrated through homogeneous expression of immune markers HBME 1, CK-19 and GAL-3, that are associates to TC.

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Prevalence of the papillomavirus infection in squamous-cell carcinomas (SCC) of the oral cavity and oropharynx in a spanish population.

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The association between oncogenic HPVs and cancer has been well established for uterine cervical carcinomas and other anogenital cancers. Substantial molecular evidence suggests a role for human HPV in the pathogenesis of a subgroup of SCC of the head and neck. The prevalence of this association varies broadly, depending on the population studied, geographical area and type of histological samples tested. The aim of this study was to investigate the association between SCC of the head and neck and HPV infection and its prevalence.

A retrospective study of 83 paraffin blocks diagnosed of SCC was included. Data regarding sex, age, anatomical localization, and toxic habits were analyzed. Samples were tested for HPV DNA by PCR and genotype identification was performed by RFLP.

HPV DNA was detected in 12 out of 83 (14.5%) samples. Of the 12 positive cases 8 were genotyped as HPV16 and the remaining 4 could not be classified. In our series, most carcinomas were classic SCC (78 cases) and also included some variants; 2 basaloid, 2 verrucous and a papillar one. HPV DNA was detected in the two basaloid and in the papillar one. Regarding anatomical location, 10 out of 47 cases from oropharynx and 2 out of 36 from oral cavity were positive for HPV. Mean age at the time of diagnosis in positive cases was 49.6 years. No significant association was found between the presence of HPV and other risk factors, including tobacco use and alcohol use.

Our prevalence is in agreement with the IARC multicontinent studies. HPV-16 is the most frequent type detected in our series and 14.5% of oral SCC was positive for the virus. In the Spanish studied population HPV associated SCC was higher in oropharynx than in the oral cavity and patients with HPV positive tumours were younger than negative ones.

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LOW GRADE ADENOCARCINOMA OF EAR LOBE WITH APOCRINE DIFFERANTIATION (CERUMINOUS ADENOCARCINOMA?): A CASE REPORT

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CONTEXT: Ceruminous glands are tubuloalveolar apocrine glands in the external auditory meatus. Ceruminous gland tumors are rare neoplasms. Histologically auricle essentially a cutaneous structure composed of keratinizing, squamous epithelium with associated cutaneous adnexial structures that include hair follicles, sebaceous glands and ecrine sweat glands. Apocrine glands in ear are located external ear canal. Apocrine adenocarcinoma of ear lobe is uncommon.

CASE REPORT.: 42 year old man was presented with 3-month history of painless mass at the right ear lobe. No history of trauma was noted. On physical examination nonulcerated mass was seen on external ear lobe. A wide local resection was performed. Histological examination showed low grade apocrine adenocarcinoma with occasinal mitotic activity. Immunohistochemically tumor showed cytokeratin 7, S100, GCDFP-15 expression.

CONCLUSION: There was no apocrine carcinoma on ear lobe at literature. Ceruminous adenocarcinomas located external auditory meatus. Apocrine and ceruminous adenocarcinomas posses glandular structure with evidence of apocrine differantiation. Only ceruminous carcinomas show dense fibrosis that seperates neoplastic proliferation. In our case localization is uncommon. Histological apperarence resembled ceruminous carcinomas. But in literature there was no apocrine glands at ear lobe; so it was difficult to name this tumor.

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CLINICOPATHOLOGICAL FEATURES OF ORAL CARCINOMA: ANALYSIS OF 240 CASES

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BACKGROUND Oral carcinoma (OC) is a neoplasia of old men. Tumors may arise in any part of the oral cavity and the most common sites vary geographically. The aim was to analyze clinicopathological features of a series of OCs and to evaluate outcome according to the location and tumor stage.

METHOD A series of 240 consecutive primary OCs collected from 1985 to 2005 was investigated for following parameters: age, sex, anatomic site, size, macroscopic appearance, histotype, grade, type of surgery, surgical margins, stage and follow up. RESULTS: OCs were more frequent in males (76%), with an average age at diagnosis of 63.1 years (vs women age 70.1 ys). Most frequently tumors were ulcerated (73%), the size ranged from 0.4 to 7 cm (average: 2.3 cm) and surgical margins were free in 87.1% of the cases. SCC was the most common histotype (93%) and the majority of cases were G2 (62.9%) or G1 (23.8%) tumors. Early stages (T1 and T2) were most commonly observed (83%) and cervical node involvement was present in 36/88 (42%) patients.The locations of OCs were tongue (38.3%), mouth floor (16.6%), lip (15%), palate (10.4%), retromolar pad (7.5%), gingiva (4.5%), cheek (2.5%) and oral cavity NOS (4.5%); subsites mostly involved were lateral tongue and lower lip. Retromolar tumors showed a male predilection, while gingival ones were more frequent in females. The survival rate was 51% (mean follow up:62.2, range: 3–96 months), while 19% of patients died of disease and 30% of other cause. OCs of lip, cheek and gingiva showed a favourable outcome while retromolar, oral cavity NOS, tongue and floor tumors had a worst prognosis.

CONCLUSION OC is more frequently observed in early stages, in males over age of 60 years, the more common site is the tongue. The site of presentation may play a role in survival outcome.

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Activated MAP-kinases show coordinated expression in salivary gland adenoid cystic carcinoma

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Background: Little is known regarding the mitogen-activated protein kinase(MAPK) activation state in salivary gland adenoid cystic carcinoma(ACC).

Methods: We evaluated expression of the phosphorylated forms of ERK(pERK), JNK(pJNK), P38(pP38), and Ki67 by immunohistochemistry in 61 paraffin-embedded tumour specimen. The median value of the H-score (the proportion of stained tumour nuclei multiplied by the staining intensity of nuclei) and of the percentage of the Ki67 tumour nuclei were used as cutoff for classifying tumours as with high or low expression.

Results: The sex ratio was 15 men to 46 women. The median age was 50 years. Forty-two out of 61 tumors

(69%) developed in the minor salivary glands. The median tumour size was 20 mm. The 23 cases with stage IV presented with bone infiltration (17), skin or facial nerve extension (3), or metastases to the lungs (3). pERK, pJNK, and pP38 were expressed at high levels in 57%, 79% and 63% ACC, respectively. The rankings of scores for pairs of activated MAPK across tumours were correlated (pERK, pP38 tau 0.38 p=0.0004;pERK,pJNK tau 0.33 p=0.002; pP38, pJNK tau 0.45 p<0.0001). The Ki67index was high in 33/60 ACC.Among clinicopathologic characteristics, perineural invasion was correlated with high pJNK and high Ki67 index (p=0.005 and p=0.03, respectively). In the 42 patients with follow-up complete data for more than 5 years, pP38 (adjusted risk ratio 0.91, 95% CI 0.86 - 0.97) and Ki67 (adjusted risk ratio 1.05, 95% CI 1.00–1.10) could be used to fit a Cox regression model to overall survival (p=0.009). In univariate analysis, patients with pP38 positive tumours survived longer than those with pP38 negative tumours.

Conclusion: This study provided evidence that the activated MAPK pERK, pJNK and pP38, were coordinately expressed in salivary gland ACC. Perineural invasion was correlated to high Ki67 index and high pJNK expression.

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Release of the soluble alpha chain of IL-15 receptor: a new tumor evasion mechanism associated with poor clinical outcome in head and neck cancer patients.

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IL-15 is a proinflammatory cytokine, as it induces the production of cytokines (IL-6, TNFα, IL-17...) which play a role in various inflammatory processes. A correlation between high intratumoral IL-15 concentrations and poor clinical outcome in lung and head and neck cancer patients has been recently reported. The purpose of this study was to investigate the role of the soluble chain of IL-15 receptor (sIL-15R), a natural regulator of IL-15, in head and neck cancer. Increased serum sIL-15R concentrations were found in head and neck cancer patients, as mean concentrations of sIL-15R were 5.8 pM for head and neck cancer patients and 0.35 pM for healthy donors (p<0.0001). Serum sIL-15R levels were significantly correlated with both locoregional control and survival. The locoregional control rate at 12 months was 78% for head and neck patients with no detectable serum sIL-15R versus only 48% for cancer patients with detectable sIL-15R (p=0.04). In addition, the 12-month survival rate in patients with no serum sIL-15R levels was 89%, versus 69% for patients with detectable sIL-15R levels (p=0.04). Multivariate analysis, including tumor stage, TNM grading, serum sIL-2R levels - considered as a powerful prognosis marker –, showed that the only significant prognostic factor independently related to locoregional control was the serum sIL-15R level (P=0.0065; relative risk, 1.078). On Cox multivariate analysis, only serum sIL-15R levels influenced overall survival probability (p=0.0059; relative risk 1.086). From these clinical results we tried to characterize this sIL-15R , determine its origin and propose some mechanisms to explain its association with poor prognosis. sIL-15R was mainly produced by tumor cells which express IL-15R *in situ* and *in vitro* both at protein and mRNA levels. This expression of IL-15R was found in 17 out of 48 tumors tested. In contrast, normal epithelium did not significantly express IL-15R Various arguments strongly suggest that sIL-15R is produced via proteolytic cleavage of IL-15R mediated by ADAM-17. A correlation was observed between ADAM-17 expression in tumor cells and serum sIL-15R concentrations. Tumor cells coexpress IL-15R and ADAM-17 *in vivo*. Surprisingly, sIL-15R did not act *in vitro* as an IL-15 antagonist but rather as an enhancer of IL-15-induced proinflammatory cytokines (IL-6, TNF , IL-17) that may promote tumor progression. This new tumor evasion mechanism based on amplification of the intratumoral inflammatory reaction is probably not restricted to head and neck cancer, as other tumors have been shown to release sIL-15R . Overall, these results support for the first time an original role of sIL-15R in inflammation and cancer.

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Immunoexpression of hgfa in different degrees of invasion of carcinoma ex-pleomorphic adenoma

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Carcinoma ex pleomorphic adenoma (CXPA) is a salivary gland tumour derived from the malignization of a benign tumour, the pleomorphic adenoma, usually from a long-standing or a recurrent pleomorphic adenoma. Hepatocyte Growth Factor (HGF) is a cytokine that induces many biological functions in not only hepatocytes but also in many epithelial cells. It plays roles in promoting cell migration and proliferation, angiogenesis, and apoptosis inhibition. Immunoexpression of HGFA has been investigated in CXPA using sixteen cases in different degrees of invasion– intracapsular, minimally and frankly invasive carcinoma. To this aim, we have analyzed positive cells in different areas. In residual pleomorphic adenoma, only the cells in the condroid and myxoid areas were positive. *In situ* areas of CXPA presented strong expression in all luminal cells. In intracapsular type, immunoexpression was detected in 2 of 4 cases, with positive cells only in focal areas next to the capsule. Minimally invasive ones presented increased area of positive cells, with variable pattern among the tumours, including areas of in situ carcinoma and small nests in invasive areas. In frankly invasive CXPA with only epithelial component, 2 cases were negative and 3 positive. An increased number of positive cells was observed when compared with the previously described subtypes. In well-circumscribed large cellular blocks, most of the cells were strongly positive for HGFA. In the small nests, strong immunostaing was observed even in individually detached cells. Some small nests with basaloid cell profile were negative. In all the four cases of frankly invasive CXPA with epithelial and/or myoepithelial component studied, the cells were negative. HGFA was present in CXPA with epithelial component, with increased number of positive cells following tumour progression. This fact may indicate a possible role of the HGFA in the invasion progress.

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Angiogenic switch in salivary carcinomas with and without myoepithelial differentiation

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BACKGROUND: Tumor angiogenesis has been considered a putative target for cancer therapy. In a previous study we have shown salivary carcinoma with myoepithelial component arising in pleomorphic adenoma presented lower angiogenesis than those without such differentiation. The process of angiogenesis is influenced by the net balance between angiogenic factors that stimulate and those that inhibit vessel growth, and in vitro syudies have suggested that myoepithelial cells may inhibit angiogenesis. In order to investigate the hypothesis whether other salivary carcinomas (non ex-pleomorphic adenoma) with and without myoepithelial differentiation could also present difference regarding degree of angiogenesis, we compared tumor vascularization between mucoepidermoid carcinomas (neoplasias without myoepithelial component) and adenoid cystic carcinomas (neoplasias with myoepithelial component). These tumors are considered the two more frequent salivary carcinomas. METHOD: In 37 mucoepidermoid carcinomas (MEC) and 31 adenoid cystic carcinomas (ACC) tumor vascularization was assessed by measuring microvessel density (MVD) in sections stained for CD34 (a pan-endothelial marker) and CD105 (a neoangiogenesis marker). For both markers MVD was calculated in three hot spot areas in intratumoral as well as in peritumoral regions. RESULTS: Whereas the CD34 values did not differ among the tumors, MVD for CD105 was significantly higher in MEC than in ACC (p=0,0004). In both carcinomas, the intratumoral region was the site with more marked angiogenesis (MEC - mean 12.91; ACC - mean 5.10). Regarding histological tumor grade MVD for CD105 did not differ significantly between: a) high grade and intermediate/lowgrade MECs and b) solid and tubular/cribriform ACCs. CONCLUSIONS: The antibody CD105 reflects better the salivary carcinoma's angiogenic switch than CD34. The degree of neoangiogenesis differs significantly between MEC and ACC suggesting that tumors with myoepithelial component, such as ACC, possibly have an angiogenic phenotype that leads to a low tumor-induced angiogenesis.

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Chondromyxoid fibroma of the zygoma

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Background: Chondromyxoid fibroma (CMF) is a rare benign neoplasm of bone with an extremely unusual involvement of facial skeleton, most often the mandible and maxilla. Zygoma involvement is even more uncommon and to date only two cases have been reported in this location. We report a case of CMF arising on the zygomatic arch in a young woman.
Method: Diagnosis was established following the WHO definition. Immunohistochemistry (IHC) was performed using antibodies against smooth muscle actin (SMA), CD 34 and S-100 protein.

Results: A 34 year-old woman presented with light expansion over her right zygomatic region of 6 month duration. She revealed a radiolucent well-circumscribed lesion of about 3 cm in diameter in the right zygoma with scalloped borders, expansion and apparent disruption of the cortical bone. A surgical curettage was performed. Light microscopy showed a tumor with a lobular-like arrangement. It was composed of hypocellular myxochondroid areas with randomly arranged stellate or spindle shaped cells. The tumor cells were devoid of pleomorphism, atypia or mitoses. The lesion also revealed densely cellular regions containing sparse osteoclast-like multinucleated giant cells. No areas of necrosis were identified. Focal coarse calcification was observed. By IHC the tumoral cells expressed SMA and showed negativity for S-100 protein and CD 34. The patient has no symptoms or signs of recurrence 3 years after the curettage.
Conclusion: CMF is an important consieration when diagnosing facial chondroid tumors, particularly in young patients. It should be distinguished from myxoid chondrosarcoma, especially in small biopsies.

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Fetal rhabdomyoma of the head and neck: clinicopathologic features of one case with comparative genomic hybridization (CGH) analysis

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Background: Extracardiac rhabdomyomas are exceptionally rare benign tumors that have a proclivity to occur on the head and neck region. Cytogenetic study of an adult rhabdomyoma has demonstrated clonal chromosome (Chr) abnormalities (reciprocal translocation between Chr15 and 17 and abnormalities of the long arm of Chr 10). CGH analysis of rhabdomyosarcoma has identified chromosomal gains and several regions of loss, such in Chr 16. We report a fetal intermediate rhabdomyoma analysed by CGH. **Method:** Freshly frozen tumor samples were obtained. CGH was performed as described by Kallioniemi, reagents and protocol modifications from Vysis® and preparations were analyzed using Isis™ software, (Metasystems®). Gains and losses were determined when the 95% confidence interval of the immunofluorescence ratio did not contain the value 1.0. Positive and negative controls were included. **Results:** A 30-year-old man with a 17-year history of an asymptomatic, solitary mass on the filtrum crest of the nose. Physical examination disclosed a whitish, firm nodule, 2×2 cm in diameter. Magnetic Resonance Imaging revealed a soft tissue mass, ruling out the presence of cartilage or bone involvement. A surgical excision was carried out. Histopathologycal examination disclosed a tumor with broad fascicles of spindled cells simulating a smooth muscle tumour and strap-like striated muscle cells without cytological atypia. Immunohistochemical studies revealed immunoreactivity for vimentin, muscle specific actin (HHF35), desmin, smooth muscle actin and bcl2. Moreover, the cells showed focal expression of myoglobin, CD117, and were negative for CD34, CD31, EMA, S100 Protein, HMB45 and CAM 5.2. No chromosomal imbalances in the entire tumor genome were detected by CGH. **Conclusions:** The histopathologycal features of this case correspond to a fetal intermediate rhabdomyoma. No gains and losses of chromosomal regions were detected by CGH analysis of the case. However, balanced translocations cannot be excluded utilizing CGH technique.

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The characteristics of inflammatory restoration period in different skin wounds of facial area during experiment

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Optimizing factors in treating wounds of mandibular-facial area is rather urgent, that's why our *aim* was to give morphofunctional estimation of skin wound edges and surrounding tissues depending on the mechanism of injury, changes in adjacent and remote tissues, location, depth and direction of wound defect, it's form and degree of wound edge tension, time of putting stitches. *Materials and methods.* Experiment was carried out on adult white lab rats of both genders, weight about 300 grams. Under aether anaesthesia (with the help of anaesthesia mask developed for rats) animals were inflicted different kinds of wounds. Operative treatment, including mobilization of tissues with the following putting stitches with different materials, was carried out under aether anaesthesia in early stages after trauma (15 minutes – 4 hours) and late stages (1–7 days). Animals were not excluded from the experiment; taking tissues for morphological investigation was performed under local anaesthesia with novocain during surgical intervention; it was also performed on decapitated animals on the same stages after putting stitches. *Results of investigation.* With the help common histological, histochemical, immunohistochemical, enzyme-chemical, cytological, morphometrical methods of investigations we revealed a certain dependence of wound process on the original stages of wound defect edges, surrounding and remote tissues, degrees of wound edges tension, type of stitch material, time of putting stitches. *Conclusion.* Morphofunctional investigation of inflammatory restoration

process in skin and soft tissues characteristics in experimental animals will help to increase efficacy of surgical treatment for patients with different wounds and their locations, in particular in mandibular facial area.

P-299

Evaluation of EGFR, Her-2/neu and Cyclin D1 Protein and Gene Copy Number in Head and Neck Squamous Cell Carcinomas

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Background The EGFR signalling pathway is considered to be important in controlling the growth of squamous carcinomas of the head and neck (HNSCC) and is a potential therapeutic target.
Methods Tissue microarrays of 200 cases of HNSCC (45 oral cavity, 72 pharynx and 83 larynx) and their nodal metastases (83 cases) have been constructed. This study has evaluated the immunocytochemical expression of epidermal growth factor receptor (EGFR), phosphorylated EGFR, Her-2 and cyclin D1 proteins, and of EGFR and HER-2 gene expression by FISH. Protein expression was defined as high or low according to the median value of % positive cells. Gene amplification was present when the ratio of signals for the gene and centromeric probes was >2. Minimum clinical follow-up is five years and the main clinical end points were nodal metastasis and survival.
Results Median survival was 14 months, and overall survival was longer in cases without nodal metastasis (log-rank, $p=0.011$). Expression of EGFR was observed in 86%, Her-2/neu in 37% and cyclin-D1 in 94% of cases by immunocytochemistry. High EGFR and cyclin-D1 expression were associated with poorly-differentiated carcinomas ($p<0.037$; <0.001 respectively); high cyclin-D1 expression was associated with pharyngeal site ($p<0.001$) and nodal metastasis ($p<0.013$).
EGFR and HER-2 gene amplification were seen in 13 and 11 cases respectively. Polysomy for centromeric probes was seen in 84% cases for chromosome 7 and 53% for chromosome 17. Protein and gene expression for EGFR and HER-2/neu were significantly correlated in primary carcinomas ($p<0.018$, 0.033 respectively) and in nodal metastases. Gene expression was not associated with prognosis.
Conclusions Immunocytochemical labelling for cyclin-D1 is of prognostic value in HNSCC. FISH analysis of EGFR and HER-2 does not provide additional prognostic value.

P-300

Unusual Localisation of a Bronchogenic Cyst

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Bronchogenic cysts are rare, most commonly seen in the skin and subcutaneous tissues of just above the sternal notch: usually on the anterior of the neck or on the chin.
In our case, the cyst lined with pseudostratified columnar epithelium with cilia and goblet cells, was located on the eyelid. The patient is 19 years old and nothing know about the lesion until this time.

P-301

BUCCOMAXILLOFACIAL LESIONS IN THE ELDERLY: A STUDY OF 545 CASES

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Background: studies of the lesions in the buccomaxillofacial area in elderly patients were not frequently related. The aim of this study was to describe the epidemiological aspects of buccomaxillofacial lesions in elderly patients. Method: the archives from the Oral Pathology Laboratory of the Federal University of Goiás were reviewed and the data obtained were from 1956 to 2006. Results: of a total of 4885 cases, 11.1% (545) were elderly patients ≥60 years old. The median age was 65 (60 to 95) years, and seventh decade of life (60 to 69 years) being the most frequent (42.4%, 231). There was a predominance of females in 63.1% (344) of the cases. The maxilla was the most affected (30.7%, 151), followed by the mandible (26.6%, 131) and jugal mucosa (19.1%, 94). Inflammatory lesions were the most frequent (62.9%, 343), being 76.7% (263) fibroepiteliais hyperplasias (traumatic lesions). Tumors were the second most frequent category, 9.2% (50) of the cases affected by malignant tumors and 6.4% (35) by benign tumors. Conclusion: among the elderly studied the lesions in the buccomaxillar area predominated in women and in the maxilla. The inflammatory lesions were the most frequent.

P-302

INTRAORAL MINOR SALIVARY GLAND TUMOR: AN EPIDEMIOLOGICAL STUDY OF 85 CASES

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Background: salivary gland tumors are uncommon, and they represent 2 to 6.5% of all head and neck neoplasms. Among them, intraoral minor salivary gland tumors corresponding to 9 to 23%. Few studies have shown epidemiological data of intraoral minor salivary gland tumors. The aim of this study is to present an epidemiological study of intraoral minor salivary gland tumors in a Brazilian population. Methods: the demographic data and diagnosis was reviewed in the anatomo-pathologic archives and corresponded to period of 10 years (1996 to 2005). Results: of 599 salivary glands tumors retrieved, 87 cases (14.5%) affected minor salivary glands. Women were the most affected with 61% (53 cases) in a male:female ratio of 1:1.6. The age ranged from 15 to 87 years old (with median age of 48 years). The palate was the most affected site with 70% of cases (61), followed by jugal mucosa with 6.9% (6) and tongue with 5.7% (5). Malignant tumors were the most frequent with 57.5% (50) of the cases, being the mucoepidermoid tumor and the adenoid cystic carcinoma the most frequent histopathologic types with 32% (16) of cases. Among benign tumors, pleomorphic adenoma was verified in 86.5% (32) of cases. Malignant tumors were more frequent in women with 62% (31) of the cases, $p>0.05$. Both adenoid cystic carcinomas and mucoepidermoid tumors predominated in women with 68.7% and 62.5% of cases, respectively, $p>0.05$. Conclusion: an epidemiological study of intraoral minor salivary gland tumors in a Brazilian population is presented. The palate was the most frequent location; malignant tumors predominated and women were more affected.

P-303

Tracheal chondrosarcoma

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BACKGROUND The most frequent tracheal malignant tumour is squamous cell carcinoma (80–90%). Respiratory tract chondrosarcomas represent just 0,2% of all malignant neoplasias; despite this, it is the most common mesenchimal tumour. Cricoid cartilage is its most usual location.
METHODS We present the case of a 73 year-old male whose scanner showed a 32 mm solid mass with numerous inner calcifications, infiltrating anterior wall of the trachea, destroying cartilage and growing into the lumen. Resection of the lesion is performed.
RESULTS Pathological examination reveals a rounded lesion of 45 mm in its greatest dimension, hard and well delimitated. Surface cut is shiny, gray-white with calcified areas. Histologically it corresponds to grade 1 chondrosarcoma that affects resection margins.
COMMENTS Tracheal chondrosarcoma is an extremely rare tumour. In our literature review we have found just 15 published cases. In most of them the patients are males, the tumour is low grade and clinical presentation is airway obstruction. Main differential diagnosis is with chondroma, which is a less celular benign tumour, with no atipia or infiltration, never larger than 20 mm, as recommended by WHO.

P-304

HUGE RETROPHARYNGEAL LIPOMA CAUSING OBSTRUCTIVE SLEEP APNEA: A CASE REPORT

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Lipoma of the retropharyngeal space is a very rare benign tumor often causing unspecific clinical symptoms. The most common symptoms are dysphagia and/or respiratory disturbances. The clinical diagnosis may be difficult. The radiological imaging techniques (CT and MRI) can provide adequate information with regard to the composition and extension of the tumor, although final histological confirmation is essential. Surgery is the treatment of choice. We present a case of 40-year-old male patient complaining of obstructive sleep apnea symptoms (respiratory disturbances, excessive daytime somnolence, morning headache). The radiological examination (CT) showed a huge (11.7× 7.2 cm) lipoma of the retropharyngeal space extending from the nasopharynx to the superior mediastinum. The tumor was removed via transcervical approach with complete amelioration of symptoms.

P-305

Tight junction claudin-7 downregulation is implicated in advanced stages of oral squamous cell carcinoma

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Background: Claudins, a large family of essential tight junction (TJ) proteins are abnormally regulated in human carcinomas, suggesting that they might represent potential targets for cancer detection, diagnosis and therapy. In a previous study, we have showed that several claudins are altered in the oral squamous cell carcinoma (OSCC) cases analyzed and that there is an association between these alterations and clinico-pathological characteristics of these tumors. **Method:** In this study, using a Tissue Microarray (TMA) comprising 133 cases of OSCC, we have analyzed the expression of claudin-7 by immunohistochemistry. We have also studied the expression of claudin-7 mRNA transcripts and methylation status of the claudin-7 promoter region. **Results:** Claudin-7 was almost absent in the majority of the cases - 9.1% of the cases showed strong expression of claudin-7 and 90.9% of the cases showed mild or no expression of claudin-7. Loss of claudin-7 was associated with advanced stages of OSCC ($p=0.044$) and tended to be more frequent in moderately/poorly differentiated tumors ($p=0.055$). Loss of claudin-7 was also associated with depth of invasion higher than 3 mm ($p=0.020$). Disease-free survival is significantly shorter in claudin-7 negative patients ($p=0.015$). Expression of the mRNA transcript of claudin-7 gene was assessed and down-regulation of claudin-7 transcripts was detected in 77.78% of the cases analyzed. As methylation is one of the mechanisms involved in downregulation of claudins, the methylation status of the promoter region of claudin-7 was investigated. We found that treatment of O28 cells (that did not express claudin-7 mRNA transcripts) with 5-Aza-2′-Deoxycytidine (5-Aza-dC) leaded to the re-expression of claudin-7 mRNA transcript. **Conclusion:** Our data suggests that loss of claudin-7 expression might be associated with the tumorigenic process of OSCC and it is associated with a poor prognosis. Furthermore, claudin-7 downregulation is problaby due to its promoter hypermethylation.

P-306

Primary oral mucosal melanoma: analysis of tetraspanin CD63 in a tissue microarray of 35 cases

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Background: Tetraspanins comprise a family of proteins with functional roles in a wide array of cellular processes and have been reported to be associated with the biological behaviour of solid tumors, especially with their metastatic potential. CD63, a tetraspanin membrane protein, has been suggested to play important role in the regulation of skin melanoma progression, being strongly expressed in early stages of the disease and downregulated in advanced stages. Little is know on the adhesion and molecular mechanisms of oral mucosal melanomas due to the rarity of the lesions. These melanomas are in general very aggressive and have poor prognosis. Modulation of CD63 may be involved with the aggressive behaviour of oral mucosal melanomas, but no studies investigated this possibility. **Method:** The present study analyzed the expression of the tetraspanins CD63 is oral melanoma using immunohistochemistry. Tissue Microarray (TMA) of 35 cases of oral melanoma and 09 normal tissue of oral cavity were evaluated immunomorphologically and semi-quantitatively, and results were compared to the clinical-pathological features and qui-square test were used for statistical analysis. **Results:** Expression of CD63 was observed in the cytoplasm of all melanocytes in the controls of normal oral mucosa. Downregulated/negative expression was observed in 23/35 (66.0%) of oral melanomas included in the array. Loss/downregulation of CD63 in oral melanoma was statistically significant when compared with the normal controls ($p<0.001$). There was no correlation between expression of CD63 and clinical-pathological parameters analysed, such race, age, sex, metastasis, recurrence, histological grade and size of tumor. **Conclusion:** Our results suggest that reduction or loss of CD63 expression is involved with the complex mechanisms of oral melanomas, and possibly contribute with other mechanisms that culminate with disease progression.

P-307

Transforming Growth Factor beta 1, beta 2 and beta 3 in human developing teeth: immunolocalization according to the odontogenesis phases

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Background: Transforming Growth Factor beta (TGF beta) is a multifunctional growth factor that has several biological effects in vivo, including control of cell growth and differentiation, cell migration, lineage determination, motility, adhesion, apoptosis, synthesis and degradation of extracellular matrix, and plays an important role in regulating tissue repair and regeneration. **Method:** Our study analyzed the participation of TGF beta 1, beta 2 and beta 3 in the different stages of morphogenesis and differentiation of human developing dental organ using immunohistochemistry. For this purpose maxilla and mandible of 10 human embryos ranging from 8 to 23 weeks of gestation were employed, according to the approval of the Ethical Committee. **Results:** TGF beta subunits – beta 1, beta 2, beta 3 were present in the various stages of tooth development, but the expression varied according to the differentiation stage, tissue and TGF beta subunit. TGF beta-1 was strongly expressed in the enamel epithelium from dental lamina up to late bell stage where it was detected in the external epithelium, stellate reticulum, stratum intermediate, preameloblasts and ameloblasts. TGF beta-2 presented mild positivity from bud stage, mainly in the enamel organ epithelium and this positivity was maintained up to bell stage. TGF beta-3 was mainly negative, with sparce positivity detected only in the dental papilla and odontoblasts. **Conclusion:** Our results indicated that TGF beta-1 is closely related to differentiation of enamel organ and initiation of matrix secretion, TGF beta-2 to cellular differentiation and TGF beta-3 to mineral maturation matrix.

P-308

Human salivary gland morphogenesis: expression of galectins-1 and -3 during the different developmental phases

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Background: Galectins are members of animal lectins that bind b-galactosides through the sequence elements of carbohydrates recognition-binding domain (CRD). Fourteen members of galectin family have been identified and galectins 1 and 3 (Gal-1 and Gal-3) are the best-studied members. They participate in biological processes such as development, differentiation, cell adhesion, cell/matrix interactions, growth, apoptosis, tumorigenesis and metastasis. Expression of these proteins has been described in salivary gland neoplasms, but their participation in salivary gland morphogenesis, which involves co-ordinated events and molecules have not yet been determined, and may be an important key for the understanding of tumorigenesis. **Method:** The present work analysed the expression of Gal-1 and Gal-3 during developmental stages of human salivary glands using immunohistochemistry. The specimens were obtained from foetuses ranging from 8 to 24 weeks of gestation, according to the approval of the Local Ethical Committee. **Results:** In adult fully developed salivary glands (controls) Gal-1 was detected in the cytoplasm of acinar cells and Gal-3 was expressed in ductal cells along the entire extension of the ductal system (from intercalated duct up to excretory portion). In human developing glands, Gal-1 was detected in the glandular stroma in all phases of morphogenesis and in the cytoplasm of rudimentary acinar cells. Gal-3 was not detected in the bud stage of gland development but its expression was increasingly observed in epithelial cells during canalization and branching phases and its greatest expression occurred in the ductal system of the advanced stages of salivary gland development (advanced cytodifferentiation). **Conclusion:** Expression of gal-1 and gal-3 in developing human salivary glands is gradual and complementary during the different phases of glandular morphogenesis. This pattern is probably related to the fully developed salivary gland functions.

P-309

Alveolar rhabdomyosarcoma of the maxillary sinus in an adult

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Background: Sites in the head and neck region are among themost frequent locations of rhabdomyosarcoma (RMS) in patients younger than 15 years. However, comparable neoplasms in adults are very uncommon and often of embryonal type. We report a case of alveolar RMS of the maxillary sinus in an adult with a review of the main

findings concerning pathology, molecular biology and differential diagnosis.
Methods A 31-year-old female, with no significant medical history, complained for paranasal sinus tumefaction associated with ophtalmic manifestations. CT scan documented a maxillary sinus mass involving the orbital floor. There was no evidence of direct extension into the orbit. Surgical biopsy of this mass was achieved.
Results: The pathological examination of the mass biopsy was consistent with an alveolar RMS which demonstrated the presence of round to oval cells arranged predominantly in sheets. Immunohistochemically the cells were positive for desmin and myogenin. The molecular biology showed the characteristic PAX3-FKHR fusion gene. Despite combination chemotherapy and radiation, the patient died of her illness within one year with disseminated metastases.
Conclusion: RMS, notably of the alveolar type, rarely present in the head and neck of adults, but should be considered in the differential diagnosis of a small cell neoplasm in patients during the third and fourth decade of life. Genetic testing may be successfully used for diagnosis and may guide therapy in future clinical trials. Despite the poor prognosis, prompt diagnosis and palliative radiotherapy may improve the qualityof life for patients with terminal disease.

P-310
TELOMERASE CATALYTIC SUBUNIT HTERT EXPRESSION IN PATIENTS WITH PRECANCEROUS LESIONS OF THE LARYNX

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Telomerase is a specific enzyme which functions as a reverse transcriptase. It synthesizes telomeric repeats at the chromosomes' endings, protecting them from excessive shortening and damaging important genes. The correlation between telomerase activity during cellular immortalization and reactivation in tumours has led to the view that telomerase reactivation is required for tumour growth. Nevertheless the telomerase reactivation in precancerous lesions is still unclear. The diagnosis and treatment of premalignant lesions of larynx has been frustrated because of failure to adequately define the histologic changes that may predict the potential for progression to invasive squamous cell carcinoma.
The aim of our study is the evaluation of catalytic subunit of telomerase (hTERT) expression in precancerous lesions of the larynx as well as its impact on possibility of neoplasm progression.
Patients and methods: The research was conducted on the group of 104 patients with precancerous lesions of the larynx, which were operated in direct microlarygoscopy. Immunohistochemical staining of paraffin sections was performed using monoclonal antibody NCL-hTERT (Novocastra) against telomerase protein and avidine – biotin – peroxydase detection system ABC.
Results: Telomerase expression was detected in 100% patients with precancerous lesions with the intensification between 5% and 75% as nuclear reaction in the form of brown granulations. The mean hTERT expression equalled 18,4% in simple hyperplasia, 24,8% in abnormal hyperplasia, 46% in atypical hyperplasia and 55,7% in carcinomas in situ.
Statistically significant dependence in hTERT expression was stated between simple hyperplasia in relation to abnormal and atypical hyperplasia as well as to carcinoma in situ ($p=0{,}001$; $p=0{,}002$; $p<0{,}01$). Similar observation was made between abnormal and atypical hyperplasia ($p=0{,}003$), whereas no statistically relevant dependence was found in telomerase expression between atypical hyperplasia and carcinoma in situ ($p=0{,}07$).
Conclusion: Telomerase expression increases in conjunction with the progression of the precancerous lesion and is potential factor which will facilitate diagnostication and making a prognosis in patients with precancerous lesions and cancer of the larynx.

P-311
POTENTIAL PROGNOSTIC IMPORTANCE OF MMP-1, 2 AND 9, AS WELL AS TIMP-1, 2 AND 3 IN PATIENTS WITH LARYNGEAL SQUAMOUS CELL CARCINOMA

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Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes implicated in the invasion and metastasis of many cancers, because of their ability to degrade components of extracellular matrix. Their activity is regulated by specific tissue inhibitors of matrix metalloproteinases (TIMPs). MMPs and TIMPs was identified in many carcinomas, including cancers of head and neck region, lung, breast and colon. In most of the researches a strong

dependence between MMPs and TIMPs expression and clinical course of the disease was observed.
The aim of the research was to evaluate the expression of MMP-1, -2 -9 and TIMP-1, -2 -3 in laryngeal cancer cells and to investigate the prognostic impact of these factors in patients with laryngeal cancer.
Material and method: 104 patients with laryngeal cancer, that underwent surgical treatment were included in the study. Only cases with at least a 5-year follow-up were included. Immunohistochemical studies were performed on formalin fixed, paraffin embedded sections by using monoclonal antibodies against MMP-1, -2, -9 and TIMP-1, -2, -3 antigen and ABC detection system.
Results: For all of the markers diffuse, cytoplasmatic staining of tumorcells and some surrounding stromal cells was observed. There was a correlation between TIMP-2 and TIMP-3 overexpression and advanced carcinomas (p= 0,037 and p=0,022, respectively). Regarding the otherenzymes investigated we could not find any correlation between their expression and tumor's stage. We observed a strong association between MMP-2 expression and the presence of nodal metastses (p=0,013). It was found that the TIMP-2 overexpression was significantly more frequent in the tumors of patients facing lymph node metastases during the follow-up period compared with those having no nodal relapses (p=0,05). No correlation was observed between other enzymes expression and nodal status and nodal or local recurrences. We found that positive immunostaining of MMP-1, -2, -9 and TIMP-1, -2 was significantly related with higher tumor grade. Specially strong correlation was seen between TIMP-2 and differentiation (p<0,001). There was no association between TIMP-3 expression and grading. We found that tumors expressing TIMP-2 were significantly associated with a poorer prognosis. Patients with TIMP-2 positive expression had diminished both overall and disease-free survival (p= 0,049). Besides the overall survival was shorter in cases with positive MMP-9 expression. This correlation was statistically borderline (p=0,07). The survival analysis revealed no difference between overall and disease-free survival time of patients in groups with positive and negative expression of MMP-1, -2 and TIMP-1, -3.
Conclusions: Our data suggest the existence of correlation between expression of MMPs and TIMPs and clinicopathological features of laryngeal carcinoma. Moreover TIMP-2 is a significant prognostic parameter for patients with laryngeal cancer.
We observed a strong association between MMP-2 expression and the presence of nodal metastses (p=0,013). It was found that the TIMP-2 overexpression was significantly more frequent in the tumors of patients facing lymph node metastases during the follow-up period compared with those having no nodal relapses (p=0,05). No correlation was observed between other enzymes expression and nodal status and nodal or local recurrences. We found that positive immunostaining of MMP-1, -2, -9 and TIMP-1, -2 was significantly related with higher tumor grade. Specially strong correlation was seen between TIMP-2 and differentiation (p<0,001). There was no association between TIMP-3 expression and grading. We found that tumors expressing TIMP-2 were significantly associated with a poorer prognosis. Patients with TIMP-2 positive expression had diminished both overall and disease-free survival (p=0,049). Besides the overall survival was shorter in cases with positive MMP-9 expression. This correlation was statistically borderline (p=0,07). The survival analysis revealed no difference between overall and disease-free survival time of patients in groups with positive and negative expression of MMP-1, -2 and TIMP-1, -3.

P-312
Fhit and Wwox Expression in Mucoepidermoid and Adenoid Cystic Carcinomas

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Background: Mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC) are salivary gland neoplasms with divergent morphological features and clinical behaviour. ACC is a basaloid tumor whereas MEC is a glandular-epithelial neoplasm. FHIT and WWOX are tumor suppressor genes that encompass the FRA3B and FRA16D fragile sites at chromosomes 3p14.2 and 16q23.3–24.1, respectively. In our previous studies, we have shown concordant loss of Fhit and Wwox expression in breast cancer, significantly more in basal like phenotype. To test if there is a similar association in salivary gland neoplasms, we designed this study on tissue microarrays (TMA).
Methods:TMAs were constructed from 25 MEC and 19 ACC of salivary gland. Fhit, Wwox, CK14, CK5/6, CK19, SMA and p63 immunostainings were performed on TMAs. Expression in more than 10% of tumor cells was accepted as positive for CK14, CK5/6, CK19, SMA and p63. Expression of Fhit and Wwox in more than 50% of neoplastic cells were assessed as positive while any other expression level was accepted as reduced. Correlations between immunohistochemical markers and their relation with histological type were determined.

Results: Reduced Fhit and Wwox expression was significantly more common in ACC (p=0.012, p=0.003, respectively). There was a negative association between Fhit and Wwox with SMA (p=0.004, p=0.01, respectively). The relation between Fhit and Wwox expression and CK5/6, CK19 and p63 were not significant. Wwox and CK14 expression related negatively (p=0.02) while there was no relation between Fhit and CK14.
Conclusion: Our results suggest that reduced Fhit and Wwox expression might have a role in the pathogenesis of basaloid differentiation in salivary gland neoplasms similar to breast cancer. Studies designed on basaloid tumors of other organ systems may show similar findings and these results may have implications on treatment modalities designed for basal like tumors.

P-313
INTERGRIN LINKED KINASE (ILK) AND p-Akt EXPRESSION IN LARYNGEAL CANCER.

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Background Laryngeal carcinoma has an ominous progression because of local invasiveness and nodal metastasis. It is evidenced in cases of many epithelial cancers that the ILK is a central molecule in many pathways that are being activated during the cancer formation which endow the cancerous cells with molecular and phenotypic traits that potentiates the cell to metastasize. Moreover p-Akt seems to be a downstream intervener of the ILK-activated molecular pathway which regulates many crucial events for the cancerous cell, like altered adhesion, inhibition of apoptosis and induction of angiogenesis.
Methods In order to examine the role of ILK and p-Akt in the pathogenesis of laryngeal cancer we evaluated the expression of these molecules using immunohistochemical methods in paraffin embedded tissue samples from 97 (n= 97) squamous laryngeal cancers. Statistical analysis was performed by SPSS for windows. The correlation between the expressions of the two proteins was evaluated by Spearman statistical test.
Results It was detected an increase expression of ILK and p-Akt in 81% and 76% of tumor specimens respectively, comparing to the normal controls. The correlation of expression pattern between ILK and p-Akt was statistical significant (p=0,003).
Conclusion The nodal role of ILK for the transition of a cancerous epithelial cell to a cell with all the proper molecular mechanisms that potentiates it to invade has been already described in cancer. This was the first time, to the best of our knowledge, the enhanced expression of ILK in laryngeal cancer to be described. Besides we have evidence that its action is applied probably through the already described pathway of PI3K-pAkt to which are attributed many of the aggressive characteristics of a metastatic cell. By making progress to the comprehension of the molecular mechanisms of the metastatic process, the nodal role of molecules, like ILK and p-Akt, are highlighted.

Uropathology

P-314
Extracellular matrix alterations in conventional renal cell carcinomas by tissue microarray profiling influenced by chronic low-dose ionizing radiation exposure in humans

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Background. The accident at the Chernobyl power plant in Ukraine on April 1986, introduced for the first time the problem of chronic persistent low-dose exposure to ionizing radiation (IR). Redox homeostatic processes in the microenvironment, involving interaction between cell-cell and cell-extracellular matrix (ECM), asociated with cell-cell comunication in conventional (clear cell) renal carcinomas (cRCCs) in patients living more than 20 years after the Chernobyl accident in Cesium (137Cs) - contaminated areas of Ukraine.
Material an method. The ECM major components such as fibronectin, laminin, E-cadherin/β-catenin complexes as well as p53 tumor suppressor gene protein and transforming growth factor beta 1 (TGF-β1) were immunohistochemically (IHC) evaluated in cRCCs from 59 Ukrainian patients, wich represented 18 living in non-contaminated areas and 41 from 137Cs - contaminated areas. A control group of 19 Spanish patients with analogue tumors were also investigated. For IHC evaluation a tissue microarray technique was used.
Results. Decrease or loss and abnormal distribution of fibronectin, laminin, E-cadherin/β-catenin complexes accompanied by elevated levels of p53 and TGF-β1 were detected in the Ukrainian cRCCs from 137Cs- contaminated areas with statistically significant differences.
Conclusion. Our study suggests that chronic long-term, low-dose IR exposure migth result in global remodelling of

ECM components of the cRCCs with disruption in peri-epithelial stroma as well as epithelial basement membranes that leads to loss of cellular differentiation. These factors may also become relevant targets for novel therapeutic strategies.
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ALTERATION OF APOPTOTIC REGULATORY MOLECULES IN CONVENTIONAL RENAL CELL CARCINOMA INFLUENCED BY CHRONIC LONG-TERM LOW-DOSE IONIZING RADIATION EXPOSURE IN HUMANS REVEALED BY TISSUE MICROARRAY*

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Background. During the 20-year period subsequent to the Chernobyl accident the morbidity of malignant renal tumours in Ukraine has increased from 4.7 to 9.0 per 100,000 of total population. Cesium 137 (^{137}Cs), which accounts for 90% of the internal radioactivity in the Ukrainian population exposed to long-term low-dose radiation and 90% of the more labile pool of ^{137}Cs, is excreted via the kidneys. The goal of our present study was to evaluate the status of pro- and anti-apoptotic regulatory molecules in conventional renal cell carcinomas (cRCCs) in Ukrainian patients.
Materials and Methods. Bcl-2, Bcl-x, BAX, death receptor (DR5), and transcriptional nuclear factor kappa B (NF-kB, with p50 and p65 subunits) were immunohistochemically (IHC) investigated using a tissue microarray technique in cRCCs from 56 Ukrainian patients, consisting of 18 patients living in non-contaminated areas and 41 patients from ^{137}Cs-contaminated areas. As a comparison, 19 Spanish patients with analogue tumours were also investigated.
Results. Our findings showed that BAX and DR5-positive cRCCs tend to increase among the Ukrainian patients living in the radio-contaminated areas along with the suppression of anti-apoptotic molecules (Bcl-2 and Bcl-x) and with p65 and p50 over-expression in these same tumours, with statistically significant differences.
Conclusions. Our study suggests that chronic long-term, low-dose radiation exposure might result in the alteration of apoptotic regulatory mechanisms, which in turn, could lead to enhanced tumour progression and resistance to apoptosis. These factors may also become relevant targets for novel therapeutic strategies.
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P-316

Gene amplification and protein expression of epidermal growth factor receptor (EGFR) in prostatic adenocarcinoma after hormonal therapy

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Background: Previous investigations have provided support for the contention that EGFR may be responsible for prostate cell growth. Evidence that over-expression of EGFR in hormone refractory prostate cancer may provide a rationale for new therapeutic strategies.
Methods: The current study was conducted to investigate whether there is significant EGFR over-expression and gene amplification in a population of patients treated with hormonal therapy using fluorescence in situ hybridization and immunohistochemical staining method.
Results: Immunohistochemically, EGFR expression was demonstrable in 57 of 71 tumors. Membranous immunostaining for EGFR was observed in >75% of tumor cells in 11% of cases, in 51–75% of tumor cells in 20% of cases, in 26–50% of tumor cells in 21% of cases, in 11–25% of tumor cells in 21% of cases, and in 1–10% of tumor cells in 7% of cases. No immunostaining for EGFR was seen in 20% of cases. FISH for EGFR gene amplification was performed in all 71 cases. EGFR gene amplification was present in 1 of 71 tumors. Polysomy (more than two copies of CEP7 and EGFR/CEP7 ratio < 2) of chromosome 7 was present in 24 of 71 tumors. Disomy was present in the remaining 46 specimens. There was no statistically significant association between the EGFR expression and other clinicopathologic characteristics including patients' ages,

different hormonal therapy regimens, and metastatic status. There was no correlation between EGFR protein expression and gene amplification.

Conclusion: Our findings provide additional evidence that EGFR plays a significant role in the progression of prostate cancer to an androgen-independent state. Future studies are needed to evaluate the overall significance of the amplification of EGFR and its potential use in anti-EGFR therapies.

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Standard Gleason grading system vs 2005 WHO/ISUP modified grading: A comparative study based on 172 needle prostatic biopsies.

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Background: In 2005, the Gleason grading underwent its first major revision. The aim of our study was to compare the concordance of pattern and change of prognostic group between these two readings. Additionally, to check any disciminative power of the modified grading we compared the time of biochemical (PSA) progression-free outcome according to prognostic groups.

Method: The study was based on 172 biopsies of patients that were subsequently submitted to radical prostatectomy (RP). Four prognostic Gleason grading groups were considered: scores 2–4, 5–6, 7, and 8–10. Several clinicopathologic variables were studied. The second reading based on the modified grading was done by the same reviewer (AB). Time to biochemical progression was studied using the Kaplan-Meier product-limit analysis.

Results: Comparing the two readings, there was concordance in 83.14%, 63.3%, and 68.02% for Gleason primary pattern, Gleason secondary pattern, and Gleason score, respectively. The second reading reflected in a change towards a higher Gleason grading group in 46/172 (26.74%) patients. The distribution of the prognostic groups for the standard reading was 1 (0.58%), 117 (68.03%), 44 (25.58%), and 10 (5.81%) biopsies, respectively, for the groups 2–4, 5–6, 7, and 8–10; for the modified reading was 0 (0%), 85 (49.42%), 68 (39.53%), and 19 (11.05%), respectively. The Kaplan-Meier curve showed no significant difference among patients stratified according to prognostic groups with the standard Gleason reading (log-rank, $p=0.524$) but a significant difference in outcome for patients stratified according to the revised reading (log-rank, $p=0.010$).

Conclusion: The highest impact of the modified grading is on the secondary pattern resulting in a change towards higher prognostic groups resulting in a significant biochemical progression outcome following RP in our study. The findings favor that the WHO/ISUP modified grading is a valuable refinement for the standard Gleason grading system.

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Clinicopathologic findings and biochemical (PSA) progression following radical prostatectomy of patients with microscopic bladder neck involvement: A study in Brazil.

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Background: It is controversial whether microscopic invasion of the bladder neck has a high risk for biochemical progression following radical prostatectomy. The TNM classification for prostate cancer considers bladder neck involvement to be pT4 disease, however, it does not specifies whether the invasion is macroscopic or microscopic.

Method: The study was based on 290 whole-mount consecutive radical prostatectomies. Biochemical progression was defined as PSA $\geq$0.2 ng/mL. Time to biochemical progression-free outcome was compared using the Kaplan-Meier product-limit analysis. To assess individual variables for risk of biochemical progression we created a univariate Cox proportional hazards model, and to assess the influence of several variables simultaneously, we developed a final multivariate Cox proportional hazards model of the statistically significant covariates.

Results: Bladder neck invasion was present in 55/290 (18.96%) surgical specimens and absent in 235/290 (81.03%). Patients with bladder neck invasion had significantly higher preoperative PSA ($p<0.001$), higher Gleason score ($p<0.001$), higher positive surgical margins ($p=0.049$), and a more advanced pathological stage ($p=0.002$). At 5 years, the progression-free outcome was 42%, 44%, and 28% for patients with microscopic bladder neck invasion, extraprostatic extension, and seminal vesicle invasion, respectively. The relative risks for PSA progression by univariate Cox analysis were 1.60, 1.85, and 2.35 for the same variables, respectively. The multivariate Cox proportional hazards models determined that the PSA progression relative risk in patients with bladder neck involvement was not significant when combined either with extraprostatic extension or seminal vesicle invasion.

Conclusion: Patients with bladder neck invasion have significantly higher preoperative PSA, higher Gleason score, higher positive surgical margins, and more advanced pathological stage compared to patients without bladder neck invasion. However, the outcome following radical prostatectomy compared either to patients with extraprostatic extension or seminal vesicle invasion do not favor considering microscopic bladder neck invasion as stage pT4.

P-319

Histological, histochemical, and immunohistochemical study of normal Skene's glands: An aid for establishing the origin from these glands of a case of adenocarcinoma of the female urethra negative for prostate-specific antigen.

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Background: Female primary urethral tumors are rare. Detailed microscopic descriptions of normal Skene's glands are not found in histology textbooks as well as in descriptions of tumors originating from these glands. A detailed histological, histochemical, and immunohistochemical study of normal Skene's glands was the clue for establishing the origin from these glands of an adenocarcinoma negative for prostate-specific antigen (PSA) in a 43-year-old female.

Method: We studied the histology of normal Skene's glands from autopsies and noninvolved glands in periurethral tumors. The histochemistry and immunohistochemistry study included: PAS, mucicarmin, alcian blue (pH 2.5), alcian blue (pH 0.4), alcian blue (pH 1), alcian blue/PAS (pH 2.5), colloidal iron, colloidal iron/PAS, bcl2, S-100, Ki-67, estrogen and progesterone receptors, 1A4, AE1/AE3, CK7, CK20, p53, p63, CD10, 34betaE12, CEA, and PSA.

Results: The female urethral mucosa is lined in most of its extension by a squamous nonkeratinizing epithelium that is continuous to the invaginated Skene's ducts. After a short distance, this epithelium is abruptly substituted by a transitional type epithelium lined in its luminal border by columnar eosinophilic cells. At the every end of the ducts, the columnar cells form alveolar outpouchings of clear cells. The strongest reactions showed positivity for CEA and AE1/AE3 (squamous nonkeratinizing epithelium); p63 and 34betaE12 (transitional type epithelium); CK7 (columnar eosinophilic epithelium); and PAS, alcian blue/PAS (pH 2.5) and colloidal iron (clear secretory cells). Only scattered secretory clear cells in some glands showed PSA positivity in the luminal part of the cytoplasm.

Conclusion: The microscopy of the periurethral adenocarcinoma reproduced all tissues of normal Skene's glands as well as the correspondent histochemical and immunohistochemical findings. Mucinous stromal pools stained similarly to the secretory cells of normal glands. In spite of negative PSA, the findings of the tumor were consistent for an origin from Skene's glands.

P-320

Malignant Solitary Fibrous Tumor of the Urinary Bladder. An Immunohistochemical and Electron Microscopic Study of 1 case.

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Background: Solitary Fibrous Tumor (SFT) may occur at any site, but it has been most frequently described in the pleura. In a search of the literature, we could only identify 11 cases of SFT arising in the urinary bladder.

Method: SFT was determined to have malignant component if there was focal areas of marked increased cellularity (≥ 5% of the tumor), with greater than 4 mitoses/10 HPF, with or without foci of necrosis. Immunohistochemical studies were performed using the Dako Envision + Kit and the following primary antibodies: pan-keratin (CK22), CK AE1/AE3, EMA, vimentin, smooth muscle actin (SMA), muscle specific actin (MSA), calponin, desmin, S-100 protein, ALK, CD34 and bcl-2. Tissue for electron microscopy was also taken and fixed in 2.5% glutaraldehyde and processed according to standard methods.

Results: a 74-year- old man presented with a recent history of gross hematuria. The patient underwent cystoprostatectomy without complications. Grossly, the tumor was a polypoid white mass that measured 10 cm and was attached by a short pedicle to the bladder wall. Microscopically, the tumor was comprised of densely packed spindled cells with a high mitotic rate (>30/10 HPF), wich form interlacing bundles resembling fibrosarcoma. The tumor cells stained diffusely for vimentin, CD34 and bcl-2, and focally for SMA by immunohistochemistry but were negative for CK22, CK AE1/AE3, EMA, MSA, calponin, desmin, S-100 and ALK. Ultrastructurally, the tumor cells had spindle or ovoid nuclei with irregular nuclear contorns and small nucleoli. The cytoplasm contained ribosomes, mitochondria, and dilated rough endoplasmic reticulum. These features were suggestive of primitive mesenchymal or fibroblast-like cells.

Conclusion: SFT is a rare mesenchymal tumor of the urinary bladder, but should always be considered in the

differential diagnosis of spindle cell neoplasms encountered in the lower urinary tract.

P-321

Nuclear fractal dimension in the study of prostate cancer.

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Background: Fractal dimension provides a measurable, precise and reproducible approximation of nuclear structure properties and especially nuclear shape complexity. Considering the applications of fractal analysis in the field of histopathology many studies have been published evaluating fractal dimension as an indicator of complexity and irregularity of tumor tissues. However, extended fractal analysis studies of prostate cancer are lacking.
Methods: Archival pathologic material from 42 radical prostatectomy specimens was examined and the pertinent medical files were reviewed. Histological sections from the 42 carcinomas as well as from non-malignant adjacent areas from 12 cases were stained with Feulgen for nuclear complexity evaluation. Two randomly selected high power fields from each case were digitally archived. These files were automatically transformed to binary images suitable for fractal analysis, through a non-supervised histogram-based clustering procedure. The nuclear fractal dimension (FD) on each image was estimated by using an implementation of the widely used box-counting algorithm in a specially designed application.
Results: Prostate carcinomas presented higher mean values of nuclear FD compared to areas of normal prostate. The difference was statistically significant ($p<0.01$). Well-differentiated neoplasms had significantly lower FD values than poorly differentiated ones. FD was significantly and positively correlated with nuclear size morphometric parameters ($r=0.67–0.75$, all $p<0.05$), as well as with Gleason score ($R=0.61$, $p<0.05$).
Conclusions: Prostate cancer nuclear FD seems to be an important morphometric parameter possibly valuable for the diagnosis as well as for the future development of an unbiased and reproducible image analysis system for the study of prostate cancer pathology.

P-322

Collecting duct renal cell carcinoma with divergent sarcomatoid components: a case report with potential implications in the pathogenesis of renal cancer dedifferentiation.

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BACKGROUND: Sarcomatoid components in renal cell carcinomas (RCC) has been described in up to 10% of cases and considered a high grade lesion in terms of tumor differentiation related to poor patient outcome. Collecting duct carcinoma represents only 1% of all histologic types in RCC. We present a case of a 75-year-old man with a collecting duct RCC presented in our hospital with abdominal pain and hematuria. Magnetic resonance imaging (MRI) and sonography revealed both a heterogenous tumor of the left renal parenchyma 11 cm in diameter with tumor areas resembling osteo- and chondrosarcoma in addition to the more frequent fusiform and storiform areas resembling a fibrosarcoma. METHODS: After radical left nephroureterectomy all tumor fragments were formalin-fixed and paraffin-embedded, and representative sections were stained with hematoxilyn & eosin and, immunohistochemically, with vimentin, CD10, 34betaE12 antigen, S-100, SOX9, osteonectin, osteocalcin, actin, desmin, COX2, CD117 (c-KIT) and Her2-Neu. RESULTS: The tumor demonstrated areas displaying irregular ducts characteristic of collecting duct RCC. Tumor areas with collecting duct appearance show specific immunoreactivity against 34betaE12 antigen along with reactivity against mesenchymal antigens (osteonectin, osteocalcin and SOX9) and vimentin. Otherwise, dedifferentiated fusocellular zones presented specific immunostaining with mesenchymal antigens such as osteonectin, osteocalcin, SOX9 and vimentin), whereas areas of typical osteosarcoma and chondrosarcoma demonstrated the presence of osteoid material and intense immunoreactivity with osteogenic antigens in the former, and intense reactivity against S-100 and SOX9 in the latter. Otherwise, dedifferentiated fusocellular zones presented specific immunostaining with mesenchymal antigens such as osteonectin, osteocalcin, SOX9 and vimentin), whereas areas of typical osteosarcoma and chondrosarcoma demonstrated the presence of osteoid material and intense immunoreactivity with osteogenic antigens in the former, and intense reactivity with S-100 and SOX9 in the latter. Interestingly, this neoplasm showed an intense Her2-Neu immunostaining in all histologic subtypes in contrast to the diffuse lack of immunostaining with c-Kit and COX-2.

CONCLUSION: Our study appears to demonstrate, at least in the current case, that in dedifferentiated types of RCC the epithelial component demonstrated a specific cytokeratin immunophenotype, whereas the both epithelial and sarcomatoid components expressed indistinctly mesenchymal markers at the immunohistochemical level.

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Atheromatous embolization (AE) diagnosed on bladder transurethral resection (B-TUR) specimens: a report on nine cases

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Background: Atheromatous embolization (AE) is an underrecognized medical disorder due to vascular migration of cholesterol crystals from atherosclerotic plaques. It is though to be associated with a high mortality rate. Since it has been very rarely reported on B-TUR specimens its true clinical relevance for this group of patients is largely unknown.

Objectives: To assess the clinical profile of patients and the prognostic relevance of finding AE on B-TURs performed for bladder cancer.

Methods: Review of clinical charts and pathology slides from patients codified as having AE on B-TUR specimens between 1988 and 2007 at the Department of Pathology, Hospital Universitari de Bellvitge.

Results: Nine patients were identified. All of them were male, aged 51 to 87 years, and all had some risk factor known to be associated with AE. Eight were or had been smokers. Four patients were under treatment for hypertension. Three had received anticoagulant or antiagregant therapy. One patient had a past history of cardiovascular surgery. In five patients AE appeared to be a spontaneous phenomenon with no precipitating factors. No patient had evidence of AE involving other anatomical sites. AE was seen as needle-shaped cholesterol clefts occupying the lumen of small arterial vessels mainly in the deep subepithelial tissue or submucosal layer of the bladder. There were two deaths, due to tumor progression, one and three years after the diagnosis of AE in bladder carcinoma. No deaths attributable to cardiovascular causes were recorded till date.

Conclusion: AE is an unusual, though not exceptional, incidental finding on B-TUR performed for bladder cancer. It is often a spontaneous phenomenon, with more than half of the patients lacking an obvious precipitating factor. In contrast with AE involving other organ systems, AE as an isolated finding on B-TUR specimens does not seem to have prognostic impact on survival.

P-324

Unusual Presentation of an abdominal seminoma

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45 years old man presented with 3–4 months history of progressive abdominal pain and lower quadrant mass. CT scans showed 9×8×7 cm.s diameter well-defined bulky mass with smooth border and compressed bladder base. It was supposed to be a mesenteric cyst in abdomen. Macroscopically a gray-cream homogenous lobulated mass, composed of multiple distinct nodules, the biggest of which was 7 cm in diameter. Microscopically, tumor showed a histopathology of a seminoma. Mitoses were numerous in tumor cells. It was emerged on undescended testis and limited in the testis, and there wasn't any lymph node and other metastases until this time. Although he has got no testis and seminal vesiculum at right side, he wasn't infertile.

P-325

Squamous cell carcinoma of the urinary bladder. A clinical immunohistochemical study.

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Background: Squamous Cell Carcinoma (SCC) arising in the urinary bladder consists 2–7% of all urothelial infiltrating cancers and is thought to arise through a process of squamous metaplasia. In areas were Schistosomiasis is edemic this percentage rises up to 70%.

Methods: We re-evaluated 102 cases of urinary bladder carcinomas in a two year period (2006–2007) and we reported 3 cases of SCC of the urinary bladder. The majority of the cases concerned Papillary Urothelial Carcinomas (PUC) (97 cases- 96.08%), one case was metastatic from the prostate (0.98%) and 3 cases were pure SCC (2.94%). The mean age of patients with SCC in this series was 66 years and the male to female ratio was 3:0. Hematuria was the presenting symptom in all the cases.

Results: Grossly the first two cases were biopsy tissue samples from the urinary bladder measuring 1.7 cm and 3 cm respectively, while the third case was a total cystectomy. In the latter case the tumor located in the

bladder neck adjacent to the paraurethral side of the prostate and measured 4.8×4.5×2 cm. All cases infiltrated the smooth muscle wall of the urinary bladder and were staged pT3b(i) the first 2 cases and pT3b(ii) the 3rd. According to Bergkvist grading system the two first cases were grade IV, while the third was grade III with extensive keratin formation. No squamous metaplasia was found adjacent to the tumors. The immunocytochemical analysis showed strong positivity for Cytokeratin (CK) 7, 8 and Pankeratin, while CK20 was not immunoreactive. In situ hybridization for HPV was performed.

Conclusion: Because none of the patients had a history of Schistosomiasis or irradiation, we suspect that SCC of the urinary bladder maybe associated with chronic irritation and inflammation. One of our patients had multiple surgical procedures which maybe set the stage for subsequent development of SCC.

P-326

A case of nephrogenic adenoma in the female urethral diverticulum.

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INTRODUCTION. Nephrogenic adenoma is a rare benign lesion that can occur at several sites along the urogenital tract that is typically preceded by some form of genitourinary injury. It is composed of small glandular structures that develop along the urothelium. Their pathogenesis is controversial. Generally presumed to be a metaplastic process of the urothelium, recent evidence suggest that it may be derived from detached renal tubular cells implanting along the urothelial tract in previously injured areas. It is important to recognize this entity and to be aware of its histologic characterisitcs to avoid misdiagnosis of malignant neoplasms occurring at the same sites.

CASE REPORT 44 year old female with a history of periurethral pain for the last 3–4 years. A urethral diverticulum was observed by magnetic resonance and cistoscopy and partially resected. Histologic examination revealed a diverticulum with an urothelial epithelium, squamous metaplasia and chronic inflammation. In some sites we saw clusters of glandular or tubular proliferation, along the urothelium lining, in an edematous and inflammatory stroma, without significant cytologic atypia and few mitoses. The CAM 5.2 expression on basal cells confirmed that it was a benign process.

CONCLUSION The most frequent localisation of Nephrogenic adenoma is in bladder trigon (79%), ureter (19%) and urethra. It is more frequent in males than in females (3:1). It can be confused with malignant neoplasms occurring at the same sites as clear cell carcinoma, nested or microcystic variants of urothelial carcinoma and prostatic adenocarcinoma. There is no specific immunohistochemical profile to distinguish this lesion from its malignant mimickers. Clinicopathologic correlation with careful attention to morphology is the most important way to achieve a correct diagnosis.

P-327

Development of a testicular germ cell tumor model in nude mice for the study of tumor biology and resistance to cisplatin treatment

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Introduction: Testicular germ cell tumor (TGCT) is the most frequent cancer in young males. Chemotherapy based on cisplatin together with surgery, leads to a very high curation rate. Nevertheless, 15–20% of patients, mainly in the non-seminomatous germ cell tumors (NSEGCT) group, will no achieve a complete remission after treatment. Most of these patients are refractory to cisplatin and die of disease. Little is known about the mechanisms of resistance to cisplatin. ***Aim:*** To identify genes involved in the development of TGCT and in the resistance to cisplatin-based treatment. ***Results:*** We have developed a model for the study of NSEGCT, based on orthotopic implantation of primary human NSEGCT in nude mice. Eleven of 28 NSEGCT (39.3%) grew as orhotopic implants in nude mice, maintaining the pathological features of primary tumors. Five tumors developed metastasis 6–8 months after orchiectomy performed when tumors raised a palpable size. Whole genome analysis (Nimblegen microarrays, 60.000 bp resolution) and expression profiles analysis (Affymetrix microarrays) were performed in perpetuated tumors as well as in paired cases of tumors/metastases obtained after orchiectomy in mice. Our genetic analysis confirms previous results, identifying recurrent regions of gain and loss in TGCT. Presence of gene mutations and epigenetic methylation changes were studied for a subset of

genes. *In vivo* response pattern to cisplatin was established in 9 tumors. Five of these tumors became resistant to cisplatin after exposure to increasing doses of the drug. Comparative whole genome and expression profiles analysis are being performed in paired cisplatin-sensible/resistant tumors in order to identify genes involved in the acquisition of resistance. ***Conclusions:*** We have developed a new model for the study of NSEGCT biology and the mechanisms of resistance to cisplatin treatments. This is a suitable preclinical model to assay new therapeutic approaches for improving prognosis of patients refractory to cisplatin.

P-328

Testicular tumour with an unusual histological pattern

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BACKGROUND Polyembryoma is a distinctive form of mixed germ cell tumour of the gonads composed of embryonal carcinoma and yolk sac tumour, sometimes with teratomatous components that are arranged in a pattern resembling the presomitic embryo. This tumour was first described by Peyron at 1939.

METHODS We present the case of a 19 year-old male with a testicular painless mass of 7 cm in its greatest dimension, predominantly solid.

RESULTS Diagnose was mixed germ cell tumour constituted by 20% seminoma and 80% polyembryoma (yolk sac tumour + embryonal carcinoma + teratoma, with abundant embryoid bodies). The embryo-like formations consist of a central "germ disc" composed of embryonal carcinoma epithelium and two cavities, a dorsal one recapitulating the amniotic cavity and a ventral one recapitulating the yolk sac cavity. The later is separated from the germ disc by a thin layer of yolk sac epithelium. Surrounding the embryoid bodies there is a loose, primitive, extraembryonic mesenchyme, giving rise to a very distinctive low-power appearance.

COMMENTS These unique histological features lead some authors to categorize it as a separate tumour. However, in the WHO histological classification of testis tumours it is classified as a tumour of more than one histological type. We agree with the opinion that is preferable to consider polyembryoma as a unique and distinctive form of highly organized mixed germ cell tumour, given that it behaves and is treated like other mixed germ cell tumours with similar components. Moreover, as in our case, polyembryomatous foci always comprise a part of a mixed germ cell tumour, and to our knowledge, polyembryoma has not been reported in pure form in the testis.

P-329

Lymphoepithelioma – like Carcinoma of the Urinary Bladder

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Background: Lymphoepithelioma-like carcinoma (LLC) of the urinary bladder is a rare type of infiltrating urothelial carcinoma with only 53 cases reported to date. We report an additional case of a pure LLC which was recently diagnosed in our hospital.

Method: A 50-year old man with a history of heavy smoking was admitted with heamaturia and with the diagnosis of an urothelial carcinoma (T2, Grade III). The patient underwent a partial cystectomy and excision of the outer right and outer left iliac lymph nodes.

Results: Grossly we received 6 tissue specimens measuring in total 2.3×4.5×5.3 cm, of soft consistency and brownish color. Microscopically a tumor developed in the subepithelial connective tissue of the bladder invading the superficial muscle and focally the deep muscle of the bladder. No conventional solid invasive transitional cell carcinoma and no urothelial dysplasia or carcinoma in situ were identified. The tumor composed of nests and sheets of undifferentiated large cells with pleomorphic nuclei and prominent nucleoli. A prominent lymphoid stroma was present throughout the tumor and consisted mainly of small lymphocytes and eosinophils. All the excised lymph nodes were free of metastases.

Immunostaining with antibodies against pankeratin, cytokeratin 7, 8 and P53 showed positivity within carcinoma tumor cells and negativity for cytokeratin 20. The lymphocytes between the tumor cells stained with antibodies against CD3 and CD 20.

Conclusion: The undifferentiated character of the tumor, the heterogeneous nature of the inflammatory infiltrate and the epithelial nature of the tumor cells establish the diagnosis of LLC. Our case was grade IV according to Bergkvist classification or undifferentiated carcinoma according to WHO. It has been suggested that LLC of the bladder has a relatively favorable prognosis and should be treated by stage like other bladder carcinomas.

P-330

Pathologic analysis of 1000 cases of transrectal ultrasound-guided systematic prostate biopsy: establishment of new sample processing method and diagnostic utility of immunohistochemistry

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Background: We developed a new processing method for extended prostate needle biopsy, and evaluated diagnostic utility of routine immunohistochemistry in 1000 consecutive unselected cases of transrectal ultrasound-guided systematic prostate biopsy. **Materials and Methods:** Four to five biopsy cores were embedded in one paraffin block. All the biopsy cores were immunohistochemically stained with basal cell markers. **Results:** The new sample processing method was technically perfect for making a diagnosis from extended prostate needle biopsy. Among 1000 cases, there were 323 cases (32.3%) of adenocarcinoma, 5 cases of other malignant tumors, 9 cases of high-grade prostatic intraepithelial neoplasia without a carcinoma, and only 8 cases of atypical small acinar proliferation. Among the 323 cases of adenocarcinoma, there were 38 cases (11.8%) of microcarcinomas <0.1 cm and 101 cases (31.3%) of small adenocarcinomas <0.3 cm in length. In the needle biopsy specimens, 59 cases (18.3%) were classified as clinically insignificant carcinomas. Among them, 37 cases underwent radical prostatectomy, which turned out to be clinically significant carcinomas in 24 cases (64.9%). **Conclusions:** Routinely performed immunohistochemistry combined with the new sample processing method is very effective for detecting microscopic carcinoma foci as well as differentiating carcinoma from benign conditions mimicking cancer.

P-331

Morphology of testis at atherosclerosis

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Background.Up to now in the field of morphology of reproductive system of men the problems of testis involution and morphogenesis at atherosclerosis haven't been thoroughly investigated.

Methods.We have investigated histologically and ultrastructurally testicles of 63 died patients aged 36–89 with general atherosclerosis and testicles of 38 men aged 36–89 with minimal manifestations of atherosclerosis died in an accident.

Results.The absolute volume of rete of testicle of patients being ill with atherosclerosis decreases by 65–80% in comparison with the age control. Absolute volume of convoluted seminiferous tubules and interstitial tissue decrease by 24–27%. The lumen of arterioles also progressively decreases. Together with it at atherosclerosis there is Leidig cells volume expansion to 80%. At general atherosclerosis in testis there are zones of focal sclerosis of seminiferous tubules, whose area goes as far as 12.7% of shear section area. It was revealed that on the periphery of sclerosis zones there is a reduction of transaction area of seminiferous tubules by 20–33% with Sertolli cells quantity reduction by 17–24%. Ultrastructural investigation of Sertolli cells has revealed zones of dissociations of endoplasmatic reticulum and activization of lysosomes with a great number of lipid inclusions of various electron density. It has been stated the downward changes in index of spermatogenesis in convoluted seminiferous tubules by 63–65.6%, accounted for the quantity reduction of all kinds of cells. In some visual fields we revealed the destruction of spermatogenous cells up to the syndrome "only Sertolli cells" and intensifying processes of spermatogenous cell endoreproduction in seminiferous tubules.

Conclusion.Morphological changes of testis at general atherosclerosis consist in focal sclerosis of convoluted seminiferous tubules, reduction of transaction area of remaining seminiferous tubules, Sertolli cells quantity reduction and downward changes in index of spermatogenesis. These changes characterize «atherosclerotic testiculopatie», caused by chronic ischemia of testis.

P-332

Primary diffuse large B-cell lymphoma of the urinary bladder

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Primary urinary bladder lymphomas are very uncommon. Among them low grade lymphomas mainly MALT type occur most frequently, and they don't create important diagnostic problems because cell morphology is easily distinguished from urothelial neoplasms. High grade lymphomas from the urinary bladder are extremely rare, and can simulate undifferentiated carcinomas. Distinction is important as chemotherapy will replace usual surgical treatment.

We report a case of a large B-cell lymphoma developed in a 42-years-old woman. She initially presented hematuria, and cystoscopy showed an ill defined non-papillary mass. Small biopsies were taken, with broad necrotic areas and isolated

large atypical cells. No epithelial nest were found, although small pseudocordonal patterns suggested a poorly differentiated carcinoma. A new transuretral resection was performed, and histological study revealed a neoplasm composed of large occasionally epithelioid cells, with pale or clear cytoplasm, and atypical irregular nuclei. High mitotic rate was found and normal or slightly hyperplastic superficial urothelium was present in some areas. There was wide parietal infiltration with muscular invasion and again necrotic and hemorrhagic regions.
Immunohistochemically neoplastic cell expressed intensely B-cell markers (CD20 and CD79a). All low and high molecular weight cytokeratins were negative. Blc2, bl6, CD30 and ALK were also negative. Ki-67 (mib-1) expression was around 30%
Imaging revealed no pelvic lymphadenopathy and extension study of the patient couldn't find dissemination of the disease at the time of diagnosis. Low number of cases and studies makes optimal management still controversial. Our patient was treated with chemotherapy, and although follow up is still short no complications non recurrence have been detected.

P-333

Pathological features of latent prostate cancer: an autopsy study with complete prostate embedding and alpha-methyl-coacetate-racemase staining

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BACKGROUND Prostate cancer (Pr Ca) is the second cause of cancer-related death in males of Western countries. Previous autopsy studies suggested that Pr Ca might be more prevalent than it seems by its clinical detection rate. Thus, the concept of latent Pr Ca was coined mostly to refer to cases detected at autopsy, in patients dying from unrelated causes. The purpose of this ongoing project is to investigate the real incidence and the features of latent Pr Ca in a detailed clinico-pathological study.
DESIGN A total of 21 autopsy prostate specimens (age range: 56–87 years; mean age: 73.9 years) are the subject of this report. All the prostates were completely embedded. The tumor foci were measured and mapped. Doubtful cases were confirmed with α-methyl-CoA-racemase and p63 staining.
RESULTS The prevalence of Pr Ca in this preliminary series is 61.9%. PSA level is < 4 ng/ml in 71.5% of these cases. The most common Gleason pattern is 3+3 (53.8%) followed by 3+4 and 3+5 patterns (23.1% each). From these cases, 53.85% meet the criteria of insignificant Pr Ca, while the remaining 46.15% would have been considered clinically significant if previously detected. The age distribution was similar in the insignificant and significant subgroups. Overall prevalence of high grade PIN (hg PIN) is 76.2%. Pr Ca was found in 62.5% of patients with hg PIN and in 60% of patients without hg PIN.
CONCLUSION The prevalence of latent Pr Ca in our series is similar to those reported in the literature. Most of the tumors appeared in men older than 65 years and were most frequently insignificant, with Gleason pattern 3+3, but there was a remarkable proportion of potentially significant and higher grade tumors. The proportion of cancer was similar in patients with and without hg PIN. Besides, PSA levels were clinically low in the majority of patients with tumor. Therefore, hg PIN and PSA levels do not seem good predictors of latent Pr Ca. Nevertheless, a high number of cases must be included in order to draw more definitive conclusions on latent Pr Ca.
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P-334

Basal cell hyperplasia of the prostate with unusual morphologic pattern

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Background. Basal cell hyperplasia (BCH) is a microglandular proliferation of the prostatic transition zone, usually easy to diagnose. However, in rare cases this well-described lesion displays unusual morphologic patterns, causing confusion and difficulties in differentiating it from malignant lesions.
Method. We report the case of a 58 years old man who underwent adenomectomy for urinary tract obstruction symptoms. The histologic evaluation was made on haematoxyline-eosine stained sections. The presence of a focus with unusual morphologic pattern imposed an immunohistochemical study with monoclonal antibodies against Prostatic Specific Antigen (PSA), high molecular weight cytokeratin 34βE12 (HMWCK), S-100 protein, P504S and p63.

Results. On a backgroud of a benign prostatic hyperplasia (BPH) we noticed a circumscribed nodule of BCH which had the usual aspect at the periphery and a cribriform pattern in the centre. The cribriform structures were medium sized, centred by small luminal spaces and separated by an unremarkable stroma. At higher magnification cells were small, with scant cytoplasm and regular hyperchromatic oval nuclei. No mitotic figures, no necrosis and no infiltration were noticed. In immunohistochemistry prostatic basal cells markers (HMWCK, p63) were positive, but only as a single layer at the periphery of the glands. In the cribriform structures all the other cells were negative for these markers. Some adluminal cells expressed PSA marker. S-100 protein and P504S were negative in all cells.
Conclusion. The proposed terminology for this lesion is adenoid cystic-like BCH. This diagnosis is sustained by the identical bland morphology of the cells of both cribriform and classical BCH, even if not all these cells expressed basal cell markers. Cells labeling PSA markers represented residual secretory cells.Adenoid cystic-like BCH is a florid benign histologic variant of the BPH, important to recognise and to distinguish from cribriform PIN and the rarest adenoid-cystic and basaloid carcinoma.

P-335

Outcome prediction for renal cell carcinoma in adults: a ten-year review with 150 tumours

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Background: A wide of histopathological parameters have been investigated for their prognostic significance in renal cell carcinoma. Recent investigations have focused upon tumor diameter, invasion within the renal sinus, and the presence of tumour necrosis.
Methods: To study the significance of these parameters in the cases from our hospital, 150 nephrectomy specimens (from 1995 to 2005) were evaluated, assesing the pT classification, histologic subtype, tumor size and grade, presence of histologic tumor necrosis, perirenal, sinus, adrenal and vascular invasion (microvascular peritumoral and renal vein).
Objectives: Statistical analysis were performed to asses the morphologic features associated with metastasis-free survival and cancer-specific survival in our series and compared with literature.

P-336

IMMUNOHISTOCHEMICAL QUANTITATIVE ASSESMENT OF THE EXPRESSION OF GSTP1, iNOS AND COX-2 IN PIA, PIN AND CARCINOMA IN THE PROSTATE GLAND

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Background: The term “proliferative inflammatory atrophy” has been proposed for the first time by De Marzo and Marchi in 1999 to unite the common atrophy and postatrophic hyperplasia, combined with chronic or acute inflammation.It has been hypothesized that the proliferative inflammatory atrophy (PIA) is a neopastic precursor of the prostatic intraepithelial neoplasia (PIN) and of the prostatic carcinoma(PCa).The present study was intended to quantitatively asses and compare the levels of expression of the GSTP1, iNOS and COX-2 in PIA, PIN and PCa.
Materials ant methods: Archived tissue sections obtained after radical prostatectomy from cases (n=30) comprising of PIA, PIN and PCa were testet using immunohistochemistry with monoclonal antibodies against GSTP1, iNOS, and COX-2 (Labvision) and visualised by biotin-streptavidin-alkaline kit (DAKO LSAB 2 kit).The chromogen abundance was qantified using modified quantitative immunohistochemistry approach and measured as eu/pix ± standard error of mean.
Results: The mean values for the intensity of the expression GSTP1 were 122,1±8,64 for PIA, 69,4±11,2 for PIN and 53,8±12,5 for PCa.The mean values for the intensity of the expression iNOS were 78,1±10,16 for PIA, 17±13,12 for PIN and 45,7±11,36 for PCa.The mean values for the intensity of the expression COX-2 were 154,7±4,14 for PIA,113,2±3,95 for PIN and 80,5±4,63 for PCa.
Conclusion: The statistically different levels of expression provide opportunity these markers to be utilized as differential diagnostic and prognostic tools.

P-337

Multiple synchronous renal cell tumors

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The use of the nephron-sparing surgery demand research of multiple renal cell tumors.

We conducted a retrospective analysis of nephrectomies and autopsies performed in our clinic.
Of 240 renal cell tumors 52 (21, 7%) were multifocal. Most of the patients were men. 24 cases were present where the histological subtype of the largest tumor was clear cell. Satellite tumors were clear cell carcinoma (14), papillary adenoma (10), angiomyolipoma (7), renomedullary fibroma (4), Bellini duct carcinoma (1). 12 multiple renal cell tumors were found with T1, 3 with T2, 9 with T3A primary tumors. 8 patients had bilateral neoplasms. 28 cases were present only multiple papillary adenomas and carcinoma. Usually there were small tumors no more than 3 cm in diameter. In 15 cases multiple tumors originated on a background of nephrosclerosis of various genesis (chronic pyelonephritis, gouty nephropathy, amyloidosis).
When conservative surgery is performed, it should be remembered that 20% patients with renal cell tumor have multiple neoplasms which may cause local recurrence. The origin of multiple renal cell tumors can be bound up with hereditary predisposition or nephrosclerosis creating multifocal tumor field.

P-338
PROSTATE NEEDLE BIOPSY FINDINGS IN PATIENTS WITH BIOCHEMICAL FAILURE AFTER BRACHYTHERAPY

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Introduction. Brachytherapy (BT) is gaining favor as a treatment option for organ-confined prostate carcinoma (PC) with excellent survival results. As a consequence of that, growing numbers of PC patients treated with BT are eventually going to be biopsied because of Prostate Specific Antigen (PSA) levels rises during follow-up.
Objective. To summarize the histological findings in needle biopsies of the prostate in patients with biochemical failure after BT for PC.
Material and methods. Retrospective analysis of the pathology material of patients with a previous history of prostate BT performed at the author's institution.
Results Fourteen BT patients with biochemical failure (according to ASTRO 1997 criteria) were found to have been biopsied. One patient was biopsied twice. So, fifteen biopsies were available for review. All the biopsies showed radiation-related changes in non-tumour tissue, mainly consisting of acinar atrophy, pseudo-infiltrative duct distortion, marked duct cell atypia and stromal fibrosis. Six biopsies from 5 patients were negative for tumour while carcinoma was seen in the other 9 biopsies from 9 patients. Carcinoma was bilateral in 4 out of 9 cases. A Gleason pattern was not reported in 6 cases. The other 3 were classified as Gleason 3+3; 3+4 and 4+3. Immunohistochemical stains for Cam 5.2 and CK903 were performed for diagnostic purposes in six cases (3 of which were finally reported as positive and 3 as negative).
Conclusions. Biopsies are found to be positive for cancer in two thirds of patients with PSA relapse after BT. In nearly half of these cases the tumour is bilateral and in two thirds the Gleason pattern cannot be estimated. Radiation induced changes are found in non-tumour prostate tissue in all cases. Difficulties in the interpretation of these post-BT biopsies are illustrated by the frequent (40%) use of immunohistochemical stains.

P-339
APPROACH TO ETIOPATHOGENIC STUDY OF BLADDER'S TRANSITIONAL TUMORS. CLINICAL-HISTOPATHOLOGICAL AND IMMUNOLOGICAL CORRELATION

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FOREWORD
Bladder cancer is the second urological neoplasm more frequently found in men worldwide.Several factors are involved in the epidemiology of this neoplasm as follows:
Eventhough their relation is thoroughly established in some of them (such as in schistosomiasis and bladder's epidermoid carcinoma), in others such as transitional carcinoma, which is the most often histological type, there's no evidence whatsoeverregarding their activating factors which are stated above.
There seem to be unanimity regarding their function as cocarcinogenics, though.
METHOD We are carrying out a study comprising all patients affected by bladder's TCC (transitional cell carcinoma) for the last fifteen years at Eighth HealthCare Area from Comunidad Valenciana (Spain). This widens through a 3,979.82 km2 area and gets a population of 58,414 dwellers.
We compare the implication of serial exogenous factors as: chemicals (tobacco,agriculture plaguecides, industrial and grape-growing toxics), physicals(Lithiasis, BPH, nuclear radiations), biologicals (parasites, bacteria, viruses.);and endogenous factors as: immunized state both tumoral Igs

(IgA) as cellular(T4/T8) and hormonal state in order to check if there is any relation among them.

RESULTS We will proceed to show and discuss the preliminary results out of the study related to geographical localization since we deal with a settled population inland Comunidad Valenciana (Spain) where a nuclear power station (Cofrentes) was founded 30 years ago.

We will set its relationship to a range of both clinical parameters (size, localization,multiplicity, recurrence, response to immunotherapy) and morphologic ones(grade, stage, 'CIS" association and immunohistochemical (ki67, p53, p16)

CONCLUSION TCC aetiology study reveals a multifactor behaviour, however, the fact of having checked a moderate increase of its incidence besides "CIS", makes us believe the presence of possible cocarcinogens in the studied area.

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Hybrid renal cell carcinoma with papillary and clear cell features. Case report

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Introduction: Clear cell (CRCC) and papillary (PRCC) renal cell carcinomas (RCC) are the two most frequent subtypes of RCC. RCC displaying an hybrid morphology with areas of PRCC and CRCC or which contained papillary structures with clear cell changes (CCC) are rarely reported.

Case report: We report a new case of hybrid renal cell carcinoma with papillary and clear cell features. 73-years old man a heavy smoker, who presented with a history of presenting with left back pain associated with episodes of total macroscopic haematuria. Imaging showed a mass in the upper pole of the left kidney. Grossly, there were tow distinct tumor the first we found a well-circumscribed, greyish-white tumor the second lesion was nodular lesion; which protrudes beyond the normal contour of the kidney. Its cut surface was variegated, composed of soft, bright yellow parenchyma with areas of hemorrhage and necrosis. Histologically, the first tumor was composed of a typical PRCC the second one was consisting with CRCC. In the papillary areas, the tumor cells were arranged around delicate fibro-vascular tissue fronds containing foamy histiocytes or lymphoplasmocytic infiltration. The cells covering the papillae commonly showed an eosinophilic cytoplasm. Both tumors were of nuclear grade 2. The CRCC, was typically formed by a network of a small blood vessels witch invests alveolar clusters of carcinoma clear cells. Immunohistochemistry showed very intense labeling of tumour cells of both component with cytokeratins: KL1 and vimentin while p504 showed an intense labeling of PRCC component only. The diagnosis of hybrid renal cell carcinoma with papillary and clear cell features was made.

Conclusion: Few authors studied and identied RCC with papillary structures and a possible admixture between CRCC and PRCC. It seems to be important to recognize this type of tumor with hybrid morphologic features of typical PRCC and CRCC due to its more aggressive biologic behavior compared to classical PRCC. Its recognition may also facilitate the classification of RCC with ambiguous morphologies.

P-341

AN USUAL VARIANT OF PRIMITIVE NEUROECTODERMAL TUMOR

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Primitive neuroectodermal tumors (PNET) are rare and aggressive tumors seen predominantly in young adults. PNETs may include small round cell tumors showing different degrees of neuroectodermal differentiation and some histological variants. Between 10 and 20% of neuroepithelioma will display spindled areas that ressemble a primitive fibrosarcoma or malignant schwannoma.

We repoet a case of PNET arising in the third cervical nerve root of a 28-year-old woman who's presented with neck mass evolving for two months. Clinical examination found a neurological disturbances with right hemiplegia, left hemiparesia and parasthesia. Neuroimaging schowed a voluminous mass measuring 56×30 mm intersting the left posterior cervical space extending to the cerviccal canal with cord compression evoking in first a tumor of nervous origin. Histological examination of the surgical specimen revealed a malignant prolifferation of spindle cells dissociated by necrotic areas. Immunohistochemical analysis showed positivity for CD99, NSE, Vimentin and PS100 and negativitycytokeratin, EMA, CD34 and desmin.

Through this case and a review of the litterature, we discuss the microscopic features of PNET with spindle cells as well as differential diagnoses.

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STUDY OF KIT EXONS 9,11 AND PDGFRA EXONS 12,18 IN SARCOMATOID RENAL CELL CARCINOMA

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Background: Sarcomatoid renal cell carcinoma (SRCC) represents high-grade transformation in different Renal cell Carcinoma subtypes. It is associated with poor prognosis because of local aggressiveness, high metastatic rate and absence of effective therapy. Imatinib a selective tyrosine kinase inhibitor of ABL-BCR, KIT and PDGFRA has recently been incorporated for the treatment of neoplasms such as Chronic Myeloid Leukemia (CML) and Gastrointestinal Stroma Tumour (GIST). Immunohistochemical expression of KIT and PDGFRA in the sarcomatoid part of SRCC has been found suggesting the promising use of Imatinib for this neoplasm. Different studies support the concept that mutation-mediated activation of these two tyrosine kinase receptors is a condition for successful therapy with Imatinib as it has been described in GIST. The aim of our study was to sequence exons 9,11 of KIT and 12 and 18 of PDGFRA, where gain of function mutations have been most commonly found in GIST.

Method: Six SRCC cases that showed immunohistochemical expression of KIT (DAKO dil:1/100) and PDGFRA (Santa Cruz dil:1/100) in the sarcomatoid part were selected. Genomic DNA was extracted of formalin-fixed, paraffin-embedded blocks containing sarcomatoid differentiation. DNA was amplified by polymerase chain reaction using the primers corresponding to KIT exon 9,11 and PDGFRA exon 12 and 18 being the product directly sequenced and analysed.

Results: Neither KIT nor PDGFRA mutations were identified in any of the cases tested.

Conclusion: Lack of mutations in KIT exons 9, 11 and PDGFRA exons 12, 18 in sarcomatoid differentiation of RCC does not support the effective use of Imatinib for this neoplasm.

P-343

Molecular alterations of PTEN in prostate cancer

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Background: Prostate cancer is the most prevalent non-cutaneous tumour and the second cause of cancer-related death in males of Western countries, as well as one of the most frequent cancers worldwide. Although many different tumour suppressor genes, including *RB1*, *PTEN* and *TP53*, are thought to be involved in prostate cancer, to date no specific gene has been found to be the key in prostate cancer progression. PTEN is a tumor suppressor gene that is able to dephosphorylate the product of PI3K. It has been studied in many cancers and it is known to be deleted or inactivated in many tumor types including endometrial, breast, prostate, lung and bladder among others. Our objective is to analyze the frequency of PTEN mutations in a well-defined group of prostate tumors.

Material and methods: 56 prostate adenocarcinomas have been studied. The series consisted of 44 clinically significant (biopsy or prostatectomy) cases, 9 autopsy tumours, and 3 bone metastases. Gleason grade was ≤7 in 43 and >7 in 11 cases. DNA was extracted (Dneasy Tissue Kit, Qiagen GmbH, Hilden, Germany) by manual microdissection from selected tumor areas of formalin-fixed, paraffin-embedded tissue. Exons 5 to 8 were amplified by PCR from intronic primers and mutational analysis was performed by direct sequencing from purified PCR products.

Results: 3/56 (5,3%) tumours showed PTEN mutations. One case, with Gleason 7, had the mutation K222K in exon 7. In two cases, the mutations have been identified in exon 5. One of them, with Gleason >7, had a deletion leading to a stop codon and the other one, with Gleason <7, had the mutation P95S.

Conclusions: The frequency of PTEN mutations in this series of prostate tumors is around 5%. We have not detected differences in the prevalence of mutations among the different tumor groups. Further analysis including a lager number of cases is being carried out.

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FGFR3 alterations in prostate cancer

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Background: Prostate cancer is the second most common cause of cancer-related death in men in the western world, and its incidence is increasing. Fibroblast growth factor receptors (FGFR1–4) regulate diverse cellular processes including cell growth, differentiation, and angiogenesis. In prostate tumors, there is a differential and changing expression of the FGFRs. FGFR3 gain-of-function mutations have been reported in multiple myeloma, cervix, and bladder cancer. However, the functional role of FGFR3 and its alterations in prostate cancer are not known.Our objective is to study the prevalence of *FGFR3* genetic mutations in a large group of prostate tumors classified by stage and Gleason grade.
Method: 98 prostate tumors were recruited prospectively from the files of the Departament of Pathology, Hospital del Mar, BCN. 73 were biopsy or prostatectomy tumors, 16 were latent tumors (from autopsy), and 9 were metastases. The cases were classified as Gleason ≤7 (n=75) and Gleason >7 (n=23). DNA was extracted from formalin-fixed, paraffin-embedded tissue in which representative tumor areas were selected and manually microdissected. Exons 7 and 10 of *FGFR3* were amplified by PCR and the mutational analysis was performed by direct sequencing.
Results: 9 tumors presented *FGFR3* mutations (9%). The most common mutation was the S249C substitution (89%), followed by A393E, found in only 1 tumor (11%). 8 of 75 (11%) tumors classified as Gleason ≤7 vs 1 of 23 (4%) tumors with Gleason grade >7 carried a mutation ($p<0.05$). The F386L germline single nucleotide polymorphism was detected in two samples (2%), both of them with a Gleason grade=7.
Conclusion: In conclusion, *FGFR3* mutations are present in about 9% of the prostate cancers, with a higher prevalence in low grade tumors.
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Gastrointestinal Pathology

P-345
Clinicopathologic study of 106 gastrointestinal stromal tumors. Experience at two medical institutions in Venezuela.

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Background: Gastrointestinal Stromal Tumors (GIST) are the most frequent sarcomas of gastrointestinal tract, with variable biologic behavior. Due to the availability of molecular-targeted therapy for GIST it is important to distinguish GIST from other mesenchymal tumors of gastrointestinal tract. The aim of this study was to evaluate the clinical, morphological and immunohistochemical features in a GIST group.
Methods: Cases of gastrointestinal mesenchymal tumors between 1997 and 2007 were reclassified according to modern criteria. Haematoxylin-eosin stained sections and clinical information were reviewed. Immunohistochemistry for vimentin, CD117, CD34, smooth muscle actin, desmin, S-100 protein and epithelial membrane antigen was performed. Clinical data were obtained from clinical histories or biopsy reports.
Results: A total of 106 cases were studied. There was a slightly female predominance, with an age range between 17 to 75 years (Mean: 45.83). Gastrointestinal bleeding was the most common symptom. The tumors were located in the stomach (48%), small intestine (31%), large bowel (12%), abdominal tumor (7%) and liver metastasis (2%). Macroscopically, tumors ranged in size from 1.5 to 44 cm (mean, 11.53 cm). Cystic degeneration was present in 15% of the tumors. The cell types observed included pure spindle cell type in 71% cases, followed by pure epithelioid cell type (15%), and mixed epithelioid/spindle (14%). Intermediated and high risk tumors were the most frequent finding. CD117 positivity was detected in 96% tumors, and CD34 was found in 62% cases. 25% were positive for smooth muscle actin, 6.12% cases were positive for S-100 and desmin was present in 4.17% tumors.
Conclusions: In Venezuela, gastrointestinal stromal tumors have similar clinicopathologic and immunohistochemical features compared with other countries. Histologically, it is not always easy to distinguish GIST from other soft tissue lesions. An adequate immuhistochemical study should be used to confirm the pathologic diagnostic.

P-346
CLINICS AND PATHOLOGIC FEATURES OF PATIENTS SCREENED FOR GASTRIC CANCER IN A COLOMBIAN POPULATION DURING 2004–2005

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Justification: The gastric cancer is one of the most frequent neoplasms. In Colombia this disease is the principal cause of death by cancer. Its early diagnosis is

difficult, for that reason, the mass screening, using endoscopy and histopathological evaluation of biopsy specimens has been imposed as detection method of the early gastric cancer. **Objective:** To describe the clinical and pathological characteristics of the patients who went under screening of gastric cancer at the *Liga Santandereana de Lucha Contra el Cancer* during 2004 and 2005. **Materials and Methods:** A retrospective observational study was made, with a population that corresponds to the people who went under screening of gastric cancer at the *Liga Santandereana de Lucha Contra el Cáncer*. There were interrogated different sociodemographic, clinical and physical examination variables, besides of endoscopic and pathological findings. **Results:** 155 patients were studied, 103 men (66,5%) with a rank of ages between 15 to 93 years old, with an average of 45.43±14.15 years old. The principal referred symptoms were epigastric pain 63.4%, pyrosis 55.5%, regurgitation 26.5%, weight loss 26.5%, metheorism 18%, nausea 16%, vomit 14.8% and abdominal distention 14.8%. The most frequent antecedents were toxicological 25.5%, followed by the massive corn intake 11.6%, having A+ blood type 58% and having history of cancer in the first degree family 21.3%. The only sign found was abdominal mass in the 0.6%. Finally, the most frequent pathological findings were chronic gastritis 31.61%, chronic follicular gastritis 16.77%, peptic ulcer 1.29%, follicular peptic ulcer 1.29% and adenocarcinoma 1.29%. **Conclusions:** Sifting for gastric cancer allows detecting multiple alterations of the upper gastroenteric tract, including diverse neoplastic and preneoplastic lesions. In the studied population the most of the patients, had symptoms and/or antecedents that suggested gastrointestinal lesions. Finally, it must be encouraged the continuity in the programs of early detection of preneoplastic lesions.

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EVALUATION OF ENDOSCOPY VISUALIZATION IN THE IDENTIFICATION OF PREMALIGNES GASTRIC LESSIONS IN A COLOMBIAN PEOPLE WITH HISTOPATHOLOGIC REFERENCE TEST

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Justification. The gastric cancer is one of the main causes of mortality and morbidity in the world, its high mortality is due to its difficult early diagnosis, and so it's identified in advanced stages. Therefore diverse methods of early detection for gastric cancer and preneoplastic lesions have been studied. However, these methods have had a low cost-effectiveness. **Objective.** To evaluate the yield of endoscopic visualization, to the identification of gastric premalignant lesions, with examination of histopathological biopsy samples as a reference test, in Bucaramanga, Colombia. **Materials and Methods.** There were selected the patients of the *Liga de Lucha Contra el Cáncer*, from Bucaramanga Colombia. A statistical analysis was made by a cross-sectional sampling, to evaluate the gastrointestinal endoscopy in the diagnosis of preneoplastic gastric lesions, using the histopathological examination as a reference test. **Results.** 155 patients were studied, with a mean age of 45.43± 14.15 years and an approximated male:female relation of 2:1. The endoscopic visualization had a sensitivity of 87.84%, a specificity of 55.56%, a Positive Predictive Value of 64.36%, a Negative Predictive Value of 83.33%, a Likelihood Ratio+ of 1.98, a Likelihood Ratio - de 0.22 and a kappa of 0.4272, in the identification of preneoplastic lesions of the stomach in a population with a disease prevalence of 47.74%. **Conclusions.** The endoscopic visualization of the gastrointestinal mucosa, allows a mild identification of early preneoplastic lesions. Therefore its use as the sole test for the detection of lesions preneoplasic is not recommended, making it necessary to evaluate the gastric mucosa through biopsy.

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ASSOCIATED FACTORS WITH THE IDENTIFICATION OF GASTRIC PRENEOPLASIC LESSIONS IN LA LIGA SANTANDEREANA DE LUCHA CONTRA EL CANCER DURING 2004 TO 2005.

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Justification. Gastric cancer is one of the malignancies that cause an increased mortality and morbidity with a major impact in Colombia, however the factors associated with the identification of preneoplasic lessions have not been studied. **Objective.** To determine the factors associated with the identification of preneoplasic gastric lesions in Bucaramanga, Santander Colombia. **Materials and Methods.** A study was conducted cross-sectional in Liga Santandereana de lucha contra el cancer with a population of 155 patients who were screened for gastric cancer, who were conducts a survey on different clinical characteristics. **Measurements and Main Results.**155 patients were studied, 103 men (66,5%) with a rank of ages between 15 to 93 years, with an average of 45.43±14.15 years. We was

determined that the infection by H. Pylori (PR 1.69; IC 95% 1.15–2.5), the daily consumption of red meat (RP 1.76; IC 95% 1.265- 2.445) and daily consumption of corn (RP 1.9; IC 95% 1.459- 2.566) were significantly associated with the identification of lesions in patients preneoplasic screenings. **Conclusión.** We identified different characteristics associated with preneoplasic injuries, which are similar to those associated with gastric cancer identified by other studies, this supports the chain of events for the development of this malignancy, and indicates that its eradication would control in the development of this disease.

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PRIMARY MONOPHASIC SYNOVIAL SARCOMA OF THE DUODENUM: CASE REPORT AND REVIEW OF THE LITERATURE

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Synovial sarcoma is an uncommon malignant neoplasm of the soft tissues. It mainly affects the soft tissues of the extremities in close proximity to the joints, and less often other sites lacking synovial and periarticular structures, including the head and neck, abdominal wall, intra-abdominal cavity, intracranial cavity, mediastinum, bones, vessels, nerves and visceral organs, such as the lung, heart, kidney and prostate. The gastrointestinal tract is an unusual location with 12 cases described in the literature to date. We report a case of primary synovial sarcoma of the duodenum in a 69-year-old woman. First histologic examination was made in the endoscopic biopsy of the duodenal mucosa and showed infiltration of the tissue by a proliferation of mesenchymal spindle cells whose morphology and immunophenotype suggested monophasic SS. The patient underwent cephalic duodenopancreatectomy. Inmunohistochemical analysis revealed diffuse positivity for antibodies against vimentin, CD56 and bcl-2 expression, partial positivity for EMA and focal AE1/3 positivity. Cytogenetic analysis confirmed the diagnosis, with detection of the X;18 translocation. The patient presented postoperative complications and died at one month following the intervention. The 12 cases described in the literature occurred in the esophagus (8) and the remaining in the gastroesophageal junction, gastric antrum, distal duodenum, jejunum, and colon. Macroscopically as polypoid masses; two intramural cases have been described, one of them ours. Histologically, the biphasic type predominates over other histological types. SS of the gastrointestinal tract is unusual, and may be an underdiagnosed or erroneously diagnosed condition. The differential diagnosis for monophasic SS of the small intestine are gastrointestinal stromal tumor (GIST) and other malignant spindle cell tumors. Cytogenetic study plays a key role in the diagnosis of monophasic types arising in exceptional locations such as the small intestine.

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A CASE OF INCOMPLETE MECHANICAL BOWEL OBSTRUCTION CAUSED BY CYSTIC ENLARGED APPENDIX

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The author's team presents a case of incomplete mechanical bowel obstruction caused by cystic enlarged appendix, filled with mucus and designated mucocele, that presses the sigmoid colon. As an etiological factor for the occurrence of the appendiceal lumen is accepted the chronical obstruction of the appendiceal lumen, and the pathologoanatomically it is observed a different stage of mucosal hyperplasia, leading to accumulation of the mucus and cystic dilatation. The histological features can be benign or malign. In the complicated forms one can observe infection, perforation with peritonitis and intra abdominal dissemination of the malign form. The treatment is operative.

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INTRAEPITHELIAL LYMPHOCYTOSIS AND CD10 EXPRESSION OF DUODENAL MUCOSA IN IRON DEFICIENCY ANEMIA

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Background: Diet iron is absorbed by the proximal small intestine. In coeliac disease the greatest damage is found in this site. Iron deficiency anemia is a common finding in newly diagnosed coeliac disease. The first aim of the study was to determine the frequency of increased intraepithelial lymphocytosis (IELs) in patients with iron deficiency anemia. The second aim of the study was to evaluate CD10 expression pattern of duodenal mucosa in iron deficiency anemia and coeliac disease.

Method: Duodenal biopsies of patients with iron deficiency anemia (n=17), coeliac disease (n=15), and patients with other disease (n=8) were included in the study. Duodenal bopsies were stained for expression of CD3 and CD10 in mucosa using immunohistochemistry. CD10 expression in mucosa and IELs were scored on a five point scale. Villous architecture was scored as a three point scale.

Results: When these study groups were compared according to IELs, it was found that there were more IELs in coeliac patients ($P<0.001$). There was no significant difference in terms of CD10 staining pattern between the coeliac and iron deficiency anemia groups ($P>0.05$). A significant correlation between the IELs and flattening of the mucosa was found in these study groups ($r=0.523$, $P=0.00053$). There was no significant difference in terms of H. pylori infection between the coeliac and iron deficiency anemia groups ($P>0.05$).

Conclusion: CD10 (CALLA), is defined also as an intestinal mucinous marker and stains normal intestinal brush border continously. Various degree of CD10 staining was observed in the duodenal biopsies of iron deficiency anemia and coeliac disease. This study suggested that partial loss of CD10 staining is an early finding of small intestinal mucosal damage.

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Fibroblastic polyps of the colon and colonic perineuriomas: two names for a single entity

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Background: Fibroblastic polyps and perineuriomas of the colon are unusual mucosal lesions with identical clinical and histologic features and apparent different immunohistochemical and ultrastructural characteristics. Immunohistochemical distinction however, was solely based on results obtained with epithelial membrane antigen (EMA), an antibody whose reactivity on perineuriomas is difficult to demonstrate. Likewise, accurate ultrastructural diagnosis may be flawed by sampling error, preservation artifacts or paucity of specific diagnostic features. In a recent short communication it was suggested that both lesions may represent a single entity. To further evaluate this hypothesis we studied by immunohistochemistry and electron microscopy a series of 28 polyps with clinical and histologic characteristics of fibroblastic polyps/perineuriomas.

Methods: Twenty eight colorectal polyps with clinical and histological features of fibroblastic polyps/perineuriomas were stained immunohistochemically for 4 markers of perineurial differentiation: claudin-1, GLUT-1, collagen type IV and EMA (the latter performed using an extended protocol for antigen retrieval and a kit for signal amplification). Electron microscopy was carried out in 4 cases.

Results: EMA and claudin-1 stained 26 of 28 cases (93%) polyps whereas GLUT-1 and collagen IV were expressed by all the lesions. EMA staining was mostly focal and weak while the other markers exhibited a diffuse and strong signal. Ultrastructurally, tumor cells showed long, very thin cytoplasmic processes, discontinuous external lamina, pinocytotic vesicles and rare thight junctions.

Conclusion: Our findings support the hypothesis that fibroblastic polyps and perineuriomas of the colon represent the same entity. We suggest reclassifying fibroblastic polyps reactive to perineurial markers as perineuriomas. To reach and accurate diagnosis, we recommend employing at least 2 markers of perineurial differentiation, and performing EMA immunostaining with high antibody concentration, prolonged incubation time, and/or extended protocol for antigen retrieval.

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HIGH FEASIBILITY OF HMLH1, HMSH2 AND HMSH6 PROTEIN EXPRESSION AND MICROSATELLITE INSTABILITY ANALYSIS (PENTAPLEX SYSTEM) TO SCREEN PATIENTS WITH CLINICAL CRITERIA OF LYNCH SYNDROME.

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Background: The Lynch Syndrome (LS), the most prevalent genetic disease in hereditary non-poliposys

cancers (HNPCC), is characterized by germinal mutations in the Mismatch Repair (MMR) genes and by microsatellite instability (MSI). MSI consists of DNA insertions and deletions in microsatellite sequences. In order to select patients for further genetic studies, nowadays the techniques used are immunohistochemestry (IHC) of MMR proteins and MSI analysis by PCR, using a panel of three-dinucleotide and two-mononucleotide repeats (Bethesda Panel). A recent NCI workshop suggested a more sensitive panel of five-mononucleotide repeats.

Method: We evaluated the concordance between IHC and the presence of MSI with the new pentaplex system (five-mononucleotide and two-pentanucleotide repeats). We collected 107 patients with colorectal cancer (CCR) selected by Bethesda clinical criteria of LS. We evaluated by IHC the nuclear expression of MLH1, MSH2 and MSH6 proteins in the neoplastic areas. We extracted DNA from a paired normal-tumor samples for each patient and evalueted MSI using a pentaplex method with BAT25, BAT26, MONO27, NR21 and NR24 microsatellites enclosed in the "MSI Analysis System" kit (Promega Corp.).

Results: We detected loss of nuclear expression of MMR proteins in 18% of the samples (19/107) (MLH1: 14/19, MSH6: 3/19 and MSH2-MSH6: 2/19). About MSI analysis by PCR, we detected MSI-positivity in 13% (14/107) of the patients: 13/14 with High-MSI (in 2 or more MS) and 1/14 with Low-MSI (in BAT26). We found complete concordance between MSI-positivity by PCR and loss of expression of any MMR protein by IHC (14 cases). 5 cases was MLH1-negative by IHC without MSI.

Conclusion: The combination of MSI analysis (pentaplex-system) and IHC allows us the selection of patients who will benefit from further germinal mutation studies. Both techniques are complementary, but other factors must be considered to improve the knowledge of the difference between hereditary and sporadic CCR.

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Is acetylcholine an autocrine growth factor in human colon cancer cells?

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Background: Acetylcholine (ACh) is an ubiquitos signalling molecule in the organism, in neuronal, as well as non-neuronal tissue, binding at muscarinic and nicotinic receptors (mAChRs; nAChRs). Moreover, ACh has been suggested to serve as an autocrine/paracrine growth factor in several types of tumor or tumor cell lines, but, so far, studies of nAChRs in human colon cancer or colon cancer cell lines are sparse. The 7 homopentamer (7nAChR) convey anti-inflammatory, as well as pro-proliferative effects by ACh-stimulation, as noted in the human colon cancer cell line, HT-29.

The aim of the current study was to investigate whether ACh is an autocrine mediator in the HT-29 cells. This was undertaken by studying the expression of 7nAChRs, and the enzymes being responsible for ACh formation and degradation, choline acetyltransferase (ChAT) and acetylcholine esterase (AChE), respectively. We also investigated the effect of nicotine on the invasive-metastatic properties of the cells. This was achieved by analysis of the secretion of urokinase type plasminogen activator (uPa).

Methods: HT-29 cells were studied with RT-PCR (ChAT, AChE), Western blotting- immunocytochemistry (α7nAChRs, ChAT, AChE), and ELISA (uPa). The cells were challenged with nicotine tartrate (1–1000 nM) for 24 h.

Results:α7nAChRs and ChAT showed strong expression while that of AChE was weak. Nicotine administration to the cells resulted in increased uPa-secretion with a peak at a concentration of 10 nM.

Conclusion:ACh may serve as an autocrine mediator in the HT-29 cells, since the enzymes for its synthesis and degradation, are expressed as well as a receptor for the ligand. Previous reports showed that nicotine increases tumor cell proliferation and here, we demonstrate that this ligand causes increased invasive-metastatic properties. The current findings may point at the development of receptor blocking agents in the prevention of metastasizing colon cancer.

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HER2 over-expression and classic histological features correlation in gastric cancer

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Background *HER2* is over-expressed in a variety of solid human cancers and related to poor prognosis.

By testing for HER2 over-expression, physicians can accurately select patients likely to benefit from therapy.

There is growing interest in its role in prognosis, pathogenesis and treatment implications for gastric cancer (GC) patients, with the future introduction of trastuzumab therapy.

The aim of this study was to examine HER2 over-expression in GC and to correlate in our population with

age and classic histological features, such as: histological type and grade.
Material and methods 100.H&E-stained, formalin fixed paraffin embedded invasive gastric carcinomas tissue samples with required criteria were collected.
Routine histological parameters were assessed according to *WHO Tumor classification.*
HER-2 analysis was performed using policlonal antibody anti Her 2 (DAKO), microwave antigenic recovery, detection system EnVision (Dako) and developed with diaminobenzidine. Results were interpreted as hercepTest® guideline's.
Results Median age was 64.2 years, histological grades were: G1: 23, G2: 39, G3: 37. HER2+ was over-expressed in 13 patients (13.0%)
With multivariate analysis, age (p=0.1059) and histological grade (p=0.168) were non statistically linked with HER2+ tumors.
Conclusions HER2+ prevalence in our sample (13%) is in line with previously published ones.
Identifying HER2+ patients enables specific therapy leading to a significant change in treatment and prognosis.
Technique standardization is a key to success in identifying HER 2 over-expression in an accurate, precise and truthful way.
Due to the small sample set we couldn't correlate HER2 over-expression with age and classic histological features.

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EFFECT OF MELATONIN ON PROLIFERATION OF LARGE INTESTINE CARCINOMA CELLS SENSITIVE (LOVO) OR RESISTANT (LOVO DX) TO DOXORUBICIN IN VITRO TESTS

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Melatonin (MLT) is a pineal hormone affecting proliferation of cultured neoplastic cells. Mechanism of its action has not been fully recognized. It is known to inactivate free radicals, which are generated in the body, i.e. during turnover of anthracyclines (e.g., doxo- and daunorubicin). Also, it has been found that MLT does not alleviates the toxic action of cytotoxic drugs exerted to neoplastic cells. It seems that there exists a potential for overcoming by MLT the multidrug resistance (MDR) of neoplastic cells to doxorubicin (DOX).
This study aimed at evaluation of *in vitro* effects of MLT on proliferation of colonic-originating carcinomas sensitive (LoVo) and resistant ($LoVo_{DX}$) to DOX cells.
The experiments were conducted in *in vitro* conditions on LoVo and $LoVo_{DX.}$ cells. Cells were exposed to MLT in concentrations of 0.1 mM and 1.0 mM and to DOX in concentrations of 0.005 μg/ml (K3), 0.05 μg/ml (K2) or 0.5 μg/ml (K1). The extent of proliferation inhibition in cell cultures was evaluated using staining with SRB technique and readout of optical density in an automatic microplate densitometer (Elx 800). The type of lesions developing in cell nuclei was estimated by comet test. Statistical analysis was performed by using Mann-Whitney's test.
In both cell lines inhibition of tumor cell proliferation (%) clearly increased in parallel to growing DOX concentration but the resistant cell line proved to be definitely less sensitive. MLT significantly inhibited proliferation of LoVo and $LoVo_{DX}$ cells but increase in MLT concentration did not intensify the cytotoxic effect. However, in the case of LoVo cells MLT intensified cytotoxicity of DOX at the concentration of K3. In parallel, MLT significantly overcome resistance to DOX of $LoVo_{DX}$ line cells.
The obtained data confirmed the anti-proliferative effects of MLT on some neoplastic cell lines and its role in overcoming resistance to DOX in resistant cell lines.

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Automatic recognition of colon cells in inflammatory bowel disease

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Background: The histological diagnosis of inflammatory boweldisease and other forms of colitis may be subjective. One of the most importantcriterion is inflammatory infiltration of the lamina propria by eosinophils,plasma cells, lymphocytes and other cells. This paper presents the automaticsystem for these cells recognition and counting.
Methods: Analysis has been performed on images taken from 60 randomlyselected cases of colitis patients primarily divided by human experts into 3groups depending on subjective assessment of inflammatory infiltrationintensity: low, moderate and high. There were 6 to 12 tissue samples taken fromeach individual IBD patient The recognition of four main type of inflammatorycells has been done on photographs randomly taken from biopsy specimens. Usingthe basic morphological operations like thresholding, filtering, watershedtransformation, the individual cells are extracted and then used to diagnosticfeatures generation,

based on the texture, geometry and histograms of cellimages. Next the set of five artificial neural networks (MLP, RBF, SVM, KNN,FLD) was used for recognition of four main types of inflammatory cells. Theprocess is fully automatic.

Results: The difficulties of automatic extraction of cells-differ significantly and the probability of error increases for each casedepending on severity of inflammatory changes. Measurements has been performedon images taken from 60 randomly selected cases primarily divided by humanexperts into 3 groups depending on subjective assessment of inflammatoryinfiltration intensity. All cases were divided into: low, moderate and highintensity of inflammatory changes. The results of automatic cell typerecognition and counting were compared with the appropriate results of humanexperts. The average relative discrepancies between scores including all celltypes were as follows: for first group 11.55% (std=3.89%), for second group14.11% (std= 5.44%) and for third group 18.48% (std=5.14%).

Conclusions: The developed computer aided system hasconfirmed its usefulness at automatic processing of medical image of the colonbiopsy specimens leading to recognition and counting subsets and distributionof inflammatory cells.

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Colon Collision Tumor: Adenocarcinoma and Carcinoid Tumor. Case Report and Literature Review

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BACKGROUND A 63 year old female patient came to the hospital with a chief complain of abdominal distention and constipation. Colonoscopy revealed a 4 cm elevated mass in the ascending colon. The biopsy reported a tubulovillous adenoma. The patient underwent a right hemicolectomy and a ileotransverstomy.

METHOD The tissue obtained at surgery was fixed in 10% buffered formalin and embedded in paraffin. Routine histological sections were stained with hematoxylin and eosin. Immunohistochemical staining was performed.

RESULTS The anatomopathologic study revealed a 5× 3 cm tumoral lesion coming from the colonic mucosa, with an exophytic aspect that infiltrated the wall macroscopically up to the submucosa. Histologically, the lesion was composed by two zones. The first one contained multiple glandular structures, the second had multiple lobules composed of cells with ovoid nuclei, chromatin in a "salt and pepper" conformation, and scarce cytoplasm. Immunohistochemical staining for CK AE1/AE3 revealed strong and diffuse positivity in the cytoplasm of adenocarcinoma cells and carcinoid cells. The carcinoid component revealed positive staining for chromogranin A and synaptophysin. The twelve dissected lymph nodes were found to be negative for tumoral involvement.

CONCLUSION The histologic and immunophenotypic studies of this tumoral lesion confirmed the diagnosis of a colon collision tumor composed by a carcinoid and a moderately differentiated adenocarcinoma infiltrating up to the submucosa. This is a rare diagnosis; with few reported cases in the literature. Prognosis of these types of tumors remains unclear, with some authors suggesting it depends on the two types of isolated components, and other studies suggesting that collision tumors and composite tumors have a worse prognosis.

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Epstein-Barr virus in gastric carcinoma in situ hybridization study of 25 Tunisian cases

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Background: The Epstein-Barr virus (EBV) is a ubiquitous human herpes virus implicated in the etiology of many human lymphoid and epithelial malignancies including gastric carcinoma. The presence of EBV DNA in gastric carcinomas was first documented in 1990 using the polymerase chain reaction. EBV-associated gastric carcinoma occurs worldwide, with varying degrees of incidence in different countries. Our study is the first to describe the frequency of EBV in gastric carcinoma in Tunisia.

Aim of study: To evaluate the prevalence of EBV in gastric carcinomas through a series of 25 Tunisian cases.

Methods: Twenty-five gastric adenocarcinomas were retrospectively investigated for the presence of EBV using in situ hybridization. Control cases included samples obtained from 8 antrectomy specimens for duodenal ulcer.

Results: Our study group comprised 15 intestinal type and 10 diffuse type gastric carcinomas. In situ hybridization detected EBV in 68% of gastric adenocarcinomas and 12.5% of control cases with a statistically significant difference between the two studied groups. The proportion of EBV+ gastric carcinomas was 80% in cardial carcinomas, 75% in carcinomas of the fundus and 62.5% in antral carcinomas. Epstein-Barr virus involvement was found more in the diffuse (80%) than the intestinal type (60%) with a statistically significant difference.

Conclusion: Our findings show a higher prevalence for EBV infection in gastric carcinoma than literature. Such results need to be confirmed by other larger series and by Polymerase Chain Reaction.

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HOW DISTANT FROM THE PRIMARY TUMOR SHOULD LYMPH NODES BE RECOVERED IN COLORECTAL CARCINOMA SURGICAL SPECIMENS ?

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Background: the nodal stage of colorectal cancer (CRC) is a major prognostic factor. The total number of nodes examined from CRC surgical specimens has been associated with improved survival possibly because of increased accuracy in staging. However, systematic sampling of lymph nodes located far from the CRC is time and money consuming and few studies have addressed it's value.

Material and Methods: this is a retrospective study of lymph node status from 349 CRC where the mesocolic and perirectal fat was divided into two parts: 1/ close to the tumor (5 cm of both sides of the tumor); 2/ distant from the tumor (above 5 cm of both sides of the tumor).

Results: Tumors were located as follows: caecum (61), ascending (31), transverse (33), descending (31), sigmoid (103) and rectum (90). The median number of nodes recovered from the fat close to and distant from the tumor was respectively 13 (range 0–66) and 4 (range 0–33). There were 175 pN0, 104 pN1 and 70 pN2 cases. In all pN1 cases, metastatic lymph nodes were located close to CRC, except for 5 cases in the rectum and 1 case in the colon where metastatic lymph nodes were only present in the distant fat. In pN2 cases, the median number of positive nodes close to and distant from the CRC was respectively 5 (range 3–29) and 0 (range 0–9).

Conclusion: In the colon, lymph nodes should be recovered only from the pericolic fat close to the tumor. In the rectum, systematic sampling of nearby and distant lymph nodes is mandatory since rare cases of metastasis arise in distant lymph nodes only. This quantitative restriction of lymph node sampling for CRC surgical specimens might induce a cost effect without having deleterious effects on patient treatment and outcome.

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solitary rectal ulcer syndrom: a clinico-pathological study of 10 cases

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Background: Solitary rectal ulcer syndrome (SRUS) is a rare disorder and has a wide spectrum of clinical presentation and variable endocscopic findings. To further characterize the clinical and pathological features, a retrospective, hospital based clinico-pathological study was conducted.

Materials & Methods: All cases of SRUS diagnosed at Farwania Hospital, Kuwait between 2002 and 2007 were retrieved. The histological slides were reviewed by two authors to confirm the diagnosis. Immunohistochemical stain for smooth muscle actin (SMA) was performed. The clinical files were reviewed for clinical features and endoscopic findings.

Results: Ten cases were identified 6 males and 4 females with an age range of 22–67. Rectal bleeding, constipation and abdominal pain were the most common clinical presentation and were seen in 80% of cases. Rectal ulceration was the most common endoscopic finding seen in 70% of the cases. Thirty percent of cases had multiple ulcerations. Two patients (20%) had polyps one of them was multiple. The histological examination of 11 biopsies (1 patient had 2 sets) reveled that all cases had surface serration, fibromuscular obliteration and crypts distortion. Forty five percent of the cases had diamond crypts. Ectatic mucosal vessels were a common finding. SMA expression was seen in all examined cases.

Conclusion: SRUS is a rare disorder and only ten cases were diagnosed in Farwania hospital over 6 years period. The clinical presentation of our patients is typical for SRUS. The presence of polyps and multiple ulcerations in the endoscopic findings further support that SRU is a misnomer. Surface serration, fibromuscular obliteration and crypts distortion are the most diagnostic features. Presence of Diamond crypts is additional diagnostic feature.

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How is the importance of inflammatory cells in the pathogenesis of colitis?

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Introduction: Free radical effects are one of the known mechanisms responsible for inducing inflammatory bowel disease (IBD). In addition to inflammatory cells, tissues are another origin for free radicals. The first origin can be controlled by steroids but tissue oxidizing agenoidsts are not affected by these drugs. Identifying the role of each mechanism can lead to a better treatment for IBD. This study is conducted to identify inflammatory cells role as the basic pathogenesis for colitis and also to evaluate the efficacy and importance of steroids in IBD treatment.

Methods & Materials: In an analytical-experimental study, 30 healthy male mice were divided into 6 groups, each consists of 5 members. Group 1 had induced colitis with acetic acid enema. Groups 2 was exposed to acetic acid like group 1, after immune suppression, and in group 3 the distal of colon which was extracted, was exposed to acetic acid. All of these groups had one control group that was exposed to water injection instead of acetic acid. Following sacrificing and laparotomy, their tissues were studied and compared on the basis of histologic and biochemical factors.
Results: Considering neutrophilic infiltration there were significant differences between group 1 and its control group and also between groups 2 & 3 ($p<0.05$).
There were significant differences among groups 2, 3 and their control ones and also between groups 1 and the control of group 3 in terms of Goblert depletion.
Based on tissue originated H2O2 we found significant differences between group 1 and its control one and group 3 and also between groups 2 and the control of group 3. All the three groups were significantly different with their controls based on FRAP and such differences were seen between groups 1 & 2 and also between their control groups ($p<0.05$).
Discussion: This study not only presents that neutrophiles aren't the only cause of oxidation process and production of tissue free radicals, but also make suspicions about steroids effect in IBD treatment because of the increasing amount of H2O2 in the presence of steroids and steady state of FRAP in such condition.
Keywords: colitis, acetic acid, steroid

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COLUMNAR-LINED ESOPHAGUS AND FETAL ESOPHAGUS: IMMUNOHISTOCHEMICAL PHENOTYPE COMPARISON

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Background. Although columnar lined esophagus (CLE) attracts much attention of investigators due to its role in development of esophageal adenocarcinoma nowadays, its nature is still being discussed.
Purpose. To compare immunohistochemical (IHC) phenotype of CLE and fetal esophagus.
Methods. 6 autopsy cases of 12–24 weeks gestation fetuses and 67 biopsy cases of CLE were studied prospectively using IHC (antibodies to CK7, CK20, CK5, CK14, villin, CEA and Ki-67).
Results. At 12–16 weeks of gestation (3 cases) fetal esophagus was lined by stratified cuboidal epithelium positive for CK7 (moderately), CK20 (from weak to moderate- reaction only in distal esophagus), CK5&14 (weakly) and CEA (weakly). At 16–18 weeks of gestation (1 case) esophagus was lined by stratified squamous epithelium positive for CK7 (weakly), CK5&14 (brightly) and CEA (moderately). At 18–24 weeks of gestation (2 cases) esophagus was lined by stratified squamous epithelium positive only for CK5&14 (brightly) and CEA (moderately). Proliferation level in basal layer of esophageal epithelium in studied fetuses was low (3,6±0,3%).
CLE was divided into gastric type (28 cases), intestinal type-Barrett's esophagus (23 cases) and predominant gastric epithelium with small islets of intestinal epithelium (13 cases). CLE with gastric type was CK7 positive and CK20 negative, weakly positive for villin and CEA. CLE with intestinal type was CK7 and CK 20 positive, strongly positive for villin and CEA. We revealed positive reaction in columnar epithelium for CK5&14 in 20 cases of CLE.
Conclusions. Similarities of IHC phenotype in CLE and fetal esophagus may support hypothesis of return to fetal state of epithelium in pathogenesis of CLE. Expression of CK7&14 (markers of squamous differentiation) in CLE supports its metaplastic nature.

P-364
CAPILLARY HEMANGIOMA OF THE ILEUM PRESENTING AS AN INTUSSUSCEPTION

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Hemangiomas of the small intestine are rare benign tumors; accounting for only 0,05% of all intestinal neoplasms and 7–10%of all benign tumors of small bowel. These tumors may be solitary or multiple and usually present with overt bleeding or chronic anemia. The majority of gastrointestinal hemangioma are cavernous type and situated in the middle jejunum. We report a new case of polypoid capillary hemangioma of the ileum. A man of 20 years admitted in emergency for abdominal pain and vomiting. Abdominal ultrasound and computed tomography scan showed a large lobulated mass of terminal ileum suggestive of an intussusception. A resection of terminal ileum and coecum was made. Gross pathological examination revealed a polypoid ileal tuminal tumor mesuring 10×3×2 cm that was myxoid at section. Microscopy revealed a capillary hemangioma of terminal ileum. We discuss the differential diagnoses of vasoformative intestinal lesions and review the litterature on enteric hemangiomas.

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UNUSUAL FOLLICULAR ADENOMA OF THYROID GLAND: MUCINOUS VARIANT

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Follicular cells of the thyroid may undergo squamous, oncocytic or mucinous metaplastic changes. Of these, the mucinous change is the most unusual, and mucinous follicular adenoma of the thyroid is extrrmrly rare. It's a variant of adenoma characterised by accumulation of abundant extracell mucin, often accompanied by a microcystic growth pattern. We describe a case of mucinous follicular adenomaof thyroid gland in a 36-year-old euthyroid woman. She's presented with a 1-year history of a right anterior neck mass. The patient underwent a hemithyroidectomy; the cut surface of the 7×3,5 cm lesion was solid and tanned orange. Microscopy of the tumor showed a mucinous follicular adenomawhich follicular cells were positive for alcian blue.

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Ileoceacal actinomycosis: Diagnosis difficulties and management, a case report.

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INTRODUCTION: Actinomycosis is an unusual chronic granulomatous disease. Actinomyces israelli has been found to be related to infectious process in those patients with affected skin integrity leading to abcess formation, fistulae or mass lesions. Actinomycosis mainly presnts in three forms cervicofacial (20%), gastrointestinal (20%) and thoracic (15%). Primary bowel involvement is rare, although it has been increased in frequency over the last years. The most common sites of the disease are the transverse colon and the caecum with the appendix.
CASE REPORT: The patient was a 55 year old man who presented with abdominal discomfort and a palpable right lower quadrant mass defined on CT scan. He underwent en bloc resction of the mass for a presumed diagnosis of tumour of uncertain type with intestinal involvement. The diagnosis was reversed, when histology revealed filamentous organisms consistent with actinomyces. He was treated with high dose penicillin for several weeks and was discharged from the hospital taking penicillin.
COMMENTS: Preoperative diagnosis of abdominal actinomycosis is difficult. An accurate diagnosis is always obtained in a histological or microbiological examination, often requiring surgical resection. Recognition is important because successful treatment requires combined surgery and prolonged penicillin treatment. The diagnosis and management of abdominal actinomycosis will be discussed through a review of the literature and a case report from our own institution.

P-367

Carcinogenetic pathways in synchronous or metachronous colorectal carcinomas

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Background: Synchronous or metachronous (S/M) neoplasms arising in the same organ system of an individual provide an interesting model to unravel the mechanisms of carcinogenesis. The aim of our study was to elucidate whether those pathways are differentially involved in S/MCRC according to inheritance pattern, microsatellite instability (MI) status, location, or stage.
Method: Expression of p53, β-catenin, PTEN, p21, p16, cyclinD1, hMLH1, hMSH2, and hMSH6 was immunohistochemically studied in a series of 43 S/MCRC from 20 patients (P) (14M/6F; age range, 41–88 years). Nineteen tumors (T) were located in the right side and 24 in the left. Thirteen T were confined to the intestinal wall (T1+T2), whereas 30 were advanced T (T3+T4). Twenty-one T showed negative lymph nodes (N0), while 22 has metastasized to lymph nodes (N1+N2). Formalin-fixed, paraffin-embedded tissue from these 43 T was used to build a TMA block. Different expression patterns were evaluated for β-catenin, p21, and p16. Expression intensity was semiquantitatively evaluated as 0=negative; 1=low; 2=moderate; 3=high. Medical records were searched for a history of familial cancer.
Results: Four P fullfied Amsterdam criteria for familial CRC. All their 10T were unstable. The remaining 16P showed sporadic CRC. Of their 33T, 4 were unstable and 29 were stable. An identical expression patterns of all markers investigated was only observed in 15 sporadic T from 7P. Nevertheless, a correlation was found between evidence of β-catenin mutation and expression of both p53 and cyclinD1 ($p=0.028$ and 0.055, repectively) when analyzing all T as a single group.
Conclusions: S/MCRC do not show a specific pattern of cell cycle abnormalities even when they are segregated according to type of inheritance, MI status, location, or stage. Random factors, therefore, must be operative in the

development of these T, which fail to reveal a differential involvement of the known carcinogenetic pathways of CRC.

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Expression of cell adhesion molecule CD44 in gastric adencocarcinoma and its correlation with cliniclopathological characteristics and survival time

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BACKGROUND: Gastric carcinoma is one of the leading causes of cancer death worldwide. The invasion depth and lymph node metastasis result from the polygenes and their protein expression in gastric carcinoma. Two types of gastric adenocarcinoma can be distinguished histopathologically: diffuse and intestinal type. We have examined the cell surface molecule CD44, which is attracting interest because of reports that isoforms are associated with metastasis. This transmembrane glycoprotein is involved in cell-cell and cell-matrix interactions and in cell trafficking and, thus, may play a role in tumor metastasis and/or local invasion. In the present study we measured the expression of CD44 in diffuse and the intestinal type adenocarcinoma and its effect on survival time in the patients.

METHOD: Paraffin wax embedded gastric adenocarcinoma cases were selected from the Omid University Hospital (Mashhad, Iran) pathology archive and were assessed by immunohistochemistry method. The patients comprised 74 males and 26 females, who had undergone curative surgery without any prior treatment between 2000 and 2006. Their mean age was 63.31 years (from 26 to 82). Adjacent normal mucosa was used as control.

RESULTS: A total of 100 gastric adenocarcinoma cases were included in the study. The tumors were categorized into intestinal (74%), diffuse (21%) and mixed type (5%). CD44 staining showed that, 64% of patients were positive (51 patients had CD44 only on surface membrane of the tumoral cells and in 13 patients CD44 was detected both in membrane and cytoplasm) and 36% were negative for CD44. 100 normal gastric mucosa showed no CD44 staining. There was a relationship between histological type of tumor and CD44 staining. 70% of intestinal types, 42% of diffuse types and 80% of mixed type gastric cancers showed positive CD44 staining. CD44 was more expressed in intestinal types (P=0.02) and there was a significant difference between well differentiated types in compare with others. The survival analaysis has shown that CD44 cause a poor prognosis for the patients (P=0.05).

CONCLUSION: The CD44 expression rates in three types of gastric cancer were different significantly (P<0.05). Expression of CD44 was related to the genesis and prognosis of gastric cancer, and is one of the biological markers indicating metastasis and poor prognosis.

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Gastrointestinal stromal tumors recognized as ovarian neoplasms before surgical operation.

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Gastrointestinal stromal tumors (GISTs) are the most commonneoplasms of mesenchymal origin ofstomach and small bowel. They derive from precursors of Cajal's cells andexpress positive reaction with CD117 antibody. This immunohistochemicalexamination is currently a base of recognition them and allows to treat with imatinib – they are resistant to classic chemotherapy and radiotherapy. Theymetastatic mainly to the liver and peritoneal cavity. Surgical resection is the most important treatment of them, butit is very often is impossible toremoved them completely. In such cases treatment with imatinib reduces size oftumors, and makes possibility to resect them completely. Aim of the study was histopathologic analysis of tumors diagnosedpreoperatively as ovarian neoplams in women, whereas postoperative pathologicrecognition revealed, that they were gastrointestinal stromal tumors (GISTs).

Materials and methods Between 2002 and 2007 in Department of Pathology of Cancer Center inWarsaw there were diagnosed 130 gastrointestinal stromal tumors of small bowelin women confirmed by positive reaction of CD 117. The postoperative materialoriginated from gynaecological department of our Institute, but most of them became from anotherhospitals from surgical and gynaecological wards for consultation.

All GISTs in women	GISTs of small intestine in women	GISTs recognized as ovarian neoplams preopratively
130cases	44cases	16 cases
100%	34%	12%

Results Grossly the tumor's diameter ranged from 10 to 15 cm, cream - coloured,sometimes with many haemorrhages. Most of them were attached to small bowel. Afew of them were connected to large intestine. In two cases they looked likebig leiomyomas of uterus. Microscopically: the spindle cells were seen mostlymixed with epitheliod and

signet –ring ones. There were no big atypia..There were skenoid fibers in a few tumors. Mitotic figures were counted in 50high power fields. They ranged from 5 to 20. Neoplams expressed a positivereaction with CD 117 antibody. The most of them were no resected completely, sothey demanded postoperative treatment.
Conclusions In case of uncharacteristic tumor of ovarian with similarmorphology to GIST, it is necessary to make immunohistochemical examination with antibody CD117, to make wellrecognition and in a consequence to introduce therapy with imatinib or othersinhibitors of kinase tyrosinase..

P-370
DECIDUOSIS IN A CASE OF AN ACUTE APPENDICITIS MIMICKING HISTIOCYTIC NODULES OF MALAKOPLAKIA

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Deciduosis is a rare event, usually located in the ovaries, cervix and uterus, but may also be located on peritoneal surfaces of pelvic organs. Usually it is an incidental finding that has not been associated with clinical symptoms. Our patient had chronic abdominal pain that was increased in pregnancy. She was taken into an operation with a clinical diagnosis of an acute appendicitis. Pathological examination of the appendix showed nodules of decidual cells containing intrcytoplasmic blue vacuoles, in a dense acute inflammatuar stroma. These mucin vacuoles may mimic calculospherules that are characteristic of malakoplakia. Calculospherules appear refractile and stain with iron and calcium but decidual cells do not. They may also suggest adenocarcinoma however the vacuoles within the decidual cells contain acidic rather than neutral mucin.

P-371
TUMOR ANGIOGENESIS IN GASTRIC CARCINOMA: CORRELATION WITH CLINICO-PATHOLOGICAL FACTORS AND SURVIVAL OF THE PATIENTS

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Aim: To evaluate the relationship between microvessel density (MVD), VEGF expression, clinico-pathological factors and survival in patients with gastric cancer.
Methods: We performed a prospective study regarding the outcome and aggressiveness of gastric carcinoma on a five year period, including 61 patients (43 men, 18 women), mean age 59,34 years. For the immunohistochemical assessment of tumor angiogenesis, we used the monoclonal antibodies anti-CD34 and anti-human VEGF by LSAB technique. Angiogenesis was quantified by measuring MVD. Immunohistochemical expression of VEGF was assessed by using a score representing the sum between the percentage of positive cells and the intensity of the staining.
Results: Gastric carcinomas were characterized by an intense neoangiogenesis, especially in the front of invasion, with numerous microvessels and CD34 positive "hot spots" among the malignant structures. MVD in gastric carcinomas ranged between 12 and 65, with a mean value of 38,7± 24,3, significantly increased in comparison with normal mucosa (12,5±9,8, $p<0.001$ ES). Increased MVD correlated with diffuse type of gastric cancer, poor differentiation, presence of vascular invasion and advanced TNM stage. For patients with MVD>38 (34 cases – 55,7%), the 5-year survival rate was significantly decreased in comparison with patients with MVD<38 (7,4% vs. 23,5%). We obtained positive immunostaining for VEGF in 40 cases of gastric carcinomas (65,6%), significantly more frequent than gastric normal mucosa (6,5%, $p<0.0001$ ES). For gastric carcinomas with VEGF +~++, the overall 5-year survival rate was 12,5%, significantly decreased than for patients with VEGF negative carcinomas (23,8%).
Conclusions: Our study demonstrates a strong correlation between VEGF expression and MVD, these two markers of neoangiogenesis playing an important role for the biological behavior, progression and prognosis of gastric carcinoma.

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RELATIONSHIP BETWEEN THE EXPRESSION OF CYCLOOXIGENASE-2, VEGF, TUMOR ANGIOGENESIS AND PROGNOSIS OF THE PATIENTS WITH GASTRIC CANCER

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Aim: To assess the immunohistochemical expression of cyclooxigenase-2 (COX-2) in gastric carcinomas and peritumoral mucosa with different lesions, by analyzing the correlation with clinico-pathological factors, angiogenesis and survival of the patients.
Methods: Our prospective study included 61 patients operated for gastric cancer (43 men and 18 women, mean age 59,34 years). COX-2 and VEGF immunohistochemical expressions were evaluated by semi-quantitative analysis. We assessed microvessel density (MVD) and VEGF

expression in COX-2 negative group and COX-2 positive group of gastric carcinomas.

Results: COX-2 immunostainings were observed significantly more frequent in gastric carcinomas, in comparison with peritumoral normal mucosa (57,4% vs. 4,9%). Areas of dysplasia surrounding carcinomas of intestinal type expressed COX-2 in 35,5% cases, significantly more frequent than normal mucosa (p=0.000384 ES). COX-2 immunostainings were significantly more frequent in the intestinal type of gastric carcinomas than diffuse type (68,4% vs. 29,4%, p<0.001 ES). COX-2 expression correlated with the dept of tumor invasion, presence of nodal metastasis and advanced TNM stage, but didn't influence the 5 year survival rate of the patients. Mean MVD values (39,4) was significantly higher in COX-2 positive carcinomas (35 cases). We observed a strong direct correlation between COX-2 and VEGF in gastric carcinomas (r=0.562, p<0.001 ES). VEGF negative carcinomas expressed COX-2 in only 19% of cases, in comparison with those VEGF positive, associated with COX-2 immunoreactions in 77,5% cases.

Conclusions: COX-2 immunoreaction appears to be in our study an early event of the sequence involved in the development of the intestinal type of gastric carcinoma. Our results showed a strong correlation between the immunohistochemical expression of COX-2, VEGF and MVD in gastric carcinomas, demonstrating an intense angiogenesis in COX-2 positive tumors.

P-373
IMMUNOHISTOCHEMICAL EXPRESSION OF E-CADHERIN IN GASTRIC CANCER: CORRELATION WITH CLINICO-PATHOLOGICAL FACTORS AND SURVIVAL

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Background: To investigate the immunohistochemical expression of E-cadherin in gastric carcinomas and in adjacent mucosa (normal or with lesions of chronic atrophic gastritis, intestinal metaplasia or dysplasia).

Methods: We included 61 patients with gastric cancers operated in Clinical Emergency County Hospital Timisoara. We analysed the E-cadherin immunohistochimical expression, the correlation with clinical and pathological factors and the outcome of the patients. The positive homogeneous pattern of staining for the cellular membranes is considered normal. Aberrant E-cadherin expression was represented by the negative or the heterogeneous pattern (both in the cytoplasm and on the membrane).

Results: Areas of chronic atrophic gastritis and intestinal metaplasia presented a normal pattern of immunostaining for the membranes. Aberrant E-cadherin expression was noticed in 30 cases of gastric carcinomas (49,2%) and in 11 cases (35,5%) of epithelial dysplasia, significantly more frequent in comparison with the surrounding normal mucosa (p<0.001 ES). Our results showed no correlation between E-cadherin expression and gender, age, tumor location, pT, pN, pTNM and lympho-vascular invasion. Aberrant E-cadherin immuostainings were significantly more frequent in diffuse-type carcinomas in comparison with the intestinal-type carcinomas (82,4% vs.31,6%)(p= 0.000491 ES). Signet-ring carcinomas and anaplastic carcinomas presented a high proportion of aberrant immunostainings (82,4% and 100%), as well as poor differentiated carcinomas (61,5%). Carcinomas with distant metastasis presented significantly more aberrant immunostainings than those without metastasis (71,4% vs.42,6%). The 5 year survival rate was significantly lower in patients with aberrant E-cadherin expression in comparison with the patients presenting normal staining (10% vs. 22,6%).

Conclusions: Our data suggest a strong correlation between Lauren classification of gastric carcinomas and E-cadherin immunohistochemical expression. Assessment of the survival curve of the patients highlighted the role of prognostic factor for the aberrant immunohistochemical E-cadherin expression.

P-374
Monocarboxylate transporter expression correlates with CD147 and HIF-1α in gastric cancermonocarboxylate transporter expression correlates with CD147 and HIF-1α in gastric cancer

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Background: Acid efflux through *M*ono*C*arboxylate *T*ransporters (MCTs) constitute one of the most important mechanisms involved in the maintenance of tumor intracellular pH and, as a result, MCTs constitute an attractive target in cancer therapy, which has not been explored yet. Recently, it was demonstrated that CD147 is required for proper expression of MCT1 and MCT4 at the cell surface as well as for catalytic activity of the transporters. On the other hand, silencing studies showed that maturation and cell surface expression of CD147 depends on MCT1 and MCT4 expressions. Also, it was described that MCT1 is induced by hypoxia and that MCT4, but not MCT1, is up-regulated by the hypoxia-inducible factor-1-α (HIF-1α).

Method: Gastric samples, including normal, metaplastic, tumoral and metastasis tissues, were obtained from the Faculty of Medicine, São Paulo University (Brazil). Analysis of the expression of MCT1, MCT4, CD147 and HIF-1α was performed by immunohistochemistry with specific antibodies, using the avidin-biotin-peroxidase complex assay. Immunoreactions were evaluated both qualitative and semi-quantitatively.
Results: We found significant correlations between CD147 expression and both MCT1 and MCT4 expressions. Also, and as supported by the literature, HIF-1α expression correlated with MCT4 expression but not with MCT1. CD147 expression was also associated with HIF-1α expression and nodal metastasis. In addition, CD147, MCT1, MCT4, HIF-1α expressions were associated with intestinal-type gastric carcinoma (Lauren classification).
Conclusion: Our data support the evidence that MCT1 expression is regulated by CD147 and that MCT4 expression is regulated by both CD147 and HIF-1α, for the first time in human tumour samples. These observations contribute to the understanding of the metabolic alterations in cancer and might be of value in the development of new therapeutic strategies.

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IMMUNOHISTOCHEMICAL EXPRESSION IN GASTROINTESTINAL STROMAL TUMORS (GISTs) AND EXTRAGASTROINTESTINAL STROMAL TUMORS(EGISTs).

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GISTs are the most common primary mesenchymal tumors of the gastrointestinal tract.
They are CD117 (stem cell factor receptor) - positive mesenchymal spindle or epithelioid cells neoplasms, believed to originate from interstitial cells of Cajal (intestinal pacemaker cells) or related stem cells. Most GISTs arise in the stomach and small bowel, while rare cases occured elsewhere in the gastrointestinal tract are known as EGISTs. An improvement of histopathological diagnosis is required by their malignant potential and also by recent advances in their management with Glivec.
They typically express CD117, as well as CD34, SMA and S100. Recently it was reported Nestin (intermediate filament of neuroectodermal stem cells and progenitor skeletal muscle cells) positivity.
Aim: We analized the immunohistochemical expression of CD117,CD34, SMA, S100 and Nestin in GISTs, compared with EGISTs, for establishing morfo-immunohistochemical correlations with possible therapeutic implications.
Method: 63 GISTs and 8 EGISTs were morphologically and immunohistochemically evaluated using hematoxiline-eosine standard method and immunohistochemical ABC method for CD117, CD34, ACT, S100 and Nestin.
Results: 74,6% of GISTs and 50% of EGISTs were positive for CD117. CD34 and S100 expression was 84,2% and 87, 9% while ACT was positive in 49, 2% of GISTs and 25% of EGISTs. Nestin was highly expressed in GISTs: 80,9% and correlated with CD117 expression in 70% and with CD34 expression in 69%. In EGISTs, Nestin was positive in 50% and correlates with CD117 and CD34 expression in 50%. Spindle-cells GISTs were positive for CD117 in 43% while spindle-cells EGISTs were CD117 positive in 100%, suggesting a possible better response in Glivec therapy.
Conclusions: The Nestin expression in GISTs and EGISTs could be considered a good marker together with CD117 and CD34 in establishing the tumoral variants; its positivity could support the stem cell-Cajal cell origin and also to represent new possible therapeutic targets.

P-376
CDX2 and Villin are useful markers for metastatic colorectal cancer.

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CDX2 and Villin are immunohistochemical markers that have been demonstrated to be of diagnostic value in identifying tumors derived from intestinal epithelium. CDX2 is a transcription factor expressed in cells of intestinal epithelium and is thought to play an important role in their proliferation and differentiation. Villin is a 95 kDa calcium-regulated actin-binding protein that regulates actin filament assembly. It is a major constituent of the brush border of epithelial cells, forming absorptive surfaces in the microvilli of intestinal and renal proximal tubular epithelia. Using monoclonal mouse anti-Villin (clone 1D2 C3) and a newly developed monoclonal mouse antibody to CDX2 (clone clone DAK-CDX2), we evaluated CDX2 and Villin expression in a series of primary and metastatic tumors to assess the utility of the antibodies in distinguishing metastatic colorectal cancer from tumors arising from other organs.
Method: Formalin-fixed, paraffin-embedded tissue specimens were procured from primary and metastatic colorectal, lung and breast carcinomas and from metastatic

prostate, renal and pancreatic carcinoma. Immunostaining was performed using monoclonal mouse antibodies to CDX2 (clone DAK-CDX2) and Villin (clone 1D2 C3) on tissue pretreated with Tris/EDTA target retrieval solution. Bound antibody was visualized with the Dako FLEX detection system.
Results: Anti-CDX2 and Villin demonstrated similar immunoreactivity on colorectal carcinomas, staining the majority of both primary (17/17 CDX2+: 16/17 Villin+) and metastatic (6/7 CDX2+: 7/7 Villin+) specimens tested. None of the primary breast or lung carcinomas (excluding neuroendocrine tumors) were postive with anti-CDX2, wheras Villin expression was observed in 3/17 lung carcinomas. Among the 14 metastatic tumors tested from sites other than colon, 13/14 were unreactive with anti-CDX2 and 11/14 with anti-Villin.
Conclusions: In this study we have demonstrated that CDX2 and Villin are highly expressed in both primary and metastatic colorectal carcinoma. Antibodies to CDX2 and Villin can be used to better differentiate metastatic colorectal cancers from other cancers when evaluating tumors of unknown origin.

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Idiopathic Myointimal hyperplasia of mesenteric veins: A rare cause of intestinal ischemia in young patients.

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Nonthrombotic occlusion of mesenteric veins is a rare cause of intestinal ischemia that has been previously described in association with systemic lupus erythematosus, Behcet`s disease and mesenteric inflammatory veno-occlusive disease. Idiopathic myointimal hyperplasia of mesenteric veins (IMHMV) is a rare, recently described entity that must be added to these associations. Its etiology remains unclear but previous trauma has been hypothesized in some cases
We describe the case of a 32-year-old female who consulted for abdominal discomfort and tenesmus and was diagnosed with intestinal pneumatosis by CT scan. The patient progressively worsened and was finally admitted due to severe abdominal pain with bloody stools requiring sigmoidectomy. The surgical specimen showed a severely thickened, well-demarcated segment of colon with pneumatosis as well as steatonecrosis of pericolonic fat. Additionally, there were severe ischemic mucosal necrosis and striking intimal circumferential thickening of small mesenteric veins in the muscularis propia and submucosa. These changes resulted in complete or near complete luminal occlusion and gave rise to tortuous hyalinized capillaries in the lamina propria, with frequent thrombus formation. Neither vasculitis nor arterial involvement was present.As already described in similar cases, the patient recovered rapidly after surgery.
IMHMV, an uncommon condition that mostly affects previously healthy young male patients, must be differentiated from other causes of ischemia such as vasculitis or connective tissue diseases requiring a very different therapeutic approach.

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Digestive neoplasms malt tipe - pathological study

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Background: Digestive neoplasms are a vast and freqent pathology. In this pathology are included MALT lymphomas wich represents 3% of digestive neoplasms and 7–8% of B cell lymphomas. Slight female predisposition has been reported for MALT. Its simptoms vary and they mimic other digestive deseases.
Method: Statistical study of MALT lymphoma cases new diagnosed between 2000 – 2007, in Morphopathology Department of County Hospital, Constanta, following a few parameters: sex, age, tumor localization, signs and symptoms, immunohistochemistry pattern, disease extension.
Results: From our study resulted an increasing in number of new diagnosed MALT lymphoma between 2000 - 2007 (5 cases in 2000 to16 cases in 2006), MALT lymphoma representing less than 1% of intestinal pathology diagnosed in this period. From all MALT cases the most frequent localization was stomach with 57% off all MALT cases, followed by intestinal localization (23%) and colo-rectal localization (10%). There were a few cases of gastro-intestinal localization associated with non-gastro-intestinal localization (5%). Sex ratio didn't show any preference for a sex determinism excepting gastric localization with slighted predisposition for mans patients. Medullar invasion was present in 35% of cases. Non-specific symptoms were present in 5% cases for increased LDH, 35% for weight loss, 3% for fever and 40% for anemia. Immunohistocemical analysis showed that MALT lymphomas are negative for CD43 (98%), CD5 and CD10 (100%), Ki67 (99%) and positive for CD11c (60%), CD20 (95%).
Conclusions:
- MALT lymphomas are a rare pathology.
- MALT lymphomas are more frequent at gastric level and they show a slighted predisposition for mans patients at this level.
- There are no cases of esophageal MALT lymphomas.
- Paraclinical most frequent syndrome is anemia.

– Symptoms mimic other digestive diseases implying morphological and immunohistochemical exam.
– IHC showed that MALT lymphomas are most frequent negative for CD5 and CD10 and positive for CD20.

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Enterogenous cyst of the hepatoduodenal ligament: case report.

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Introduction: Enterogenous cysts (enteric duplication cysts) are hollow, epithelium-lined, spherical or tubular structures that are tightly attached to some portion of the gastrointestinal tract. They are located in or adjacent to the wall of part of the gastrointestinal tract and found most commonly in the distal ileum, the posterior mediastinum, and the third part of the duodenum. Rarely, enteric cysts can occur distant from the gut.

Case report: A 47-year-old woman presented with an incidentally found abdominal mass. The preoperative diagnosis was a cystic neoplasm the head of pancreas. Laparotomy showed a cystic mass in the hepatoduodenal ligament. It had no connection to the pancreas, stomach, small bowel, or large bowel. The gross specimen showed a cystic lesion with smooth and red-gray external surface 3,3×3×2,5 cm. On cut section, the mass expelled turbid fluid. The inner surface was relatively smooth and the wall of the cyst was thick (1 cm). Histopathologic findings showed that the cystic wall composed of irregulary oriented smooth muscle, and the cyst is lined with mucin-secreting columnar epithelium gastric and intestinal types with mucous glands.

On immunohistochemistry, the majority of cells were positive for CK7, CK8, CK18, CK19, CK20, MUC1, MUC2, MUC4. A few cells also immunoreactivity with MUC5AC. The final diagnosis of a completely isolated enterogenous cyst was made.

Conclusion: No communication or connection with the adjacent alimentary tract but the presence of a typical histopathologic and immunohistochemical features of a enterogenous cyst has been described as an isolated enterogenous cyst.

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PROGNOSTIC IMPORTANCE OF ANGIOGENESIS IN GASTRIC CANCER

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BACKGROUND: The new blod vessels which generation tumor inducer is a important in process of growth, prognosis and metastasis of tumors. We investigate the relationship between the angiogenesis and clinicopathologic features to related with prognosis in gastric cancer patients.

METHODS: To asses tumor angiogenesis, microvascular density (MVD) were analyzed immunohistochemically in 83 primary gastric cancers. Any single brown-stained cell with Factor VIII, that indicates an endothelial cell, was counted as a single vessel. Branching structures were counted as a single vessel unless there was a discontinuity in the structure. Vessels were counted in 4 regions, with the highest vascular density, at 200-magnification, and the average number of microvessels was recorded.

RESULTS: The microvascular density for 83 tumor specimen ranged from 17,5–51,5 with a mean MVD of 33,17± 7,95. Mean microvascular density was chosen as the cut-off point, 38 patients were categorized as a low MVD and 45 patients as a high MVD. There was no significant relationship between angiogenesis and age, sex, and histological type of gastric cancer. A significant correlation was found between the angiogenesis in early and advanced gastric cancer, and between gastric cancer with and without regional lymph node metastasis. Angiogenesis was significantly associated with poor survival. Multivariate survival analysis showed that stage of dissease and angiogenesis were independent prognostic factors.

CONCLUSION: Angiogenesis is one of the most important prognostic factors for gastric cancer patients, and therapeutic inhibition of angiogenesis may advance poor prognosis of gastric cancer patients.

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ANGIOGENESIS AND VESSELS' MATURATION GRADE IN COLORECTAL CARCINOMAS

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Background: It is very difficult to identify, based on the immunohistochemically (IHC) aspects, the cases with colorectal carcinomas (CRC) in which the angiogenesis inhibition could prolonge life. In CRC the double immu-

nostain (DIHS) with CD105 (endoglin) and SMA (Smooth Muscle Actin) was studied in a few papers.
Methods: 20 CRC surgical specimens were IHC studied utilizing the antibodies CD31 and CD105. The DIHS realized with CD105 and SMA.
Results: With DIHS we identified more type of vessels. The endothelial isolated cells were represented by non-irrigated endothelial cells, did not have lumen and marked only with CD105 in DIHS. The immature vessels presented lumen, were marked only with CD105 and were not have pericytes. The intermediary vessels were marked with both CD105 and SMA. The wall was very thin, the lumen was small, they did not have pericytes neither smooth muscle cells. The mature vessels were marked only with SMA, they had pericytes and a smooth muscle layer. CD105 in DIHS was correlated with CD105 in simple immunostain (SIHS) at 400× but not at 200× magnification. CD105 in both SIHS and DIHS was correlated with CD31 immunostain at 400× magnification. The microvascular density of intermediary vessels was correlated with the histological grade of non-mucinous CRC. The positive area of intermediary vessels was higher in well differentiated CRC (2±0,03) than in moderate (0,14±0,02) or poorly differentiated ones (0,07±0,01). Their high density was also observed in displasia and polypoid CRC. This type of vessels could not be identified with SIHS and we believe that this is the argument for using DIHS in study of CRC angiogenesis.
Conclusions: The density of intermediary vessels, evidentiated only with DIHS, could be one of the reasons for success or failure of antiangiogenic treatment. The DIHS could be utilised for the study of vessels' maturation grade.

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Cyclooxygenase (COX-2) and Matrix Metalloproteinase 9 (MMP-9) expression in human colonic angiodysplasia

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BACKGROUND: Gastrointestinal angiodysplasia (AGD) is an acquired vascular pathology of the elderly and a major cause of chronic or recurrent bleeding. We previously showed that angiodysplasia strongly express vascular endothelial growth factor, a key effector molecule of angiogenesis. Angiogenesis is tightly controlled by multiple effector molecules and pathways. Among them, COX-2 and MMP-9 appear to play a role in the signaling pathway that mediate angiogenesis in colorectal cancer.
AIMS: To investigate whether these molecules participate in the process of vascular remodeling of human colonic angiodysplasia we examined the immunohistochemical expression of COX-2 and MMP-9.
METHODS: Immunohistochemistry was performed in sections of formalin-fixed, paraffin-embedded specimens obtained from 20 patients with colonic angiodysplasia and from seven patients with colon cancer and its adjacent, histologically normal margins of resection, as controls. We used affinity-purified rabbit polyclonal antibodies for COX-2 (4H12, Novocastra) and MMP9 (2C3, Oncogen Research) at 1:100 and 1:200 dilutions with the Envision method.
RESULTS: Strong vascular immunoreactivity for COX-2 was seen in 9 (45%) specimens of angiodysplasia and in 5 (71%) of colon cancer. In contrast, no immunoreactivity was found in normal colon. In angiodysplasia, staining for COX-2 was homogeneously distributed in the endothelial lining of blood vessels. Positive specimens were mostly graded as 2+ or 3+ intensity. Semiquantitative analysis revealed that the intensity of vascular staining in angiodysplasia (2.0+0.2) was similar to that of colon adenocarcinoma (1.6+0.2) (p=NS). Vascular immunoreactivity for MMP-9 was seen in 10 (50%) specimens of angiodysplasia and 4 (57%) of colon cancer, but not in normal colon. Vascular staining for MMP- 9 in angiodysplasia was focally distributed in endothelium as well as in pericytes, and was similar to that of colon cancer
CONCLUSION: Induction of COX-2 and MMP 9 expression support their role in the development and/or progression of human colonic angiodysplasia and provides potential therapeutic targets for the prevention of bleeding from these vascular lesions.

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Podoplanin expression and lymphatic microvessel density in colorectal carcinomas. Correlation with histopathologic types

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Background. Podoplanin expression in human colon cancer is less studied. Some data suggest that level of podoplanin is greater in colon tumors with lymph node metastasis compared with those without it. There are controversies concerning the presence of intratumoral lymphatic vessels. The involvement of podoplanin in the metastatic process is supported by expression in tumor cells

at primary and metastatic sites from many tumors but is not certified in colon neoplasms. The aim of the present study was to describe the podoplanin expression in tumor cells, lymphatics and stroma components of colon tumors. **Methods.** Specimens from 30 cases with colorectal carcinomas were evaluated for microscopic diagnosis on routine stained slides. Immunohistochemistry for podoplanin was performed using anti-podoplanin antibodies, clone 18H5 followed by application of catalyzed signal amplification working system. **Results.** In colon dysplasia podoplanin was expressed weakly in glandular cells with cytoplasmic granular pattern. An intense staining for podoplanin was observed in tumor cells of adenocarcinomas. Mucinous adenocarcinomas showed strong reaction in tumor cells, stroma, and high number of positive lymphatic vessels closely linked with mucus quantity. A more intense expression was found in undifferentiated tumor cells. Clusters of tumor cells invading stroma at distance from the core of the tumor also expressed podoplanin more intensely than primary tumor cells. Positivity in tumor cells was associated with low intratumor lymphatic vessels density. Lymphatic vessels were more numerous at distance from the tumor. Positive stromal cells were grouped between tumor areas with and without necrosis or scattered between stromal components. **Conclusions.** Podoplanin expression in tumor cells was mainly found in mucinous adenocarcinoma and undifferentiated tumors. Our results suggest that podoplanin could be used as prognostic marker of invasion and metastasis in colorectal carcinomas.

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Mutation and Expression of APC/β-Catenin/E-cadherin in Colorectal Adenocarcinoma

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Purpose: The pathogenesis of colorectal adenocarcinoma is described as a sequential multistep accumulation of genetic alterations. Adenomatous polyposis coli (APC) somatic mutations are the earliest known abnormalities in the development of sporadic colorectal tumors. Recently, accumulation of β-catenin by β-catenin mutation in colorectal carcinoma cell lines without APC mutation was demonstrated, which also occurred in some sporadic primary colorectal tumors. Most research into the development of colorectal cancer has focused on the detection of mutation of particular gene, but the elucidation for the interaction of these mutated genes with cell adhesion molecules is also important to understand the colorectal tumorigenesis. Methods: To elucidate the role of APC/β-catenin/E-cadherin in the colorectal adenocarcinoma, we identified APC/β-catenin/E-cadherin mutation and expression in forty two colorectal adenocarcinomas. Results: Mutations of APC exon 15 and β-catenin exon 3 were detected in eleven cases (26.2%) and one case (2.4%) out of forty two colorectal adenocarcinomas. But there was no evidence of aberrant shift in E-cadherin exon 6–9. APC and E-cadherin promoter methylations were found in five cases (11.9%) and twenty five cases (59.5%) out of forty two colorectal adenocarcinomas. The membranous staining of APC and E-cadherin was decreased in thirty cases (71.4%) and thirty seven cases (88.1%) out of forty two colorectal adenocarcinomas. The β-catenin expression was increased in the membranes in thirty four cases (81.0%), cytoplasms in seventeen cases (40.0%) and nuclei in twenty one cases (50.0%) out of forty two colorectal adenocarcinomas. At the invasive areas of tumor growth, nuclear expression of β-catenin was common. The degree of reduced E-cadherin expression and the grade of tumor differentiation were statistically significant. Conclusion: Down-regulation of APC and E-cadherin by genetic mutation or epigenetic change such as methylation as well as β-catenin mutation was closely related with increased expression of β-catenin protein in colorectal adenocarcinoma. Decreased expression of APC and E-cadherin and increased β-catenin expression were commonly found in colorectal adenocarcinoma, which may be helpful to understand the pathogenesis of colorectal adenocarcinoma. But increased β-catenin expression is caused by secondary change arising from APC inactivation or competitive binding of β-catenin to APC and E-cadherin than by carcinogenic effect directly involved in tumorigenesis of colorectal adenocarcinoma.

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hMLH1 Abnormality in 180 Sporadic Colorectal Carcinomas

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Colorectal carcinomas in South Korea is increasing due to the increase of lipid-rich diet and meat intake. Pathogenesis of colorectal carcinomas has known to be involved with numerous chromosomes and genetic abnormalities. One of them is microsatellite instability (MSI) due to defective DNA mismatch repair (MMR) genes (hMLH1, hMSH2, hMSH6, PMS1, PMS2). The MMR gene abnormality is associated with promoter methylation as well as genetic mutation. Recently immunohistochemical detection of DNA MMR protein is able to suggest of the presence of MMR gene abnormality. The aim of this study was to

evaluate the role of immunohistochemical stain of hMLH1 as a screening tool for carcinomas with defective DNA mismatch repair (MMR) genes. In this study, 180 colorectal carcinoma tissue were obtained in the fresh status for MSI phenotype, hMLH1 promoter methylation, hMLH1 mutation analysis. The tissue microarray slides made of 180 formalin-fixed, paraffin-embedded colorectal carcinomas were examined for hMLH1 immunohistochemistry. High frequency of MSI (MSI-H) indicates at least two of five standard MSI markers (D2S123, D5S346, D17S250, Bat-25, and Bat-26). Of the 180 colorectal carcinomas, nine cases (5.0%) were MSI-H, nine cases (5.0%) had hMLH1 mutation on exon 1, 7, 12, and 16. Twenty one cases (11.7%) showed loss of hMLH1 protein expression on immunohistochemistry, and sixty one cases (33.9%) were methylated at the hMLH1 promoter. Status of MSI-H was correlated with younger age below 50 years old ($p=0.004$), tumor location of right colon ($p<0.0001$), and mucinous carcinoma ($p=0.025$). Presence of hMLH1 mutation was correlated with tumor location of right colon ($p=0.022$), and mucinous carcinoma ($p=0.015$). Immunohistochemical stain of hMLH1 was correlated with only younger age below 50 years old ($p=0.029$), but hMLH1 promoter methylation was not significant correlation with any clinicopathologic feature. Average of tumor size was larger in the groups of MLH1-H and hMLH1 gene mutation than MSS/MLH1-L and none mutation. Patients' age was younger in the group of MLH-H than MSS/MLH1-L. And immunohistochemical stain of hMLH1 was correlated with the results of MSI-H (<0.0001), hMLH1 promoter methylation ($p<0.0001$), and MLH1 mutation ($p=0.039$). In conclusion, most hereditary non-polyposis colorectal carcinomas was known to be associated with DNA MMR genetic mutation, but in sporadic colorectal carcinomas hMLH1 promoter methylation (34%) than hMLH1 gene mutaion (5%) is more frequently detected. And immunohistochemical stain of hMLH1, as single simple test, is able to apply for effective detection method of MLH1 abnormality.

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The Value of Magnifying Colonoscopy In Determining Serrated Polyps In Asymptomatic Individuals

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BACKGROUND: The genetic pathway model for the pathogenesis of colorectal cancer (CRC) is mainly based upon the concept of an adenoma-carcinoma sequence. In recent years, however, a "serrated pathway" involving hyperplastic polyp-serrated adenoma-carcinoma sequence has been proposed. This prospective study was designed to evaluate the value of magnifying colonoscopy in determining serrated polyps in asymptomatic individuals.
METHODS: One hundred and seventy four asymptomatic individuals over the age of 50 years were included in the study. After identifying the lesions at colonoscopy, 0.2% indigocarmine solution was sprayed and a magnified view of the stained crypt orifice at a maximum of 100 times magnification was obtained. The observed pit patterns were classified into 6 categories (I, II, IIIL, IIIS, IV, and V) according to Kudo's classification. Types I and II were designated as non-neoplastic patterns whereas types III-V were considered as neoplastic. All polypoid lesions were histopathologically examined on a routine basis. Chi-square test was used for statistical analysis.
RESULTS: There were 93 females and 81 males, with a mean age of 58,78±9,13 years. During magnifying colonoscopy, a total of 182 polyps, including 103 serrated polyps (SP) (78 hyperplastic polyps, 23 sessile serrated adenomas, 2 traditional serrated adenomas), and 79 conventional adenomas were identified. The majority of the polyps were located in the left colon (76,2%) while 23,8% were observed in the right colon. According to the pit pattern analysis 80,4% of the polyps revealed types I-II while 19,6% showed types III-V. Types I-II were significantly more frequently observed in SP ($p<0,001$) while types III-V were significantly higher in adenomas ($p<0,001$).
CONCLUSIONS: Magnifying colonoscopy proves to be useful in differentiating serrated polypoid lesions of the colon even in asymptomatic individuals according to pit pattern analysis which seems to correlate with histopathologic subtyping.

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FOXP3+ REGULATORY T CELLS IN MICROSCOPIC COLITIS.

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Background: Regulatory T cells (Treg) contribute to the control of immunologic tolerance, autoimmunity and immune response phenomena of human diseases. Treg cells express IL-2Ralpha (CD25) and they are specifically identified by FOXP3 expression, which is the main regulating gen involved in these immunological processes. The pathogenesis of microscopic colitis is unknown, but it has been suggested an autoimmune basis as well as alterations in the immune response against several antigens.

Objective: The aim of this study is to assess the presence of Treg cells in patients with collagenous (CC) and lymphocytic colitis (LC) in comparison with patients with chronic diarrhea and nonspecific colonic inflammation.
Materials and Methods: We studied colonic biopsies from 41 patients with microscopic colitis (21 CC and 20 LC), 17 patients with chronic diarrhea and nonspecific inflammation of the colonic mucosa (NC), and 10 control patients without diarrhea (CO). Immunohistochemical stains in formalin fixed, paraffin-embedded tissue for CD3 (intraepitelial lymphocytes; %), CD25 (0–3 score), and FOXP3 (0–3) were performed. The results were expressed as mean±SEM or median and compared by the Kruskal-Wallis one-way analysis of variance, and the Freeman-Halton extension of the Fisher test for a 2×3 table.
Results: We found CD25 expression in 1/17 NC, 19/20 LC y 14/21 CC ($p<0.0001$), with a subepithelial predominance. There was no expression of CD25 in the control group (CO). Immunoreactivity for FOXP3 with predominant subepithelial distribution was observed in the cases of CC and LC with CD25 expression.
Conclusions: Treg cells CD4+/CD25+/FOXP3+ are increased in lamina propria in patients with CC y LC, suggesting that they may play a role in the pathogenesis of these diseases

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THE EXPRESSION OF SNAIL AND E-CADHERINE IN COLORECTAL ADENOMATOUS POLYPS

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Background: The Snail gene family is composed of Snail (sna 1) and Slug (Sna2). Snail gene downregulate E-Cadherine expression, which is essential for epithelial to mesenchymal transition (ETM). This process takes place in the embryonic phase, during nervous central system development and in tumorigenesis.
Snail is overexpressed in some carcinomas in both epithelial and mesenchymal component, but its expression is unknown in colorectal adenomatous lesions. To investigate expression of Snail in the colorectal adenoma-carcinoma sequence, we examined expression of different types of adenoma.
Methods: A series of 50 colorectal adenomatous polyps have been selected. Five normal colonic mucosa and 5 colorectal carcinoma has been included for comparative purposes. Immunohistochemical studies for Snail, E-Cadherine, Mib 1 and p53 were perfomed in paraffin blocks.
Results: Transcriptional factor Snail is expresssed in 10% tubular adenoma and in 56% villous adenoma. In normal mucosa, the bottom crypt cells are slightly positive for epithelium, whereas colorectal carcinoma showed strong nuclear snail expression in epithelial and mesenchymal cells. E-cadherina is overexpressed in tubular and villous adenoma with light- moderate dysplasia. However its expression is decreased in adenoma with severe dysplasia.
Conclusions: Expression of Snail protein varies according to histological subtype of adenoma, dysplasia grade and cellular phenotype (epithelial or mesenchymal), however positivity for E-Cadherine depends on dysplasia grade. The role of nuclear expression of Snail as a biomarker for progression of adenoma needs further study.

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Slow Transit Constipation and decreased Interstitial Cells of Cajal

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Background: Chronic constipation is a common pathology affecting mainly younger women. Slow Transit Constipation (STC) is the most severe form, frequently refractory to therapeutic measures, needing a surgical approach. The causes of STC are still poorly understood, but are thought to be related to abnormal colonic motility. Decrease in colonic Interstitial Cells of Cajal (ICC) has been reported in patients with STC.
Case: A 33 years old woman diagnosed with nervous anorexia from age 12 to 24. At 25 she presented severe chronic constipation, showing slow colon transit at the right colon and a neuropathic pattern in the gastrointestinal manometry. Due to non-response to medical treatments, she underwent subtotal colectomy.The ascending and transverse colon were slightly dilated without other alterations. Histological sections were normal; submucous and intermuscular plexus showed ganglionar cells normally distributed at all levels. Immunohistochemical *c-kit* stains were done at different levels to demonstrate the presence of ICC. The sections from ascending and transverse colon showed decrease of ICC, with total loss in some areas. The distribution of ICC at descending colon and terminal ileum was normal, compared with normal surgical specimens used as controls.
Discussion: ICC are thought to play an important role in the control of gut motility, acting as pacemakers. They are localized mainly at the mienteric plexus and muscular

layers along the entire colon, and express consistently *c-kit*, so immunodetection of *c-kit* in tissue is a simple and reproducible method to evaluate alterations in number or distribution of ICC in the bowel. Our case is similar to others previously reported, supporting the role of ICC anomalies in the pathogenesis of at least some cases of STC.

Conclusion: In surgical specimens, from patients with intestinal motility disturbances, immunohistochemistry for *c-kit* is very useful to disclose ICC alterations in an otherwise histologically normal colon.

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Prognostic role of HER-2/neu over expression in colorectal cancer

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BACKGROUND: The Her-2/neu (c-erbB2) oncogene is one of the members of thyrosine kinas family.this oncogene encodes a 185 KD transmembrane protein which is involved in pathways of cell proliferation and differentiation because of its extensive homology and relation to epidermoid growth factor receptor.Her-2/neu is over-expressed in 30–40% of breast and ovarian cancers. This over-expression has been shown to correlate with poor prognosis of such malignancies. Monoclonal antibody-directed therapy has been used as an effective treatment for the patients with over-expressed tumors. We investigated over-expression of Her-2/neu in colorectal cancer, and its correlation with survival of the patients.

METHODS: Over-expression of Her-2/neu was examined by immunohistochemistry in 50 patients with colorectal cancer. The patients had undergone curative sugery at Alzahra Hospital since 2002 till 2005.

RESULTS: Over-expression of Her-2/neu was detected in 52%(26) of patients. There was no relationship between over-expression of Her-2/neu and prognosis.

(Respectively the mean survival in over-expressed and non over-expressed patients, were 44.46 +/- 2.68 vs 40.47 +/- 3.95 months).

CONCLUSION: Immunohistochemistry did not reveal any correlation between Her-2/neu over-expression and survival in colorectal cancer.

Key words: colon cancer,her2-neu, prognosis.

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Mucins expression in gastric neoplasms

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Gastric cancers constitute a highly heterogeneous group of tumors with respect to epidemiology, genetics, histopathology and biological behaviour.

Epithelial mucins MUC2, MUC5 and MUC6 whose genes are clustered on chromosome 11p15.5 are involved in the mucus gel formation. MUC5 and MUC6 are the major mucin components in normal gastric mucosa. MUC5 is highly expressed in mucous cells of the superficial and foveolar epithelium, whereas MUC6 is present in mucous neck cells and in mucous glands of cardia and antrum. Moreover, MUC2 and MUC5 which are absent or barely detectable in normal gastric tissues have been reported in gastric carcinoma. MUC2 expression has been related more specifically to the mucinous carcinomas of WHO classification whereas MUC5 has been associated with poor tubular differentiation and high mucin content. However, coexpression of multiple mucins is frequently observed in gastric carcinomas. In an effort to bring additional information in understanding the diversity of gastric neoplasms,we investigated the expression of MUC2, MUC5 and MUC6 in a series of gastric neoplasms.

We studied the immunohistochemical expression of MUC2, MUC5, MUC6, gastrin, villin and chromogranin in a cohort of 28 gastric neoplasms (7 adenocarcinomas, 11 undifferentiated carcinomas, 4 neuroendocrine tumors and 6 tubular adenomas).

We found MUC2 expression in 3 (43%) adenocarcinomas, 6 undifferentiaded carcinomas (55%) and 2 tubular adenomas (33%); MUC5 was expressed in 1 adenocarcinoma (15%), 1 undifferentiaded carcinoma (9%) and in all 6 tubular adenomas (100%); MUC6 was expressed in 1 adenocarcinoma (15%), 1 undifferentated carcinoma (9%) and in 4 tubular adenomas (66%). No expression of the the 3 studied mucins was found in neuroendocrine carcinomas. These data, even if in a small cohort, suggest that mucins could be useful, together with the classical neuroendocrine markers to identify an epithelial rather then a neuroendocrine origin of the neoplasia.

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Mixed exocrine-endocrine carcinoma of the stomach associated with sarcoid-like granulomas

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Mixed exocrine-endocrine carcinomas are defined as epithelial malignant tumors characterised by a combination of a predominant exocrine component (conventional adenocarcinoma) and a neuroendocrine tumor component represented by at least one-third of the tumor area. These tumors are rare in the stomach, and only 28 cases have been reported so far. We describe a further case in a 36-year-old man who underwent subtotal gastrectomy with regional lymph node dissection for an intestinal-type adenocarcinoma on initial biopsy. However, the resected tumor consisted of two components: a poorly differentiated intestinal-type adenocarcinoma with focal glandular areas and a poorly differentiated neuroendocrine carcinoma occupying approximately 30% of the tumor mass. The latter component was composed of diffuse sheets of small cells with high nuclear/cytoplasmic ratio. The adenocarcinomatous areas were immunopositive for cytokeratin 7, cytokeratin 20 and CEA, but the neuroendocrine component was negative. Neuroendocrine markers, including chromogranin A, synaptophysin and neuron-specific enolase, were diffusely positive in the neuroendocrine portions of the tumor, and only in scattered neuroendocrine cells within glandular structures of the adenocarcinomatous component. Perigastric lymph node metastases corresponded either to neuroendocrine or adenocarcinomatous component. Our case is unique in its association with sarcoid-like granulomas existing in the entire gastric mucosa and all perigastric lymph nodes. Numerous giant cells within granulomas exhibited laminated Schaumann bodies, and some of them contained asteroid bodies, too. The abscence of any clinical manifestations and the negative results of chest radiograph and laboratory test for the serum angiotensin converting enzyme excluded the possibility of systemic sarcoidosis. The association of mixed exocrine-endocrine carcinoma of the stomach and sarcoid reaction has never been reported previously.

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Cell cycle proteins alterations in gastric cancer

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Background: Progression of the cell cycle is governed by cyclin-dependent kinases (cdks), whose activity is inhibited by the cdk inhibitors. Dysregulated expression of cyclins, cdks, and cdks inhibitors is closed linked to uncontrolled proliferation and malignant transformation of the cell. In gastric cancer (GC) increasing evidence suggests that alterations in cell cycle proteins are associated with poor prognosis but the results still contradictory. The aim of this study was determinate the expression of cell cycle proteins in gastric cancer by immunohistochemistry (IHC) and their prognostic significance. **Material and methods:** Using IHC, expression of p27, p21, p16, pRb, p53, cyclin D1, cyclin A, and cyclin B1 was evaluated in a high throughput tissue microarray containing 482 GC in duplicate. According with literature criteria, positive cases were determinate when nuclear or cytoplasmatic staining was observed in >10% of the tumor cells. **Results:** Expression for p53, p27, p16, p21, and pRb was observed in 137 out 458 (30%), 231 out 457 (50%), 50 out 463 (10.8%), 64 out 458 (14%), and in 313 out 458 (68%) GC. 220 out 449 (49%), 316 out 458 (69%), and 229 out 460 (49%) GC was positive for cyclin D1, cyclin A, and for cyclin B1. Intestinal type of GC frequently showed expression of p21 and p53 and loss cyclin B1 and pRb staining compared with diffuse type of GC. Diffuse type of GC with p53 expression showed poorer prognosis than the p53 negative cases ($p<0.001$). **Conclusion:** Alterations in cell cycle proteins expression are common events in gastric cancer. Loss of p21 and p16 staining with overexpression of cyclin D1, cyclin A, and cyclin B1 are the major findings. Poor prognosis is associated with p53 expression in diffuse type of GC. This marker could be used to identify individuals that are at high risk.

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THE WAY IMUNO-MARKERS APOPTOTIC GENES, PROLIFERATION FACTORS AND ENDOTHELIAL ANTIGENS TURN UP IN BARRETT ESOPHAGUS AND ITS COMPLICATIONS

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Introduction. The most serious complication of the chronic reflux esophagitis, or Barrett esophagus (BE), with glandular metaplasia is secondary esophagial adenocarcinoma. **Purpose:** Checking the link between the progress of Barrett's metaplasia to adenocarcinoma and the application of tumor markers involved in increased proliferation

aspects with presence of apoptotic genes (p-53, bcl-2) proliferation factors (PCNA, Ki-67) and endothelial antigenes (CD 34)

Material and methods. The study included 102 cases, mainly males, aged between 23 and 65, who were subjected to endoscopic and histopathologic investigation. Biopsies were fixed in 10% formol and parafin included.. Than ue used HE and histochemical staining for neutral and acid mucopolysaccharides and IHC staining: (indirect tristadial Avidine-Biotine-Peroxidase method).

Results: The 102 cases, diagnosed by endoscopic and histologic methods, classified as follows: 35 cases of Barrett esophagus with specialised epithelium, 11 cases of non-determined metaplasia, 9 cases of initial intraepithelium neoplasic lesions, 10 cases of advanced intraepithelium neoplasic lesions and 17 cases of moderate and undifferentiated carcinoma (one of them abounding in Russell bodies).

Immunohistochemical, Mutated tumor marker p-53 evidentiation was expressed in 61% cases (high grade of displasya of Barrett's esophagus and adenocarcionoma). The second antiapoptotic marker bcl-2 was inconstantly positive, especially in the lymphocytes of lamina propria. Monoclonal PCNA and 85% of Ki-67 antibodies were positive especially in the undifferentiated carcinoma. Endothelial antigenes (CD 34) were positive especially in the areas with the highest vascularization of well differentiated carcinoma. Follow-up studies are necessary to evaluate the prognostic value of BE complications.

Conclusion: To diagnose the distal esophagus precancerous and cancerous conditions, assess the prognosis and risk of progression and to determine effective treatment, a wider panel of tissue tumour markers could help.

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Macro and microscopic assessment of activitiy in ulcerative colitis

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Background: Medical treatments have changed morphological features ol ulcerative colitis (UC) giving unusual patterns of colonic involvement. In this setting we try to assess the ability of macro and microscopic parameters to determine the activity of UC in treated and untreated patients.

Method: 113 consecutive patients with active and inactive UC (64 men, 49 women; mean age 45 years, range 18–81 years) in their first attack (16 cases) or with established colitis (97 cases) were prospectively assessed. They underwent a complete ileocolonoscopy with sequential biopsies of terminal ileum and all colonic segments (total= 625 samples). Endoscopic and histopathological parameters were evaluated using two different scores in which acute and chronic signs of inflammation were considered separately. Five diagnostic categories (normality, remission, mild, moderate and severe disease) and the continuous/patchy extension were established. The degree of agreement between scores was characterized by kappa statistics.

Results: kappa values between scores were 0,469 (concordance=59.2%) for the different categories and 0.748 (concordance=87.6%) when only inflammatory activity was considered. Endoscopy didn't detect normality in 5.4% of samples and overdiagnosed remission in 8.8% of them. Histology revealed more inflammatory activity respect endoscopy in 3.3%. In patients in their first attack there was an excellent correlation on the inflammatory activity (k=1.000) in contrast with that seen in established colitis (k=0.529–0.758). Patchiness was determined in 38/113 (33.6%) of patients by both endoscopic and histological parameters. Histological rectal sparing was detected in 7 of 113 (6.2%) patients.

Conclusion: Correlation between macro and microscopic scores is frequently bad in longstanding UC. Endoscopy underestimates normality and inflammatory activity and overestimates remission. Endoscopic parameters are insufficent to detect normality. Rectal sparing and patchiness are frequentlly seen in treated longstanding UC.

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Clinicopathological and immunohistochemical prognostic factors in stage II (T3/4N0M0) colorectal carcinoma

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Background: Several proteins have been suggested to represent prognostic factors in colorectal cancer; however, the prognostic role of their expression in the group of stage II (T3/4N0M0) carcinomas has only rarely been studied and compared to the prognostic value of classical clinicopathological factors. We studied these relations with the use of tissue microarray (TMA) technology in a large tumor series.

Methods: Data on stage II colorectal carcinomas from 251 patients who underwent R0 resection, received no adjuvant treatment and were followed for a minimum of 5 years were retrieved from pathology reports. In addition, 2 mm cores were obtained from paraffin blocks of these tumors and used for the construction of TMAs, which were stained immunohistochemically for p53, cyclin-D1, E-cadherin,

HER2, and EGFR. Associations with overall survival were assessed by univariate (Kaplan-Meier and log rank test) and multivariate (Cox regression) analysis.
Results: The overall 5-year survival was 72.5%. Older age, presence of lymphovascular invasion, higher pT category (pT4), rectal location, lower number of examined lymph nodes (<12) and presence of perineural invasion were significantly associated with decreased survival in univariate analysis; tumor grade and patients' sex were not related to survival. In multivariate analysis, older age, male sex, and presence of lymphovascular and/or perineural invasion were the only independent adverse prognostic factors. None of the features studied by immunohistochemistry were significantly associated with survival in univariate or multivariate analysis.
Conclusion: Our findings suggest that classical clinicopathologic parameters are superior to some immmunohistochemical features in predicting survival in stage II colorectal carcinoma and should be included in future studies of potential prognostic value of new molecular markers.

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Correlation Of Clinical And Histopathologic Features Of Celiac Disease

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Aim: Endoscopic, clinical and histopathologic findings of Celiac disease is well described. In this study we aimed to investigate the relationship between these findings.
Methods: First, 420 cases were collected who had duodenal biopsies because of suspicion of malabsorption from the archives of Pathology Department. We reexamined the biopsies for histopathologic features in them. Then clinical features and serologic findings of the cases were reviewed. The relationship between the histopathologic features and endoscopic, clinical and serologic results were investigated statistically, using chi square and Multiple Comparison Tests.
Results: After the microscopic examination, 99 cases were diagnosed as Celiac disease. 20 of them had previous biopsies, among whom, 16 had complete response and 4 had no response to diet. No definite diagnosis could be given in 19 cases. Among all patients, male to female ratio was 0,52 and 102 of them were under the age of 19, while 35 were adults. The most common clinical finding is diarrhea (42 cases), followed by failure to thrive in 24, abdominal meteorism in 17 and weight loss in 13 cases. 2 cases were diagnosed as Hashimoto thyroiditis and 3 cases were diagnosed as cystic fibrosis. Endoscopic examination was diagnostic in 2 cases with the loss of folds mostly. 138 cases were gathered and 69 of them had clinical findings. Antigliadin G, A, and antiendomysium antibodies were positive in 59%, 58%, and 58% of the patients respectively. After the histopathologic review all cases were found to be have villous atrophy, and increase in number of intraepithelial lymphocytes. The results of correlations between age, gender, histopathologic, endoscopic and serologic findings were discussed.
Conclusion: In clinically symptomatic cases of celiac disease, endoscopic and serologic findings are usually suggestive, however, as the sensitivity and specifity of histopathologic features are the highest, duodenal biopsies should be always performed for correct diagnosis.

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Venous invasion in lymph node negative colorectal carcinomas

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Introduction: Patients diagnosed with lymph node negative colorectal carcinomas (UICC Stage I and II) undergo surgical tumor resection with curative intent, yet up to 30% of these patients suffer recurrent or metastatic disease within 5 years of surgery. Vascular invasion has been associated with inverse outcome but major differences in the detection rate and the impact in respect to the localization of venous invasion has been reported.
Material and Methods: We retrospectively evaluated Stage I and II colorectal carcinomas for the presence of intra- and/or extramural venous invasion ($V1_{IM}/V1_{EM}$). All available tumor sections were stained with H&E and Elastica van Gieson (EvG) and the findings were correlated with the clinical outcome.
Results: A total of 185 colorectal cancers, including 39 stage I and 146 stage II, were reanalyzed. On an average, 3.7 tumor sections were evaluated per case and median clinical follow-up was 32 months (range 0 to 153 months). Venous invasion was present in 43 (23.2%) patients (26 intramural, 17 extramural), including 37 (86.1%) cases in which venous invasion was previously not detected by H&E. Venous invasion was more common in stage II compared to stage I cancers (28.8% versus 2.6%; $p<0.001$). Survival analyses of patients with and without venous invasion showed 5-year survival rates of 65% and 69%, respectively, which did not reach statistical significance (p=

0.382). Comparison between $V1_{IM}$ and $V1_{EM}$ did not show significant differences either (5-year survival rates 62% vs. 65%, p=0.663).

Conclusion: Venous invasion is common in stage II colorectal cancers, but elastic stains to highlight vascular structures are mandatory for reliable detection. In our serie, however, the presence of venous invasion, irrespective of localization does not have a prognostic impact.

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Analyis of FGFR4 Gly388Arg polymorphism as a prognostic factor in resected hepatic metastases of colorectal cancer.

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Background: Colorectal carcinoma (CRC) is the second cause of cancer-related mortality in the Western world. In the majority of patients, CRC is detected with lymph node metastases and a significant number of cases have distant spread, mainly in the liver. To improve patient treatment it becomes important dispose of genetic markers that select better the patients. Presence of fibroblast growth factor receptor-4 (FGFR4) Gly388Arg polymorphism has been associated with a poor prognosis in several neoplasms including colorectal and breast tumors. The aim of this study is to analyze the prognosis value of the presence of Gly388Arg polymorphism in a series of resected colorectal hepatic metastases. ***Methods:*** From 2001 until 2006 a series of 156 patients harboring CRC liver metastases underwent radical surgery. Histological confirmation was made for all cases, and fresh-frozen hepatic metastases and paired non-adjacent normal liver parenchyma was simultaneously collected for all patients. Prospective clinical follow-up is avalaible for all patients. DNA and RNA was obtained for all cases by standard methods. Presence of Gly388Arg polymorphism was analized by a BstNI-RFLP (Restriction Fragment Length Polymorphism) method. ***Results:*** Presence of FGFR4 (Arg 388) allele was identified in the 42.15% of the patients, in 65 patients heterozygous and in 7 patients homozygous. In a preliminary analysis we did not identified any association between the presence of FGFR4 (Arg 388) allele and the clinicopathological variables analyzed. Similary, in our preliminary prognostic data presence of FGFR4 (Arg 388) allele seems not correlates with survival parameters. ***Conclusion:*** To improve our study we are analyzing: (i) the correlation between this polymorphism and the degree of fibrosis present in liver metastases; and (ii) establish the association between the presence of FGFR4 (Arg 388) allele and the FGFR4 expression levels determined by quantitative real-time PCR (qPCR). Our study will permit to establish the prognostic significance of FGFR4 (Arg 388) allele in colorectal liver metastases.

P-400

Intestinal graft versus host reaction in patients with Good's syndrome – case report

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60 year old patient was admitted to our Gastroenterology Unit due to bloody diarrhea of about month duration. The patient had been operated on mediastinal thymoma (AB type) two months earlier. In laboratory studies he presented hypogammaglobulinemia but no overt infection was appreciated. Stool was collected for microbiological studies but they were constantly negative for bacteria or fungi presence.

Biopsy specimen were taken from stomach, right colon, sigmoideum and rectum. According to endoscopic depiction large bowel mucosa were oedematous and hyperaemic with many ulcers and changes in vascular pattern what gave the suggestion of nonspecific inflammatory bowel disease.

In histological picture many extensive ulcers and mucosal sloughing were seen. No architectural crypt changes occurred and many apoptotic bodies were present in crypts' epithelium. These changes were so pronounced and numerous in rectal mucosa that it would correspond with what is called in literature "exploding crypt cells". Interestingly, inflammatory infiltrate, in the areas remote from necrosis, was quite sparse.

Additional the immunohistochemical studies for CMV were performed and turned out negative as the serology for cytomegalovirus was.

No histological changes were seen in gastric mucosa.

Conclusion: Graft versus host like reaction is rare complication in non-transplanted patients but should be taken into differential diagnosis of diarrhea in patients with Good's syndrome. Intestinal histological features could include, next to characteristic apoptotic bodies, quite extensive and deep ulcers. Slight distortion of crypts can be appreciated but no pronounced mucosal remodeling occurs.

Acute infectious colitis should always be the matter of differential diagnosis debate as the CMV infection is. CMV colitis could have very similar histological picture and the adjunctive immunohistochemistry or in situ hybridization use seems mandatory.

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TUMORATION IN THE JEJUNUM WITH HISTOPATHOLOGIC FEATURES OF SARCOMA OF CLEAR CELLS

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BACKGROUND Clear cell sarcoma (CCS), also known as malignant melanoma of soft parts, is a soft tissue sarcoma of the lower limb in adolescents and young adults. Other anatomical sites of this tumour such as gastrointestinal tract are extremely rare. In particular, the location in the jejunum of this neoplasm is exceptional, so we performed a review of the literature with an emphasis on its histopathological differential diagnosis.

METHOD The biopsy material, for the pathologic study was fixed in 4% formalin according to the usual methods for processing and inclusion in paraffin, and the posterior sections were stained by hematoxiline-eosine, PAS and Wilder's reticuline. Some sections were choosed for immunohistochemical study and for an evaluation of chromosome 22 translocation using FISH.

RESULTS A 41 year old male patient presented a neoplasia, measuring 7.7 cm along the major axis, in the jejunum which affected all the parietal strata with local adenopathic conglomerate metastasis. Microscopically this was a tumour with a pattern of solid growth in the form of masses, nests and ribbons which were composed of atypical epithelioid cells with eosinophile or clear cytoplasm (positive PAS) and a vesiculose nucleus with macronucleoli and frequent mitosis. There was also the presence, in some cases, of osteoclastic type cells. The tumoural cells were immunohistochemically positive for S-100 protein, vimentine and bcl-2, and negative for other markers, including CD-117, HMB-45 and Melan A.

CONCLUSIONS After a review of the literature, a fourth case of CCS in the jejunum, with a genetic study, is presented here. A differential diagnosis with GIST, malignant schwannoma melanomas and carcinomas, was made given the extremely uncommon location of the tumour.

P-402

Impact of small lymph nodes (<2 mm) on N-stage in colorectal cancers

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Introduction: Lymph node metastasis is an important prognostic factor of colorectal cancers. A minimal requirement of 12 lymph nodes has been proposed by the UICC/TNM. However, several authors feel that this number is too low and therefore a cumbersome search for small lymph nodes is initiated. In the present study, we evaluated the impact of small lymph nodes in tumour staging.

Material and methods: Retrospectively, lymph nodes of surgical specimen of colorectal cancers were analyzed.

Results: A total of 464 colorectal carcinomas were evaluated. Lymph node metastasis was detected in 216 (46.6%) of specimens (130 patients N1, 86 patients N2). Of 7659 lymph nodes, 899 (11.7%) show tumour involvement. The size of lymph nodes with metastasis was significantly larger than lymph nodes free of tumour (median diameter 5.76 mm versus 3.52 mm; $p<0.0001$). The size of lymph nodes in 65 patients with a single lymph node metastasis was also significantly larger (5.76 mm versus 3.44 mm; $p<0.0001$). Metastatic involvement of lymph nodes <2 mm was significantly less common than of larger lymph nodes (2.2% versus 13.2%; $p<0.0001$). Only in 2 patients, metastasis of a solitary lymph node <2 mm led to an upstaging from N0 to N1. Using a cut off of <3 mm, 10 (2.2%) patients would have been upgraded from N0 to N1 whereas a cut off <5 mm would understage 45 (9.6%) patients inadequately as N0.

Conclusion: A careful evaluation of lymph nodes in colorectal surgical specimen is crucial in the management of patients with colorectal carcinomas. This includes also lymph nodes smaller than 5 mm; however, lymph nodes <2 mm are seldom involved by carcinoma and rarely (0.4%) lead to an inadequate N0 staging.

P-403

Colo-rectal micropapillary carcinoma: A clinicopathological and molecular study of 27 cases.

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INTRODUCTION. Invasive micropapillary carcinoma (MC) is associated with frequent lymph node metastasis and adverse clinical outcome. It has been reported in ovary, breast, urinary bladder, ureter, lung, parotid gland and, recently, in colon. Little is known about the morphological and molecular profile of this type of colonic carcinoma.
AIM. To analyze the clinico-pathologic features and molecular findings of colorectal MC and to compare with non-MC carcinoma.
MATERIAL AND METHODS. Clinicopathological features of a cohort of 333 patients with primary colo-rectal cancer were retrospectively reviewed. All slides avalaible from each tumor were reviewed by two pathologists, looking for the presence of micropapillary pattern (small papillary cell clusters surrounded by lacunar spaces). An estimation of the proportion of this component was recorded. The parameters evaluated in each case included: age, sex, location of primary tumor, tumor size, growing pattern (infiltrative or expansive), grade, depth of invasion (pT), lymphovascular and perineural invasion, nodal status (pN) and number of positive lymph nodes Genetic assesment of microsatellite instability (MIN), chromosome 18q status, p53 and Kras mutation were performed on DNA extracted from sections of formalin-fixed, paraffin-embedded specimens.
RESULTS. Twenty seven cases (8.1%) had micropapillary component, ranging from 10 to 95% of the tumor. They showed higher frequency of infiltrative pattern, lymphovascular and perineural invasion, a higher depth of invasion, and more positive lymph nodes when compared with conventional adenocarcionoma. These differences were statistically significative ($p</=0.0001$). There were not statistical significative differences in molecular findings between the two groups, but MC had a tendency to carry p53 mutations ($p=0.03945$).
CONCLUSIONS. Colorectal MC appears to be more aggressive than conventional colorectal adenocarcinoma. They present at a higher tumor stage with frequent lymphovascular and perineural invasion and nodal metastasis compared with conventional adenocarcinoma and have more tendency to carry p53 mutations.

P-404

IMMUNOHISTOCHEMICAL AND MORPHOMETRICAL EVALUATION OF THE NEUROENDOCRINE CELLS AND PROLIFERATIVE ACIVITY OF GASTRIC EPITHELIUM IN SUPERFICIAL (gchs) AND DEEP (gch) CHRONIC GASTRITIS

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Background Neuroendocrinecells exist almost in the whole alimentary canal. These cells have an influence on secretory functions and normal gastric mucosa histostructure. Up to nowinvestigations confirmed participation of these cells in e.g. HCl and gastrinsecretion regulation and in some of the proliferative states of gastric mucosa.The aim of the study was to estimate participation of neuroendocrine cells andstatus of the proliferative activity gastric epithelim in gchs and gch.
Method Paraffin blocks from gastricoligobiopsies were the material which was collected from 35 women (of average-age-52) and 34 men (of average age-54). Chronic inflammations were gradedaccording to modified Whitehead's classification. Helicobacter pylori wasevaluated according to the system from Sydney.Neuroendocrine cells and proliferative antigen expression of gastric mucosawere counted after proper immunohistochemical stains (Chromogranin A, Serotonin,Somatostatin and PCNA) in circular, oblique short, oblique long and oblong mucosalgastric glands sections. All measurements were conducted separately for gastricmucosa prepyloric part and gastric corpus. Next we use the thresholdingoperation sequentially and apply the artificial neural network of SVM type formeasurement of the same objects. The results of quantitative evaluations wereanalysed with the use of statistic methods. Results In gastricmucosa antrum there were twice more Chromogranin A,Serotonin and Somatostatincells in comparison with corpus. Gastric mucosa inflammatory type did not havea significant influence on cells localization in gastric mucosa. The number of ChromograninA cells diminished in gchs gastric antrum among patients who were over 50 yearsold,whereas the same number increased in corpus inflammations of this type.The increase of Chromogranin A cells number was found in gchs gastric antrumwith Helicobacter pylori colonization.and higher proliferative activity of thegastric epithelium. In these cases Serotonin and Somatostatin cells numberdiminished. Any differences were found in proliferative activity gastricepithelium depending on histotopography of samples, age and Helicobacter pyloricolonization.
Conclusion

1. Chronic superficial gastritis coexists with higher proliferative activity in the prepyloric part and Chromogranin A cells number increase. In these cases Somatostatin and Serotonin cells number decreases.
2. Changes of the proliferative activity in chronic gastritis do not depend on its age and Helicobacter pylori colonization in the gastric mucosa
3. In chronic gastritis quantitative changes of Chromogranin A, Somatostatin and Serotonin cells run differently in gchs and gch both in prepyloric part and in gastric corpus.

P-405

Expression of survivin in colorectal carcinomas

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Background: Survivin is a new member of the IAP family that has a dual function as mitotic regulator and apoptosis inhibitor. Survivin is prominently expressed in transformed cell lines and in many human cancers including colorectal carcinoma. The aim of the study was to investigate the expression of survivin in colorectal carcinomas and to correlate it with clinicopathological parameters and patient survival. **Materials and Methods:** Formalin-fixed paraffin embedded tissue from 60 cases of colorectal carcinomas was stained by immunohistochemistry for survivin. **Results:** Survivin was mainly detected in the bottom of the glands of the normal mucosa and the localization was mainly cytoplasmic. No reactivity of the survivin antibody was found in the infiltrating lymphocytes, fibroblasts, smooth-muscle cells or neural tissue. Survivin predominantly cytoplasmic and minimal nuclear staining was found in 36/60 (60%) carcinomas. A slight tendency for a relationship between survivin expression and tumor differentiation (p-value=0.32) was observed. Survivin expression was not statistically significantly correlated with the others clinicopathological variables. The survival curves did not differ according to survivin expression. **Conclusion:** Overexpression of survivin suppress the apoptotic checkpoint during mitosis, promote aberrant progression through mitosis and seems to provide a survival advantage for tumor progression.

P-406

Detection of MLH1 and MSH2 expression in colorectal carcinomas of patients less than 50 years old in Costa Rica

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Background: Microsatelite instability (MSI) is detected in more than 90% of cases with hereditary non-polyposis colorectal cancer (HNPCC). Immunohistochemistry analysis of MLH1 and MSH2 expression can be used in colorectal carcinomas to identify patients with a possible DNA mismatch repair defect related with HNPCC. Besides, it has been demostrated that MLH1 or MSH2-deficient colorectal carcinomas have better prognosis. **Method:** Fourty three colorectal adenocarcinomas ocurring before 50 years of age were retrieved from the Laboratory of Pathology of Hospital México, Costa Rica. They were studied by immunohistochemistry in paraffin-embedded surgical samples for MLH1 (Biocare, clone G168–15) and MSH2 (Zymed, clone FE11) protein expression. Absence of tumor cell nuclear staining with positive internal control was considered negative. **Results:** 10 (23.3%) tumors showed loss of MLH1 or MSH2. We found significant differences regarding to patients' age (negative expression 35.6±2.8; positive expression 40±1.4). There was a higher percentage of colorectal cancer familiar history in patients negative for MSH1 or MLH2 (negative expression 30.0%; postive expression 12.0%). A trend regarding tumor size was found (negative expression 10.9 cm; positive expression 15.3 cm). The two groups were not different for tumor site, differentiation, pTNM stage, vascular and perineural invasion and 5-year survival rates. **Conclusions:** MLH1 or MSH2-deficient colorectal adenocarcinomas of less than 50 years old patients exhibit some features similar or sugestive of HNPCC. This study confirms that immunohistochemistry is a useful tool for the routine study of patients with suspicion of HNPCC in Costa Rica.

P-407

The role of the gastric biopsy for the diagnosis of the Biermer's disease, about 64 moroccan cases.

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Introduction: Pernicious anemia, also called Biermer'sdisease, is an autoimmunne gastritis limited to the fundus of the stomach. In addition to fundic atrophy, the patient presents reduced secretion of gastric acid and intrinsic factor as well as vitamin B12 malabsorption. Prevalence was estimated to 9–17 new cases/100000 H/year in the occidental countries.

Methods: We report 64 cases of the pernicious anemia colliged in 2006–2007. This study is based on the gastric biopsy specimens made for patients with pernicious anemia or for the other symptomatology.the pathological criteria for diagnosis of the pernicious anemia were intestinal metaplasia, fundic atrophy and hyperplasic of the fundic endocrine cells.

Results: There were 34 females and 30 males with a median age of 53 year. We have 18 patients recorded with pernicious anemia. 17 patients had macrocytosis. 19 patients had hyperplasia of the fundic endocrine cells. In follow up, 6 patients developed neuroendocrine tumors. Helicobacter pylori was diagnosed in 23 patients.
Conclusion: There is evidence that the gastric biopsy is an essential tool for diagnosis, prognosis and confirmation of clinically suspected cases.

P-408
Specific features of small bowell gastrointestinal stromal tumors

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Background:Small bowell Gastrointestinal Stromal Tumors (GIST) are rare mesenchymal tumors originating in the wall of the gastrointestinal (GI) tract.The cell origin of these tumors is thought to be a stem cell that differentiates toward the interstitial cell of Cajal. Aim: Different clinical and morphological characteristics of the small bowell GISTs from the stomach GISTs, are the reason for this study. **Material and methods:** We have discovered 14 small bowell GISTs on surgical material, during last five years. Paraffine sections were stained with HE, PAS, HID-AB pH =2,5 and immunohistochemical LSAB2 methods, by using the antibodies: CD117, CD34, Ki-67, Melan A, S-100 protein, Desmin, Vimentin, NSE and panCytokeratin (Galen Fokus- Kopenhagen). **Results:** 14 patients (9 female and 5 male) were identified. The mean age of patients were 52 years, range 8, 17 (two children) to 72 years. Based on microscopical features of malignant potential of GISTs, disease was localized in 5 patients, locally advanced in 7 patients and with multiple primary lesions in 2 patients, associated with recurrent disease (after 7 months from surgical therapy in one) and spreading within the abdominal cavity (in the other), but never to lymph nodes. The most impressive finding was the discover of intracellular lipofuscin-like and melanin pigments in the spindled cells that showed strong expression the specific kit tyrosine-kinase receptor (CD117). Important histological diagnostic feature of the small bowell GISTs was globoid extracellular collagen accumulations (so-called skenoid fibers). **Conclusion:** It has been concluded that small bowell GISTs behave more agressivelly than gastric tumors, produce lipofuscin-like and melanine pigments and occur in the children as well as in adults.

P-409
Both histological and immunohistochemical study of rare duodenal neuroendocrine tumor - gangliocytic paraganglioma

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Background. Duodenal gangliocytic paraganglioma is very rare, accounting for about 9% of all gut endocrine tumors. Though considered benign the disease can spread to regional lymphatics. **Case presentation.** We report a case of a 25 - year - old woman presenting with a 2-week history of melena and was found to have periampullary mass. The tumor was resected with pyloris-preserving pancreatico-duodenectomy and was found to represent an infiltrative lesion of large size (9 cm in diameter). The resection margins were free of tumor. Histologically, the tumor had triphasic cellular appearance: 1. Spindle cells resembling Schwann cells or sustenticular cells, positive for neurofilament and S- 100 protein. 2. Ganglion-like cells, in nests, resembling the “zellballen” of classical paragangliomas, positive for synaptophysin and glial fibrillary acidic protein. 3. Endocrine cells arranged in nests, trabeculae, papillae and gland-like structures, positive for pancreatic polypeptide and neurone specific enolase, but staining for chromogranine was negative. There were areas of stromal hyalinization resembling amyloid, with focal calcification. She is alive and well one year following resection. **Discussion.** The authors have reviewed the current literature pertaining to this entity and have discussed the biological behavior of the tumor.

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ADENOMYOMA OF THE GASTROINTESTINAL TRACT. REPORTS OF FOUR CASES AND REVIEW OF THE LITERATURE

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Background. Adenomyoma of the gastrointestinal tract is a rare benign tumor-like condition characterized histologically by a mixture of glandular structures lined by tall columnar epithelium with intervening bundles of smooth muscle. The pathogenesis of adenomyoma is considered to be either a form of myoepithelial hamartoma or a pancreatic heterotopia. **Reported cases.** We describe four cases of adenomyoma. One was the jejunal adenomyoma that presented with intussusception occuring in a 48-year-old man. The other was gastric antral adenomyoma found incidentally in a 62-year-old man, during cholecistectomy. It had intra-abdominal localisation, origining from the

antral subserous region. The third had just below the cardia intramuscular localisation, inducing both the cardiac stenosis and cardiac ulcer, found after gastrectomy. The fourth was the Vater's papilla adenoma, discovered during the autopsy in a 54-year-old woman. Clinical diagnosis was dysfunction of the papilla Vateri, associated with severe obstructive jaundice induced by its cancer. Formaldehyde fixed, paraffin sections were stained with H&E, Van Gieson, PAS and HID-AB methods. Immunohistochemical LSAB2 method was used for verification of epithelial, mesemchymal, smooth muscles and myoepithelial tissues as well as nuclear proliferative activity (DacoCytomation). On histological examination, the lesions consisted of hyperplastic glandular lobules of the pylorus, exocrine pancreas and jejunum, covered by single-layer epithelium of cuboidal and columnar cell type. The cells showed no atypia; some ducts showed cystic changes. The hyperplastic glandular lobules were surrounded by hyperplastic mesenchymal tissue, composed of bundles of smooth muscle. Immunohistochemically, intense expression of panCytokeratin (epithelial marker), vimentin (mesenchymal marker), desmin (smooth muscle marker) as well as actin (myoepithelial cell marker) has been found. Ki-67 activity (proliferative activity marker) was negative. **Conclusion.** Adenomyoma of the gastrointestinal tube is a rare benign lesion. The differential diagnosis from adenocarcinoma, especially in a frosen section, might be difficult. The main features are the absence of cytological abnormalities, the absence of desmoplastic stroma and the presence of smooth muscle bundles encircling the cysts.

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Cytopathology

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chordoma – preoperatve diagnosis using fine needle aspiration cytology: a cytologic, histologic, radiological & immunohistochemical correlation.

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BACKGROUND- Chordoma is a rare tumor arising fromprimitive notochord located along the axial skeleton. The fine needleaspiration biopsy findings are described, correlated with histology, radiologyand immunohistochemistry and compared with previously reported descriptions of Chordoma.

STUDY DESIGN –During years 2006 & 2007 clinically unsuspected four cases of Chordoma werediagnosed preoperatively in the department of cytology, The Gujarat Cancer& Research Institute, Ahmedabad, India. Cytological material includedsmears and cell blocks. Immunostains were performed in all four cases onhistological material. Multiple cytological parameters were studied andcompared with previously reported description of Chordoma.

RESULTS- All four cases were male patients (mean age 58.7 years, range 45–65 years)had tumor involving sacrum (2 cases) and coccyx (2 cases). Pain was thepresenting symptom in all four cases. All smears were cellular with myxoidbackground; chondroid background was noted in one case. Cells with clear,vacuolated cytoplasm constituted the predominant cellular component. Small epithelial like cells were seen inthree cases. However, true physaliphorous cells were rare. Binucleated andmultinucleated cells were present in all.

CONCLUSION- Cytomorphologic findings are characteristic and when taken in context withradiological study allow differentiation of Chordoma from other primary ormetastatic lesion in axial skeleton. Chordoma is often an unsuspected diagnosisand fine needle aspiration biopsy can lead to a correct preoperativediagnosis.FNA may be utilized to document recurrence and thus facilitate theevaluation and management of the patients with these lesions.

P-412

Low Grade Squamous Intraepithelial Lesion/Human Papilloma Virus: Retrospective Cytologyc Study of the South East of Mexico

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Background: Uterine cervix carcinoma (CCU) is the second cause of women oncologic death in the world. Human papilloma virus (HPV) is associated with low grade squamous lesions (LSIL) and these ones are associated withthe development of cervix carcinoma. The objective is to evaluate the relationship between HPV and LSIL of Mexican and foreign women from Cozumel Island and its associated risk factors.

Methods and Materials: 305 pap smears were evaluated from The Cozumel Medical Center from November/2004 to May/2005. Papanicolaou technique was selected for evaluation, and interpretation was made by Bethesda System/ 2001. We excluded gyn-citologies with other sexually transmission deseases.

Results: Respective and correspondent percentage of total cases was: ASC, 11.07%; LSIL, 4.56% and HSIL, 0.65%. 30 years old was the average for LSIL, 8 (15.7%) were foreigners and 8 (0.3%) were native patients; 2 corresponded to HSIL (1 foreign case, 35 years old and the other a local one, was 74 years old, with later diagnosis of differentiated squamous uterine cervix carcinoma).
Conclusions: Cozumel's total population is a risk one. Average of foreign patients with LSIL is higher than native patients average. Tourism would be a risk variablefor transmission and infection with HPV.

P-413

Metaplastic endometrial cells as a possible pitfall in cervical/vaginal smears

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40-year old patient with a history of uterine spotting had a cervicovaginal smear with endometrial cell clusters demonstrating cytologic features of eosinophilic metaplasia. A concurrent endometrial biopsy showed shedding endometrium with eosinophilic metaplasia including clusters of cells identical to those seen in the Pap smear. According to the 2001 Bethesda system the presence of endometrial cells should be reported in all women of 40 years of age or older. Since benign metaplastic changes can alter the morphology of endometrial cells, it is possible that the presence of endometrial cells in Pap smears can be substantially underdiagnosed. Endometrial metaplastic changes can cause endometrial cells to resemble columnar endocervical, squamous metaplastic, eosinophilic, clear or tubal cells. This report raises awareness of an unusual metaplastic change that may prevent recognition of the cells as endometrial. Reports of metaplastic endometrial cells as a possible pitfall in the diagnosis of endometrial cells and/or malignancies is severely lacking in the cytology literature. While it is admittedly difficult to diagnose endometrial metaplasia in Pap smears alone, the fact remains that endometrial cells may not appear as the "typical" three dimensional clusters of small cells with high nuclear/cytoplasmic ratio and inconspicious nucleoli in Pap smears, especially in women 40 years of age or older when endometrial metaplasia is quite common.

P-414

ENDOSCOPIC ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION OF PANCREATIC CYSTIC AND SOLID MASS LESIONS.

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EUS-FNA of pancreatic masses has shown to be a very effective diagnostic technique. We evaluated our series.
Material and Method A total of 66 EUS-FNAs in 62 patients with either cystic (21) or solid (45) pancreatic masses (40 head, 23 body, 3 tail) were performed. The mean age was 66 years (range 34–90).The final diagnosis was based on histology, clinical and/or imaging follow-up or biochemical and tumor markers.
Results: A median of 2 needle passes were performed (range, one to five). Histological correlation was available on 13 patients.The cytological diagnoses included: 30 (28 solids, 2 cystics) malignant (25 ductal adenocarcinoma, 1 mucinous cystadenocarcinoma, 1 intraductal papillary mucinous neoplasm with invasive carcinoma, 2 neuroendocrine tumors and 1 lymphoma), 28 benign (17 cyst: 11 serous and 4 mucinous cysts and 2 pseudocysts), 2 suspicious, and 6 nondiagnostic. All malignant diagnoses were confirmed.One suspicious case was a false-positive identified as chronic pancreatitis. All false-negatives (n=3) corresponding to a solid mass (1 head, 1 body, 1 tail).Six unsatisfactory cases (3 head, 3 body) corresponded to 4 ADC, 1 serous cyst and 1 mucinous cystadenoma.
Sensitivity, specificity, and positive and negative predictive values were 91,18%, 96,15%, 96,88%, and 89,29%, respectively. The diagnostic accuracy was 91%.
Conclusions EUS-FNA is an effective method for diagnosis in patients with suspected pancreatic malignancy, as well as in evaluation of cyst. The appearance and biochemical analysis of the fluid aid in precise diagnostic of cystic aspirates.The location was not in determining the suitability of the samples.False-negative diagnoses were attributed to sampling error and the false-positive were due to the presence of scant atypical cellularity. To avoid false-negative and nondiagnostic results in highly suspicious malignant lesions, we should increase the number of needles passes until we achieve the malignant cytological diagnosis on-site.

P-415

A quality control study on concordance in Pap Test among cytotechnologist (CT) and cytopathologist (CP)

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Background Quality-control is an important issue in Pap Test screening. The accuracy of Pap Test is largely dependent on CT's expertise. We performed a CT-CP concordance study to analyse areas of discordance. This could be used to target remedial actions for continuous quality improvement.
Methods An experienced staff pathologist (MB) out of three CPs, recorded in an excel datasheet the diagnoses of all cases referred to him for review by nine CTs over one year period. He compared their provisional diagnosis with his final diagnosis according to the Bethesda System 2001 and analyzed the discordant categories.
Result A total of 10,453 Pap Tests were screened by CTs during one year out of which 933 were referred to MB for final diagnosis. The most problematic diagnoses were "negative, inflammation" and "ASC-H" with concordance rates of 29% and 37.5% respectively. The range of agreement in the other diagnostic categories was 67% to 80%.
In the "negative, inflammation" category, the overall CTs-CP discordance was 71% (112/158). Among 112 misclassified cases, 84 (75%) were overcalled ASC-US and 17 (15%) cases were marked negative by CTs. For "ASC-H" the overall CTs-CP discordance was 62.5% (20/32). Among 20 misclassified cases, 13 (65%) were under interpreted as ASC-US and 4 (20%) as HSIL by CTs.
In 4 cases few malignant cells and necrotic debris were present and a definitive diagnosis of "suspicious for neoplasia" was rendered by CP. CTs diagnosed only one case correctly and called the rest as AGC (2) and negative (1).
Conclusion. We used an objective procedure to evaluate CTs's performance and observe dispersion of discordance. These data allow us to direct financial resources for education to specific diagnostic categories. The inflammatory category was the most problematic; therefore a specific education session describing inflammatory changes in squamous epithelium could be useful to improve concordance.

P-416

Liqui prep a new liquid based cervical cytology method in comparison with conventional pap smear

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Background: The aim of the study was to compare, the screening performance of a new liquid-based cytology method, LiquiPREP™,with conventional Pap in a low risk population, using colposcopy followed histology as "gold standard".
Materials and Method: This prospective study was performed in a general gynecology clinic in ValiAsr University Hospital, Tehran, Iran from February 2005 to March 2007. The split-sample method was used for preparing conventional and liquid-based cytology.A new technique of liquid-based cytology; Liqui-PREP™ was used in this study. All positive result of smears and 10% of negative results in each group were submitted to colposcopy and a biopsy taken when any atypical transformation zone was seen. Sensitivity, specificity, positive and negative predictive values, and overall accuracy of both conventional and LiquiPREP™ methods were computed in relation to histology.
Results: A total of 506 patients were analyzed by two cytology methods and in 65 (12.84%) of cases histologic diagnosis was performed. There were more adequate samples with LiquiPREP™ (94.7%) than with conventional (92.1%) smears. There was not any LSIL and HSIL report in two groups. ASCUS was diagnosed significantly more with conventional than with LiquiPREP™ smear (1.56% vs. 0.79%). Pathologically 50% of ASCUS in Liqui-PREP™ and 12.5% in CP had squamous abnormality. LiquiPREP™ had a significantly higher sensitivity (66% vs. 83%) and specificity (86% vs. 98%) than the conventional Pap smear to detect ASCUS+ at histology.
Conclusion: This study confirms the superiority of the LiquiPREP™ method to detect cervical lesions.
Key Words: Cervical carcinoma; Screening; Conventional smear, Liquid based smear

P-417

Evaluation of morphometric diagnosis accuracy in thyroid cytology

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Introduction: Decision making in pathology is changing toward quantitative criteria. Quantitative measurements obviously can demonstrate minimal differences, not readily identifiable to human vision. Morphometry is quantitative description of geometric characteristics of objects. This study tends to test feasibility of this new technique using available instruments and softwares and to select useful parameters and their thresholds for discrimination of benign versus malignant lesions of thyroid.
Materials & Methods: Cases have been selected among received fine needle aspiration specimens of thyroid in pathology department of Dr. Shariati hospital from October 2003 to March 2004. Patients whose surgical pathologic reports were not available have been excluded due to impossibility of comparing diagnoses. At least 100 cells have been measured for each patient. The cells were selected by a zone guided manner. Totally 67 patients were entered to the study, 36 with benign pathology diagnosis

and 31 with malignant lesions. In a pilot study to test reproducibility of measurements, a single cell was selected and measured for 20 times.
Result: Coefficient of variation remained below %2 for all measurements but nuclear diameter which has been excluded from analysis. Benign cells were different from malignant ones in mean nuclear perimeter (Peri), mean nuclear area (Area), nuclear area to perimeter ratio (A/P), cluster diameter, mean gray level (in 256 gray levels) and standard deviations of Peri, Area and A/P (P-values<0.05); but not in CV of nuclear area, intercellular standard deviation of gray levels, intracellular CV of gray levels and its standard deviation. a Receiver Operator Characteristic curve analysis was performed for parameters with significant difference and sensitivities and specificities calculated for different cut-off values.
Conclusion: The highest sensitivity has been reached for nuclear perimeter, nuclear area, cluster diameter and nuclear area to perimeter ratio equal to %93.55 and for cluster diameter equal to %89.55. The results are comparable to classic cytology practice thus this technique is recommended to screen thyroid FNA specimens.
Keywords: Morphometry, Cytology, Pathology, Thyroid, Needle Aspriration

P-418

Fine needle aspiration of axillary lymph node in breast carcinoma. Cytohistological correlation with sentinel lymph node.

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Introduction: Axillary lymph node metastases is one of the most important prognostic parameters in patients with breast cancer. Axillary limphadenectomy is substituted by the technique of sentinel lymph node (SLN) biopsy in patients without palpable lymph nodes. Currently the ultrasound guided fine needle aspiration of axillary lymph node (FNALN) has been proposed, to determine the best treatment for each patient, but FNALN not always is done in SLN.
Objective: To evaluate the FNALN we correlated cytological findings with SLN study, and axillary lymphadenectomy.
Material and methods: 179 FNALN were studied between 2005 and 2006, and correlated to 45 cases with SLN study and with surgical lymphadenectomy specimen.
Results: The FNALN was positive in 64 cases (35,7%), negative in 95 (53%) and inadequated in 20 (11,11%).
There were histological correlation in 46 of 64 positive cases; in 38 the diagnosis was confirmed, and in 8 cases the lymph nodes were negative, all of them treated with neoadjuvant chemotherapy.
The 95 negative cases had histological correlation in 71. In 45 of them SLN technique was made; at the cytologic intraoperative study 8 were positive and 37 negative, 5 of them showed micrometastases in the exhaustive histological and immunohistochemistry study. Of 26 cases without SLN study, 5 presented metastases in the axillary lymphadenectomy.
Excluding the 8 negative cases after neoadjuvant chemotherapy, the Efficiency of the FNALN in our experience was: Sensitivity: 66,6%; Specificity: 100%; VPP: 100%; VPN: 73,2%; and Accuracy: 77%.
Conclusions: The FNALN has moderate sensitivity, high specificity and high accuracy to detect metastases and to stage breast cancer. It is very useful to establish the best treatment for each patient, and to avoid the study of SLN in a high rate of cases.

P-419

Unusual Findings of Papillary Carcinoma in Aspiration Cytology

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FNA biopsy is highly accurate in diagnosis of Papillary thyroid carcinoma (PTC).The smear of PTC is usually cellular and it has papillary structures of various types. The cytoplasm of follicular cells are squamoid,and have septate vacuoles in PTC. In nucleus, grooved membrane,INCIs, fine pale chromatin,marginated nucleoli, are other findings in PTC. Psammoma bodies, epitheloid giant cells, gummy colloid are seen in some PTC cases.Sometimes you can't see any characteristic finding in PTC such as in our case.
In cytology, we saw irregular membranes, grooved nuclei in follicular cells arranged in flat sheets. Furhermore nuclei were much bigger than normal. Flame cells were found in some areas they were thought to be a finding of hyperthyroidi, less commonly seen ın neoplasm.Accordıng to all of these fındıng we thought benign cytology and cellular nodule which presented hyperactivite finding.
After operation, histopathological findings showed that diagnosis as papillary carcinoma with follicular varyant and tumour cells extended to surgical margins.
On conclusıon, if you see bigger nuclei with grooves ın most cells and you don't see any colloid or a little colloid, you must be careful when you writing diagnosis. Perhaps it is better to write suspicious cytology as a diagnosis

P-420

LANGERHANS CELL HISTIOCYTOSIS IN ORBIT- DIAGNOSED BY FINE NEEDLE ASPIRATION CYTOLOGY (FNA):TWO CASES REPORT

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Background: langerhans cell histiocytosis is a benign lesion that rarely occurred in orbital bone. Most cases present before the age of 20 and the majority of lesions are monostotic. FNA cytologic findings of this disorder have been well described, but using of this method in the diagnosis of orbital lesions is a recent experience. We were aimed to report two cases of langerhans cell histiocytosis in the orbital bone that diagnosed by FNA and later confirmed by routine H&E histopathology and Immunohistochemistry methods.
Material and method: Case report. We performed our study in Farbi eye hospital, a referral ophthalmologic center in Tehran University of Medical Science during two years, 2005 and 2006.
Results: The first case was a one year old boy with a left upper lid mass. CT scan showed a solid tumor with orbital bone invasion and erosion, suspicious for malignancy. The other case was a three years old boy with right lower lid edema. Radiographic study revealed a mass with peripheral condensation and orbital bone defect. FNA of both lesions showed a mixed cell population of eosinophils, neutrophils and lymphocytes admixed with neoplastic histiocytes with folded nuclei; so called "coffee- bean" nuclei. Some of these cells were binuclear or multinucleated. So the cytologic diagnosis was langerhans cell histiocytosis. After open surgery and mass excision, that diagnosis was confirmed by the routine histopathologic examination. In both cases IHC for CD1a were also performed.
Conclusion: In the hands of experienced ophthalmologists and pathologists, FNA can provides a rapid and reliable diagnostic method in orbital lesions suspicious for langerhan's cell histiocytosis.

P-421

CYTOKERATIN 20 IN URINE LIQUID-BASED CYTOLOGY SMEARS: A POTENCIAL MARKER FOR THE DETECTION OF UROTHELIAL CARCINOMA

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Background: The expression of CK 20 is useful to differenciate bening processes (such reactive urothelial atypia and degenerative changes secondary to calculi, trauma, instrumentation, infection or even intravesical terapy) of urothelial carcinoma, to avoid false positives, when the basis of morphology alone, may be difficult in some cases.
Method: 70 voided urine samples were evaluated by liquid-based cytology and CK 20 immunoexpression was detected. We reviewed using cytologic-histologic correlation, the histologic diagnosis was considered the "gold standard".
Results: Diagnosis of urine cytology were: 9 cases bening urothelial cells, 13 cases reactive urothelial atypia, 6 cases papillary lesion, 17 cases atypical urothelial cells, 7 cases atypical urothelial cells suspicious of malignancy and 18 cases of malignant cells.
CK 20 was positive in twenty-three cases, seventeen of them showed urothelial carcinoma in the biopsy, and six cases the biopsy was normal.
The fifty-seven cases with negativity to CK 20 immunostaining, twenty-three cases corresponded to cytologic diagnosis of atypical cell, atypical cell suspicious of malignancy and malignant cells, of these sixteen biopsies did not show alterations, in four cases had urothelial carcinoma, in two urothelial displasia and one case atypia of unknown significance.
Conclusions: Urothelial carcinoma shows strong immunoexpression of CK 20 in urine liquid-based cytology, except in a few cases, could be explained, because the expression is not found in 10% of cellularity. The CK 20 therefore aid to differenciate bening processes from the urothelial carcinoma, disminishing the false positives cases and increased diagnosis accurancy.

P-422

INTRA-OPERATIVE CYTOPATHOLOGY: A LITERATURE REVIEW

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Background: Intra-operative cytopathology (IOC) is a diagnostic technique used in many pathology departments around the world, alone or in association with tissue frozen section (FS). Although it was developed over 80 years before the FS, it never replaced it. Likely, the main reason for this is the inexperience of pathologists in interpreting the findings. The purpose of this study is to determine the current profile of IOC.
Methods: A literature review was performed in MEDLINE for articles published between 1927 and November 2007.

The inclusion criterion was that every specimen examined by IOC had a corresponding histologic section. A database was created cataloging the following variables: country of origin, total number of cases, organs/tissues examined, method of material collection, staining and accuracy.
Results: Two hundred ninety seven articles addressing IOC were selected. Most studies originated in the United States, Japan and Germany. In conjunction, 61,846 cases were examined, and, overall, the most commonly studied organs/tissues were central nervous system lesions (25.2%), lymph nodes (22.7%) and breast (8.7%). Within the last 15 years sentinel lymph nodes, central nervous system tumors, pancreatic lesions and mediastinal tumors became the most common evaluated tissues. In most cases, material for IOC was obtained using imprint and smear techniques, and stained by Papanicolaou and May-Grunwald-Giemsa methods. After correlation with histologic samples, the accuracy of IOC ranged between 80 and 100%.
Conclusion: The vast majority of articles recognized that IOC is a safe, simple, inexpensive and rapid technique option for intra-operative diagnosis. In addition, it is an effective way to prevent production of FS artifact in tissues. The practice acquired during IOC in examining cytomorphologic details can aid in improving fine needle aspiration diagnosis skills.

P-423

Clinical significance of diagnosis of ASC-US and ASC-H in cervicovaginal cytology: comparison between premenopausal and postmenopausal women

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BACKGROUND: Some studies in order to evaluate the clinical significance of the cytological diagnosis of ASC-H (Atypical Squamous Cells cannot exclude H-SIL) in cervicovaginal cytology of postmenopausal women, comparing with other age groups, have shown opposed conclusions regarding their posterior management.
MATERIALS AND METHODS: We have reviewed the cytohistologic follow-up during the first year after diagnosis of ASC-US (Atypical Squamous Cells Undetermined Significance) or ASC-H in our department between 1997 and 2006 in premenopausal (< 45 years), perimenopausal (45–54 years), and postmenopausal women (> 54 years). In this period, in a total of 141669 smears examined, 611 (0.4%) cases of ASC-US (568) or ASC-H (43) were identified, and 490 of them (455 ASC-US and 35 ASC-H) had cytohistologic follow-up.
RESULTS: In the follow-up, 139 of the 455 cases initially diagnosed of ASC-US evolved into Low grade Squamous Intraepithelial Lesion (L-SIL), High grade Squamous Intraepithelial Lesion (H-SIL) or Positive For Malignant Cells (PFMC), and 17 of the 35 cases initially diagnosed of ASC-H changed into H-SIL or PFMC. The positive predictive value (PPV) of ASC-US for L-SIL/H-SIL/PFCM was: 32.5% in premenopausal, 24.7% in perimenopausal, and 28.3% in postmenopausal women. The PPV of ASC-H for H-SIL/PFCM was: 38% premenopausal, 60% perimenopausal, and 66.6% postmenopausal women. We have found statistically significant differences between age and diagnosis of H-SIL/PFCM in the follow-up of ASC-H ($p=0.02$), but not in ASC-US ($p=0.35$)
CONCLUSIONS: Our experience suggests that diagnosis of ASC-H in postmenopausal women would justify a close surveillance, because these patients have risk to develop a more aggressive lesion. In the case of ASC-US it would not be justified to make differences in the management of patients with regard to age group.

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Cloacogenic (basaloid) carcinoma of the anus metastatic to the uterine cervix diagnosed by Liquid Based Cytology

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Carcinomas of the anal canal and anus comprise fewer than 10% of the carcinomas in the distal colon. Most of these are epidermoid carcinomas and only 3% constitute the cloacogenic (basaloid) subtype. Extrauterine tumors metastatic to the cervix are very rare. The most frequent primary sites are the ovaries, gastrointestinal tract and breast. Among them only 2 cases of primary cloacogenic (basaloid) carcinoma of the anus metastatic to the uterine cervix and detected by Papanikolaou test have been reported in the English literature.
We present an extremely rare case of a cloacogenic (basaloid) carcinoma of the anus metastatic to the uterine cervix incidentally detected by a routine Papanikolaou smear. A 57-year-old woman was treated for squamous cell carcinoma, cloacogenic (basaloid) subtype of the anus, stage IIIA, with partial resection of the tumor, followed by 5 courses of systemic chemotherapy and radiotherapy. One year later, a routine Papanikolaou test was performed. The material was processed by Liquid Based Cytology (ThinPrep) technique. Evaluation of the cervicovaginal smear revealed a few well circumscribed aggregates of small,

uniform squamoid cells with hyperchromatic nuclei and peripheral palisading. Immunocytochemistry showed strong positivity for Cytokeratines 8/18, CEA and p53. Infection with 'high risk' human papillomavirus (HPV) was not detected by polymerase chain reaction (PCR). Diagnosis was based on the cytomorphologic and immunocytochemical characteristics as well as the patient's history and concerned a metastatic involvement of the uterine cervix by a squamous cell carcinoma, cloacogenic (basaloid) subtype. The patient received 3 courses of systemic chemotherapy and radiotherapy and is in partial remission.

Cervicovaginal smear screening in extrauterine malignancies is of high clinical significance to monitor these patients for possible metastasis and evaluate the stage of the disease or even to detect a second primary neoplasm.

P-425
EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND PLATELET-DERIVED GROWTH FACTOR RECEPTOR (PDGFR) IN MALIGNANT ASCITIC FLUIDS OF PATIENTS WITH OVARIAN CARCINOMAS

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Background: Angiogenesis plays a crucial role for growth, invasion and metastasis of solid tumours. Various molecules and their receptors have been studied to evaluate the mechanism of angiogenesis. Among them, vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptor (PDGFR) induce proliferation of endothelial cells and promote the activation of tumour cells.

We investigated the immunoexpression of VEGF and PDGFR in ascitic fluids from various types of ovarian cancer.

Method: The study consisted of 38 ascitic fluids, including 14 cases with a cytomorphological and immunocytochemical diagnosis of type I(10 low-grade serous, 3 endometrioid, 1 clear cell carcinomas) and 24 cases of type II (19 high-grade serous, 2 undifferentiated carcinomas and 3 carcinosarcomas) ovarian malignancies. The material was processed using Liquid Based Cytology (ThinPrep) technique. Then, we applied antibodies against VEGF and PDGFR.

Results: VEGF showed granular/homogeneous cytoplasmic or membrane staining. The VEGF expression was much stronger in type II (15/24) ovarian carcinomas in comparison with those of type I (5/14). PDGFR was expressed in 23/38 of ovarian malignancies showing cytoplasmic and nuclear staining also with prevalence in type II carcinomas. Moreover, in five cases of type II ovarian carcinomas, weak PDGFR staining was observed even though VEGF was negative.

Conclusion: Co-expression of VEGF and PDGFR in tumours of the ovary suggests that the two pathways interconnect and may play an important role in angiogenesis of ovarian cancer, subsequent proliferation of tumour cells, and further tumour progression. This study supports the hypothesis that inhibition of VEGF signaling in ovarian cancer might induce suppressive effects in tumour cell proliferation. Anti-angiogenesis therapy targeting VEGF and PDGFR may be a promising therapeutic approach for patients with ovarian carcinoma, especially for those with overexpression of VEGF and PDGFR.

P-426
The role of L1 protein and of p16 INK4a gene in the prognosis of changes of the cervical endothelium.

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Introduction Immunocytochemical detection methods using HPV L1 capsid protein and the tumour suppressing gene p16 ^{INK4a} seem to help in the prognosis and follow up of precancerous changes related to the HPV (Human Papilloma Virus).

The aim of this study was to investigate the expression of the two biochemical markers in vaginal-cervical smear in the prognosis of progress of precancerous changes as well as the comparative assessment of their values in relation to the cell morphology and the follow-up.

Material and Methods: Two hundred vaginocervical samples from women (with or without changes in cell morphology) were studied. Thirty of them were followed up. The samples were processed by ThinPrep Thehnique followed by immunocytochemical detection of the L1 protein and of the gene p16 ^{INK4a}.

The results from the two techniques were compared as well as their correlation to the cell morphology and follow up, where possible.

Results: L1 was mainly expressed in low grade endothelial changes and was correlated to a good prognosis while p16 ^{INK4a} was detected in all the high grade endothelial changes indicating an unfavourable prognosis. Most of the followed

up cases showed a change of expression of the indices in accord with the cell morphology and colposcopy image.
Conclusions: This study showed that the immunocytochemical indices L1 and $p16^{INK4a}$ are two reliable aids in assessing the prognosis of precancerous changes of the cervix. Their routine application combined with the cytological examination and colposcopy can improve the treatment of women with HPV infection.
Key words: Pap-test, HPV, L1 capsid protein, $p16^{INK4a}$ gene, follow up, ThinPrep technique

P-427

IS VIRAL LOAD A PROGNOSTIC FACTOR IN LSIL CASES?

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Objective. To asses if viral load is a prognostic factor related to persistence/progression in LSIL cases.
Materials and Methods 134 patients diagnosed as LSIL using liquid based cytology ThinPrep (Cytyc), in a one-year period. The same sample was used to determine HPV and viral load using Hybrid Capture II (Digene). The patients were followed by cytology and/or biopsy.
Results: 55 (41%) cases regressed and 79 (59%) cases persisted or progressed. The mean of viral load in cases that regressed was 366.13 URL and the mean in cases that persisted was 861.5 RLU. The difference is statistically significant. If we consider the HPV positive cases when the value of RLU is 1, we obtain a sensitivity of 89% and a specificity of 22%, related to the persistence/progression of the disease. On the other hand. the specificity higher than 95% is obtained when we consider a RLU equal or greater than 1250. In these circumstances the sensitivity decreases around 30% but the predictive positive value is greater than 90%.
Conclusions: We think reasonable to suggest that patients diagnosed as LSIL with a viral load equal or greater than 1250 RLU should be submitted to surgical procedures. Using these data and based in our cases, 17% of the patients diagnosed as LSIL would be classified in this category.

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3Q (HTERC) AMPLIFICATION AS A PERSISTENCE/PROGRESSION PREDICTIVE FACTOR IN LSIL CASES. A STUDY IN FROZED MATERIAL

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Objective: To assess the relationship between 3q26 (HTERC) amplification and persistence/progression in LSIL cases.
Material and Methods: 48 cased studied using liquid based cytology (ThinPrep, Cytyc), 24 normal cases used as controls and 24 LSIL cases used as a problem cases. All of the problem cases were followed on a control 6 months after diagnosis. 12 of them showed persistence/progression of LSIL and 12 showed regression of the lesion. Using the frozed material corresponding to first diagnosis, a study of copy number of HTERC was performed applying FISH techniques.
Results: The normal cases showed a mean of 5.3% of cells with only one copy of HTERC and a mean of 3.9% of cell with more than 2 copies of HTERC. There was no differences between trofic and atroffic cases.
None of LSIL cases showed cells with only one copy of HTERC.
The mean of cells with more than 2 HTERC copies in LSIL with regression was 4.9% and the mean of cells in LSIL cases with persistence/progression, was 11.75% ($p<0.05$)
Conclussion: HTERC amplification may be used as a predictive factor for the persistence/progression of LSIL

P-429

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) AS A VERY RELIABLE DIAGNOSTIC METHOD IN BONE LESIONS: A REVIEW OF 1040 CASES, FOCUSING ON 347 METASTATIC CARCINOMA.

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BACKGROUND: FNAC is a non-invasive and very useful method and a proved cost-effective technique in the diagnosis of neoplastic lesions. This study was designed to evaluate our experience with the use of FNAC, its utility and reliability, focusing on the diagnosis of bone metastatic carcinoma (MC).
DESIGN: We reviewed 1040 CT-guided FNAC of bone lesions performed between 1990 and 2007. We selected 347 MC positive cases. Aspirated material was air-dryed for Diff-Quik stain and fixed in ethanol for Papanicolaou staining. Tissue cell block was available in 330 cases. Immunohistochemical (IHC) techniques were performed in 177 samples.
RESULT: Of the 1040 reviewed FNAC, 808 aspirates were adequate for evaluation, 209 were insufficient, 347 positive for MC, and 494 from miscellaneous entities (lymphoma, multiple mieloma, primary lesions, inflammatory and negatives results).
Foccusing on the 347 MC FNAC, the patients ranged in age from 29 to 88 years, with 216 male and 136 female. In 74/347 cases with known primary tumour 61/74 were malignant aspirates confirming the patient's primary malignancies, 13/74 reveled a second metastatic tumour. The most frequent radiological and clinical presentation were as lytic lesion, pain and nodules/masses. The spine was the most frequent aspirated site (lumbar, dorsal and cervical), followed by ilium bone, ribs and femur. Of MC cases, carcinoma with different subtypes and origin were 162 (61.1%), 139(43.84%) corresponded to adenocarcinomes, 9 (2.83%) were small cell carcinoma (neuroendocrine) and 7 (2.2%) undifferentiated tumours. The primary tumour site was breast, lung, pancreatic and gall bladder area, kidney and prostate in order of frequency.
CONCLUSION: FNA is a non-invasive and very reliable method in the diagnosis of bone lesions. The use of the thicker-gauged needles in the last few years, has improved significantly the technique in order to obtain satisfactory samples, avoid inssufficient specimens and get high diagnostic accuracy. When previous history of malignancy is unknown, performing IHC markers on tissue cell block has proved determinant to establish site origin of the primary tumour. In our experience CK7, CK20, CK19, PSA, Hormonal Receptors and TTF-1 are the most used/useful markers. To summerize, on satisfactory FNAC specimens, the cytomorphology combined with IHC techniques, viewed in a clinical-radiological context, asses high diagnostic accuracy rate over 95%.

P-430

The new protocol for cervical cancer screening in catalonia: results in women with inadequate screening history.

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Introduction Cervical cancer screening in Spain is opportunistic. Cytology covers 50–70% of the population and about 80% do so every year. However, 80% of invasive cervical cancers appear in women without a previous Pap smear. Since June 2006 in Catalonia, a new protocol of screening activities has been implemented to increase coverage and to reach a 3 years interval between screening Pap smears.
The new protocol includes HPV detection in 3 circumstances: inadequate screening history (IS), triage of ASC-US, and follow up of patients treated for cervical lesions (PT). We report the results of the first year of the new protocol on the IS group.
Material and methods Women attending primary health care centers are questioned by sentinels (nurses, midwives, medical doctors) about screening history. IS is considered when a > 40 y.o. woman doesn't have a Pap smear in the previous 5 years. HPV and cytology are offered to the women. Hybrid Capture 2 (HC2, Digene) is being performed in 12 different laboratories. Cytology is reported following Bethesda classification.
Results The new protocol has generated 7649 HPV detections, distributed as follows: 56.5% from IS, 27.8% from ASC-US, 5.3% from PT and 10.4% from other cause. From the samples corresponding to IS 72.4% (n=3127) followed the criteria and HPV was detected in 5.7% of these samples. Cytology and biopsy results from 3 areas are being recruited.
Conclusion
- The protocol has been able to identify 179 women at risk of developing cervical cancer (40 y.o. and HPV+).
- HC2 has been easily implemented in the reference laboratories.
- Follow up of these patients as well as the results of cytology and histology will determine the real impact of the new protocol in cervical cancer screening.

P-431

Fine needle aspiration cytology of large cell neuroendocrine carcinoma of the lung. A cyto-histologic correlation study of 11 cases

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Background: The classification of pulmonary neuroendocrine neoplasms is particularly controversial. Among them, large cell neuroendocrine carcinoma (LCNEC) has emerged as a separate entity. Regardless terminological considerations there is a problem that pathologists face periodically: aspirates from pulmonary tumors showing neuroendocrine features but composed of large cells. Their specific recognition is important since prognosis is worse than for other large cell carcinomas. Few cytologic studies concerning this entity are available. The objective of the present study is to describe cytologic features of LCNEC, in an attempt to separate them from other pulmonary carcinomas.
Method: A cyto-histologic study of 11 surgical lobectomy specimens classified as LCNEC. In all of them preoperative fine needle aspiration cytology material was available for review.
Results: Cytologic features were rather similar resulting in a repetitive pattern. Most smears were hypercellular with numerous single, medium to large cells. Naked nuclei were abundant but a variable subset of cells showed evident cytoplasm. Groups were three-dimensional, of variable size, some of them large. Nuclear pleomorphism, molding and mitosis were common findings. A necrotic background was evident in 6 cases. In at least 6 cases, neoplastic groups showed peripheral nuclear palisading. Rosette-like structures were present in samples from five patients. In four cases immunocytochemistry for the detection of synaptophysin was performed with positive results.
Conclusions: Our experience, with 11 FNA cases of LCNEC, has led us to believe that their cytologic image is peculiar and recognizable in many cases. Nevertheless, it is a difficult diagnosis and immunocytochemistry plays a critical diagnostic role.

P-432

Positive urine cytology in patients with lung cancer without obvious urine tract metastases.
A single center study.

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Background: Urine cytology is widely used for the diagnosis of primary tumors of the urinary tract as well as the detection of metastases in the urinary tract. The presence of positive urine cytology in patients without obvious urine tract metastases is not expected and only few cases had been reported in the literature.
Method: Our department recently reported this phenomenon in 5 such patients. They all had metastatic lung cancer and positive urine cytology without urinary tract metastases. This is an ongoing study and up to now we have studied the cases of 35 patients with metastatic lung cancer (small and non small cell lung cancer).
Results: The urine sediment cytology of those patients revealed 9 cases with positive for malignancy results. None of these patients had radiological verification of metastasis to urinary tract. The morphological appearance of the cells from the primary lung biopsy and the urine cytology were identical.
Conclusion: Urine cytology is a reliable method for the detection of malignancy. In combination with immunocytochemical and molecular techniques it can identify the exact origin of the malignant cells.

P-433

A STUDY OF ATYPICAL GLANDULAR CELLS (AGC) ON CONVENTIONAL CERVICAL SMEARS AND ITS HISTOPATHOLOGICAL CORRELATION.

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OBJECTIVE: The aim of this study is to correlate the atypical glandular cells (AGC) diagnosed on conventional Pap smears with subsequent biopsy diagnosis retrospectively.
MATERIALS AND METHODS: We retrieved 28 cases of atypical glandular cells diagnosed cytologically in conventional Pap smears from the archieves of Gulhane Military Medical Academy, Pathology Department and correlated their cervical biopsies, endocervical and endometrial curettages during the follow-up period of two years.
RESULTS: Biopsy findings of cervical smears were as follows: of 28 smears, 14 cases showed bening lesions (cervicitis, endometritis, endocervical and endometrial polyp, tubal metaplasia and squamous metaplasia, keratosis, fetal and placental tissues, ectopic pregnancy and microglandular adenosis, irregular proliferative endometrium coexisting adenomyosis, endometrial hyperplasia and cervical endometriosis), 4 cases had sguamous intraepithelial lesion (2 LGSIL and 2 high grade SIL), 4 cases showed malignancy (2 cases adenocarcinoma in situ, in which one of them was associated with low grade squamous intraepithelial lesion (LGSIL), and 2 case adenocarcinoma).

Correlation of 6 cases with histopathology and follow up was not achieved.
CONCLUSION: Biopsy follow-up of conventional cervical smears diagnosed as atypical glandular cells (AGC) revealed mainly benign reactive epithelial changes, some of them squamous cervical intraepithelial lesions and as well as glandular lesions (adenocarcinoma in situ and adenocarcinoma). The diagnosis of glandular lesions in conventional smears are often not recognized due to lack of experience. However, in our small series, one third of the cases were malignant and premalignant lesions. Therefore, a careful examination can be helpful in diagnosing the glandular lesions.

P-434

FINE NEEDLE ASPIRATION CYTOLOGY OF A PAPILLARY-CYSTIC TYPE ACINIC CELL CARCINOMA OF THE SALIVARY GLAND.

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OBJECTIVE: To impress the importance of brown hemosiderin-like pigment in fine needle aspiration cytology (FNAC) of salivary gland lesions.
MATERİAL AND METHOD: A case of acinic cell carcinoma of salivary gland diagnosed by FNAC in 20 year old male patient. Patient complained of a mass (2 cm) localized in right parotid gland. Fine needle aspiration biyopsy was performed. Microscopical examination of Papanicolaou-stained slides showed cellular aggregates arranged in sheets or papillary clusters. The cells had round to oval nuclei, some with enlarged nucleoli. The cytoplasmic vacuoles were small and some contained brown pigment. Differential diagnosis included papillary thyroid carcinoma, malignant melanoma and primary salivary gland carcinoma. In cytology slides, the pigment in cytoplasms were stained with Prussian blue and they did not express HMB45 and thyroglobulin, immunocytochemically. Final diagnosis was malignant epitelial cells present and cytological findings are consistent with malignant epithelial tumor favored acinic cell carcinoma. The excision specimen was diagnosed as acinic cell carcinoma, papillary cystic type.
CONCLUSION: Acinic cell carcinoma accounts for 12–17% of primary salivary gland carcinomas. In tissue section its morphological features are clear cut and well defined but diagnosis on FNAC is more difficult. Herein we discuss the cytological findings and hemosiderin pigment which is only seen in papillary-cystic type acinic cell carcinoma and are clue to the diagnosis.

P-435

MOLECULAR HPV TYPIFICATION IN ATYPICAL GYNECOLOGICAL CYTOLOGIES: DESCRIPTION OF OUR SERIES

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BACKGROUND: HPV is the main cause of cervical cancer. The detection and genotyping in women with atypical cytology improves their clinical management
METHODS: We have analyzed 1715 samples from 1223 patients with previous cytological abnormalities, by liquid cytology and molecular typing of HPV (Clinical Arrays, Genomica). The DNA was obtained following the manufacturer protocols.
RESULTS: 782 cases (600 patients) were positive (POS) for HPV. In decreasing order of frequency the prevalence of HPV types was: 16(19.3.%), 53 (10.2%), 6 (8.69.%), 66, 51,31, 61, 18, 11, 33, 52, 84, 59, 70, 81, 56, 83, 62, 39, 45, 54, 73, 68, 82, 72, 44, 71, 35, 89, 42, 89, 43 and 40, with 182 co-infections.The more frequent high-risk(HR) types were 16, 53, 58, 51, 66, 31, 18; low-risk(LR) 6 and 61, indetermined-risk(IR) 84 and 83. The HPV types associated to LSIL were 16 (18.4%),53,6,31 and 51. HPV 16 was predominant in HSIL(47.5%). 275 cases were POS for HPV with normal (N) cytology. The predominant types in this group were: LR: 6 (12%) and 61 (10%); HR 16 (12%) and 53 (11%); IR: 84 (4%) and 83 (1.5%). Thirteen of these 275 cases showed atypical cytology in the next control (6 LSIL, 6 ASC-US and 1 HSIL). HPV 53 and 66 have been classified as low risk in some series, in our series both are associated to atypical cytology as a single agent: HPV 53, 71% and HPV 66, 77%.
CONCLUSION: HPV typing combined with cytology is an effective method to control patients with SIL, due to its ability to detect new infections, viral clearance and co-infections. From the biological point of view, the molecular test allows to best define the oncogenic role of each type and clinical follow-up. According to our dates HPV 53 and 66 should be reconsidered of high risk group.The high sensitivity of the HPV test allows to detect infections without cytological changes

P-436

Correlation between estrogen and progesterone receptor status in fine-needle aspiration biopsies and surgically resected specimens in 74 breast cancer patients

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Background: Estrogen (ER) and progesterone (PgR) receptors in breast cancer are predictive factors for response to adjuvant hormone therapy. Hormone receptor analysis has been traditionally performed on surgically resected specimens. Therefore, the aim of the study was to correlate estrogen and progesterone receptor status in fine needle aspiration biopsies (FNAB) and surgically resected specimens from the same breast cancer patients.

Methods: FNABs from 74 breast cancer patients were immunostained for ER (Novocastra, NCL-ER-6F11, dilution 1:10) and PgR (Novocastra, NCL-PGR-AB, dilution 1:10) by automated immunostainer (Ventana, NexEs™M), without pretreatment. Immunohistochemical analysis for ER (Neomarkers, RM-9101-S, dilution 1:100, pretreatment in Tris EDTA, PT module) and PgR (Dako, M 3569, dilution 1:50, pretreatment in Dako Pascal) was performed on surgically resected specimens from the same patients by automated immunostainer (Lab Vision). ER and PgR stain was considered positive if unequivocal strong staining was detected in more than 10% of cells.

Results: ER and PgR detection in FNAB had a sensitivity of 93.4% and 91.4%, a specificity of 100% and 85.1%, a positive predictive value of 100% and 91.4%, and a negative predictive value of 76% and 85.1%.

Conclusions: Automated immunostaining for ER and PgR in FNAB samples is a safe and reliable method for assessment of hormone receptor status in breast cancer patients. This method is especially suitable for patients with inoperable breast cancer, tumours inaccessible to surgical biopsy and general contraindications for surgery. Furthermore, sensitivity of hormone receptor analysis in FNABs can even be increased by repeating FNAB and the test in ER or PgR negative patients, thus increasing the negative predictive value of the test.

P-437

CYTOLOGICAL FEATURES OF THE SEBACEOUS LYMPHADENOMA OF THE PAROTID GLAND

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INTRODUCTION Primary sebaceous tumours of the salivary glands are uncommon (0.2%). Although sebaceous metaplasia has an 11% presence in the parotid gland and a 6% presence in the submaxilar gland, sebaceous lymphadenoma accounts for only 0.1% of all adenomas in the salivary gland.

A case of sebaceous lymphadenoma, whose cytological picture suggests a differential diagnosis between a tumour of the salivary gland or adenopathy, is reported here.

RESULTS A 74 year old female patient presented a left parotid tumoration of an evolution of months. A FNA of the lesion was performed and slides were obtained which were stained with Diff-Quick and Papanicolaou.

Its removal was recommended because a suspected probable Warthin's tumour. A parotidectomy was performed and a histological study was carried out with a diagnosis of a sebaceous lymphadenoma of the parotid gland.

DISCUSSION FNA is an efficient method for the diagnosis of salivary gland lesions. The most common tumours have well established cytological features, but it is also necessary to bear in mind the less common forms, which may lead us into diagnostic mistakes.

In the FNA study, in this case, a prominent lymphoid background was identified suggesting a series of possible diagnoses such as Warthin's tumour, sebaceous lymphadenoma, lymphoepithelial carcinoma, as well as other benign possibilities like sialoadenitis, or malignant possibilities such as lymphoma or metastatic illness.

P-438

Value of the cytodiagnosis as a screening method for the periodontal disease

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Write the text... *Background:* Periodontal disease describes a large number of clinical entities that affect gingiva, gingival attachment, periodontal ligament, cementum and supporting alveolar bone. Unique ecologic ninches are

observed in most areas of the mounth such as gingiva margins and tooth surface. The aim of the study is to qualified the significance of the cytodiagnosis for the identification and evolution in the periodontal disease. *Method:* Between 2004–2005 we have selected 345 pacients with lesional and non-lesional periodontum. Periodontal disease was classified in seven major categories including periodontitis as a manifestation of systemic disease. The cytological samples were obtained by aspiration, brushing or imprints of the periodontal areas. The samples were air-dried or alcohol fixed before staining with cytological methods (Papanicolau, Blue-Polichrom-Tanin-Dragan, MGG)
The cytodiagnostic was corelated with clinical data and the histodiagnosis by histochemical methods (Alcian Blue, PAS, Silver impregnation) or immunohistochemical stainings (Cks, S100protein, Vim, Desm, Lymphoid markers and Ki-67).
Results: According to clinical examination we have found 3 groups of lesions: C1-C2-C3 (a,b) depending of the inflammation, tissue destruction, bone resorbtion and impaired tissue regenerationIn C1-C2 samples most of the examined cells were epithelial scuamous with different nucleo-cytoplasmic alterations. In C3a-C3b samples, according with the cytological features we have found numerous conjunctive-inflammatories cells. The periodontal disease has a grading of severity from level I to III, coresponding with the deepness of the tissue loss (1 mm to 3 mm). Bacterial flora is qite similar to all the 3 categories of lesions depending on the host individual defence. *Conclusion:* We agreed that the cytodiagnosis is an important method for the screening and the management in periodontal lesions with a sensitivity of 82%, specificity of 76% and false-negative fraction of 12–15%.

P-439

Cytological aspects in the hyperglucidic stress - study of the mesenchymal stem cells, dental pulp cells and on oral samples from diabetic patients

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Background. Extracellular matrix with volume and inappropriate composition for the cellular metabolism influences the K+, Na+ and Ca+ ionic pumps, the intercellular junctions, the membrane pumps and the proteic synthesis. The behavior of the cells in the host organism and of the pathogenic microflora in the hyperglucidic medium is dictated by the intervention of various factors of oxidative and mechanic stress which lead to modifications of the cellular cycle partially explaining the cavities in diabetic patients and the periodontal disease.
Method In 2007 there have been taken in study 150 specimens from diabetic patients from both sexes with the age between 23 and 57 years with cavities and periodontal problems, a lot of patients without oro-dental evolutional lesions as witness lot, and samples from stem cells cultures from the pulp, collected in conformity with the ethic and asepsis regulations, from 3 subjects 7–8 years of age. The samples were harvested from the global saliva and from the gingival gap, and for the cellular cultures reactives from Sigma Aldrich, Redox Treding-Romania and Promo Cell Heidelberg Germany. The coloration of the samples was made with May-Grunwald-Giemsa and the original Blue-Polichrom-Tanin-Dragan methods.
Results We have reached the following results: at the diabetic patients without drugs control the samples were highly supurative and litical and the dominant flora is fusospirilaris and fungical on an acidic Ph background. At the stem cellsin hiperglucidic enviroment, the density, the nuclear and cellular volume are increased, we can observe citoplasmatic vacuolation and variations in shape and borders of the cells.
Conclusions: These findings suggest possible consequences of the tissue substitution in diabetic patients.

Miscellaneuos

P-440

Importance and Repercussions of Renal and Cardiovascular Pathology on Stroke in Young Adults: An Anatomopathologic Study of 52 Clinical Necropsies

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BACKGROUND: Stroke in young adults has seldom been studied in a necropsy series. The objective of the present clinical necropsy-based investigation was to analyze stroke and its relationship with cardiovascular and renal pathology in young adults.
METHODS: The protocols of 52 clinical necropsies with diagnoses of stroke in patients aged 18 - 49 years, performed between the years 1990–2006, were reviewed.
RESULTS: Hemorrhagic stroke was diagnosed in 36 patients (69.3%), whereas the remaining 16 (30.7%) had ischemic stroke. Hypertensive cardiopathy was evident in 88.4% of the cases. Chronic renal pathology, directly or indirectly related to hypertension, was observed in 55.7%

of the patients. Ischemic stroke as a result of occlusive atherosclerotic disease was seen in 50% of cases. Cardiogenic emboli were found in 25% of the cadavers. Hemorrhagic stroke was associated with hypertension in 43% of the cases, with ruptured vascular malformations in 29%, and coagulopathies in 17% of the cases. Hypertensive cardiopathy was present in patients with either ischemic or hemorrhagic stroke (81.2% and 91.6%, respectively). The most frequently observed renal ailments were chronic pyelonephritis (23%) and nephrosclerosis (21.1%). These were associated with ischemic stroke in 43.7%, and 12.5% of the cases, respectively, and with 13.8% and 25% of the hemorrhagic stroke cases.

CONCLUSION: Hypertensive cardiopathy, occlusive atherosclerotic disease, chronic pyelonephritis and nephrosclerosis are among the pathophysiologycal mechanisms that apparently and eventually interact to induce a significant number of cases of stroke in young adults. A chronic systemic inflammatory state appears to be an important related condition because it possibly constitutes an accelerant of the pathophysiologycal process.

P-441
AN EVALUATION OF INTRAOPERATIVE FROZEN-SECTION BIOPSY IN PHILIPPINE HEART CENTER: A 10-YEAR RETROSPECTIVE STUDY

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Background: Frozen section is a very helpful ancillary procedure for the surgeon in guiding him in his decision intraoperatively. Indications include: to establish the presence and nature of lesion, determine adequacy of surgical margins and to establish whether the tissue obtained contained diagnosable material. The accuracy of frozen section varied depending on the author and type of tissue.

Objective: To review and evaluate the accuracy of frozen section in the Philippine Heart Center from 1994–2005.

Methodology and Result: This is a retrospective study where we reviewed 114 lesions that underwent frozen section from 1994–2005. The most common site sent for frozen section diagnosis was the mediastinum, followed by the lung and breast. There were 47% true positive, 54% true negative and 2.7% false negative. There were no cases of false positive frozen section diagnosis. Cases with false negative diagnosis includes 3 cases of mediastinal tumors which were diagnosed as thymoma on frozen section but was later signed out as lymphoma on permanent sections. There was also a single case of breast tissue with a deferred diagnosis. Our study revealed that our frozen section diagnosis has a very good sensitivity (94.5%) and specificity (100%) in diagnosing tumors.

Conclusion: We conclude that frozen section is very accurate in diagnosing mediastinal, pulmonary and breast lesions. Since frozen section has some limitations, we recommende good communication between the surgeon and pathologist to avoid misdiagnosis.

P-442
PATOMORFOLOGY OF THE UTERO-PLACENTARY COMPLEX AT BREACH OF PATRIMONIAL ACTIVITY

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MATERIAL AND METODS: Complex research of patomorfology utero-plasentary complex was lead at 145 women: In 47 cases at weakness of patrimonial activity, in 45 supervision at premature fall off normally located placenta and in 53 - at discoordination the patrimonial activity, accompanied breaks of corpse and cervical part of a uterus. Histologic, macro-histostereometric and ultramicroscopic methods of research were used.

RESULTS: At weakness of patrimonial activity absence of adequate expansion of a gleam miometral segments of utero-placentary arteries placentary laying down and pathological immaturity fleecy of horion with development of chronic placentary insufficiency was precisely combined with weak lacunary transformation of a dividing venous sine of a body of a uterus and a formed bottom segment. These changes, being the structural precondition of decrease morfofunctional capacities of venous depot of a uterus at development of the patrimonial activity, accompanied unproductive hypotonic fights, in turn, lead to infringement of processes of maturing cervical part a uterus.

At hypertonic dysfunction of a uterus the formed uteroplacentary form of chronic placentary insufficiency, incomplete hestation reorganization both endo-, and myometrial segments of utero-placental arteries, reduction morfometrics parameters of a vascular vessel myometrium. Ultramicroscopical research has revealed law of damages of communication communications between myocytous.

At premature fall off normally located placenta authentic dependence between defective gestational transformation of utero-placental arteries placentary laying down, expressed hemoreological infringements fleecy horion, preliminary venous lacunary transformation of a vascular layer of a body of a uterus with a thrombosis of a dividing venous sine and critical increase of pressure in intervillous space with progressing hipoxia a condition at a fetus is certain.

At dyscoordination the patrimonial activity, accompanied breaks of cervical part and bodies of a uterus processes expressed lacunary transformations of a dividing venous sine of a body of a uterus and dystalis parts of the generated bottom segment with progressing reduction in a direction to it proximalis parts and cervical part a uterus not ready to deformation reconstruction develop on a background inadequate, stimulating patrimonial activity, therapies in a combination to an accompanying initial pathology cervical part of a uterus.
CONCLUSION:Research patomorfology corpse, lower segment and cervical part of a uterus, placentary laying down and placentae at infringement of patrimonial activity has allowed to reveal a morphological basis of a system pathology of a utero-placental complex.

P-443

Pathomorphology of endocrine glands during chronic drug intoxication

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Introduction. The peculiarities of morphological changes and opportunities for differential forensic-pathologic diagnostics were studied during chronic drug intoxication on the basis of analysis of pathomorphologic changes in hypophysis and adrenal.
Materials and methods. 110 autopsies, including 83 forensic and 27 postmortem examinations of bodies (from various acute or chronic recurrent infectious diseases) of individuals suffering from chronic intravenous abuse of narcotics, were carried out. Among the dead individuals were 95 men (86.4%) and 15 women (13.6%) aged between 17 and 36.
Result. During the microscopic examination of drug addicts, who abused narcotics for less than two years, morphological changes in hypophysis and adrenal were of a mainly hypertrophic, hyperplastic and dystrophic nature, with acute microcirculatory disorders as a reaction of strain. In the cases of drug abuse for up to ten years by older drug addicts, necrotic, atrophic and sclerotic changes of parenchyma and intersticium of glands associate with the aforesaid pathologies in the hypophysis-adrenal group. The atrophic changes in endocrine glands testify to lingering high functional load on these organs, which leads to exhaustion of their functions (polyendocrinopathy syndrome).
Conclusion. Thus, changes taking place in the neuroendocrine organs during chronic drug intoxication develop within realization of general adaptation syndrome [theory of H. Selye], like in all strong stresses. In this case, high-toxic drug addiction (chronic "chemical stress") plays the role of a stressor. From this viewpoint, phase change of hormonal homeostasis happens during chronic drug intoxication. The morphological differences in the examined endocrine glands are likely related in different cases to "longevity" of rug abuse, i.e., the duration of drug addiction, different effects of various doses and individually constitutional peculiarities of drug addicts.
Similar changes in hypophysis and adrenal require determination of hormonal balance and its correction in patients with chronic drug addiction.

P-444

Morphology of immunodeficiency state during chronic drug addiction

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Introduction. The peculiarities of morphologic changes taking place during chronic drug addiction were studied at its different development stages on the basis of analysis of pathomorphologic changes in the organs of immune system (thymus, spleen, glands and lymphoid follicles of intestines).
Materials and method. 135 autopsy observations of individuals, who have become victims of various kinds of violent death or died of acute or chronic recurrent infectious diseases, were investigated. All of these individuals also suffered from chronic drug addiction through intravenous abuse of narcotics (mainly opium). The dead persons were 118 men and 17 women aged between 17 and 40. Depending on the duration of drug abuse, all observations were divided into two groups: the first group – chronic drug addiction with misuse of narcotics for up to a year or two; the second group – chronic drug addiction with misuse of narcotics from two to ten years.
Result. In the first group, the observations of morphologic changes in the immune system were of a mainly reactive-hyperplastic and dystrophic nature, with acute microcirculatory disorders. The morphologic changes in the immune system at the early stage of chronic drug addiction are formed from "acute atrophy" defined lymphoid devastation of thymus and spleen caused by amplification of emigration of lymphocytes, their redistribution and, to a considerably lesser extent, apoptosis.
In the second group, morphologic changes of an atrophic and sclerotic nature were revealed during the observations of drug addicts, who committed intravenous abuse of narcotics for over two years (up to ten years). The depletion of compensatory capabilities of the immune system occurred and secondary B- and T-cell immunodeficiency states developed in these patients.
Conclusion. The reduced data confirm that during intravenous chronic drug intoxication the "reserve" forces of

organs of the immune system get activated first in order to overcome the increasing immunosuppression (compensation stage). The stages of temporary activation and resistance are followed by the late stage of immunodeficiency states (decompensation stage), i.e., lymphoid tissue of the immune organ feels real exhaustion. Morphologically, this process occurs due to decrease in the number of lymphoid cells and T- and B-zone atrophies, appearance of great number of changed cells, activation of apoptosis of lymphocytes and development of atrophic and sclerotic changes in lymphoid organs.

P-445
Withdrawn

P-446
A rare case of combined thymic epithelial tumor (thymic carcinoma and type B2 thymoma) in a 51-year-old female

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Background: Combined thymic epithelial tumors are extremely rare neoplasm having at least two distinct areas each corresponding to one of the histological thymoma and thymic carcinoma. Prognosis is dependent upon the most aggressive histologic subtype. Therefore careful diagnosis should be made especially with the presence of two distinct histologic types.
Case Methodology: This is a case of a 51-year old female with a chief complaint of right eye ptosis, right sided weakness of the face and upper extremities for three months. Chest CT scan revealed an anterior mediastinal mass measuring 3.3×5.0×6.0 cm. FNAB of the anterior mediastinum was done. Open thoracotomy with wedge resection of the lung was performed and specimen was submitted for histopathology. Patient was discharge with good condition.
Result: CT guided FNAB of the anterior mediastinal mass revealed cytomorphologic features consistent with thymoma, favor A or AB subtype. The specimen submitted for histopathology is tan-brown, irregular firm mass with nodulations and measures 7.5×6.0×4.5 cm. Histologically, the tumor was composed of a mixture of lymphocyte-poor and lymphocyte-rich component. The lymphocyte-poor component contains spindle-shaped cells with bland irregular nuclei, dispersed chromatin and inconspicuous nuclei in solid sheet and hemangiopericytoma-like appearance. The lymphocyte-rich component consists predominantly of small polygonal epithelial cells with small, round, oval or spindle nuclei showing dispersed chromatin and inconspicuous nucleoli admixed with mature lymphocytes. With these finding, a diagnosis of combined thymic epithelial tumor (thymic carcinoma and type B2 thymoma) is made.
Conclusion: A combination of thymic carcinoma and type B2 thymoma is an exceptionally rare thymic epithelial tumor accounting for <1%. In our institution it is the 1.5%. Fine needle aspiration may reveal one of the two components if not both components. Depending on the sampling, the malignant area maybe missed. Tissue confirmation is thus imperative.

P-447
PATTERN OF PLEURAL DISEASES SEEN IN EASTERN NIGERIA.A review of 75 histopathological biopsies seen over a five year period from January1, 2000-December31, 2004.

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BACKGROUND. There is a dearth of authentic information regarding pleural and respiratory lesions in our environment. I then sought to determine the pattern of pleural lesions diagnosed in our environment by biopsies sent to us at UNTH.Enugu, Nigeria over a 5-year period as a base line data.
DESIGN. A retrospective study, involving analysis and review of slides of trucut needle biopsies received and processed at UNTH, in Enugu.Nigeria, over a five-year period.
RESULTS. From a total of 75 biopsies received, males (n=49) were 68.1%, while females were 31.2%(n=26). The mean age was 42.6 years ranging from 1.5 years to 86 years.The causes of the lesions as found by biopsy were as follows. Tuberculosis (n=8) constituting 10.7%, metastatic breast carcinoma (n=7) was responsible for 9.3%, chronic non-specific bacterial pleuritis was the most common indication for pleural biopsy (n=22) constituting 29.3%. Three biopsies were false 4%, empyaema thoracis was responsible for 18.7%(n=14),with three cases of bronchogenic carcinoma; squamous cell variety was two (2.8%), while bronchial adenocarcinoma variant had one case(1.3%). Non-Hodgkin's Lymphomas was responsible for 2.7%(n=2). Metastatic prostate cancers (n=3) constituted 4%. There were two cases of haemathorax (2.7%). Two cases each of metastatic sarcomas and a metastaic

unclassified carcinoma in the females,each constituting 2.7%. Lymphogranulomatosis n=1 was 1.3%.
CONCLUSION The commonest indication for pleural biopsy here remains infective causes n=22,or its complication of empyaema n=14.Then tuberculosis n=8 and

P-448

Clinical and laboratorial findings of spontaneous bacterial peritonitis in patients of Universidade Braz Cubas- School Hospital, Mogi das Cruzes, Brazil.

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Introduction: spontaneous bacterial peritonitis (SBP) is a severe complication in patients with liver cirrhosis and ascitis, with mortality rate around 20–37%. Objectives: to review the literature and establish the clinical manifestation and laboratorial characteristics of the patients diagnosed as SBP. Patients and methods: a retrospective study of 19 medical documents in period from January, 2005 to July, 2007 was performed in Universidade Braz Cubas- School Hospital Results: the mean age of the patients was 75, predominantly men. The average hospitalize period was 9 days. The most common symptoms were: abdominal pain, ascitis and jaundice. The most common cause of chronic hepatophaty was viral Hepatitis C, present in 33% of cases. The majority of SBP was communal with leucometry over 250 polymorphonuclear leukocyte/mm^3 in 78% of cases, only one presenting positive culture of the ascitic fluid, isolating Staphylococcus aureus, whereas 50% of the hemocultures were positive, with Escherichia coli isolated. Only one case presented bacterioscopic exam positive, with Gram positive coccus. The laboratory blood exams present the following average results: leukocyte count 9216,67/mm^3 with 75,33% neutrophil, prothrombin time was 54,3%, creatinine 1,58 mg/dl and albumin 2,44 g/dl. The treatment used was third and fourth generation cephalosporins per 10 days. The outcome of the patients was: upturn and released from hospital in 17 cases, 1 death and 1 case hospital transference. Conclusions: findings correspond to the literature, less the fact of the ascitic fluid collection were done by routine method. Nowadays, the best method used to collect ascitic fluid is direct inoculation of ascites into hemoculture bottle method with a higher positivity of 49,8%. Therefore, the knowledge about SBP is essential, as diagnosed methods and clinical manifestations looking for early treatment to reduce the mortality.

P-449

Identification of the predominance bacterial pathogen of community acquired orofaringitis (OF) at Hospital Geral in Guarulhos, Brazil.

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OFs remain the common infections diagnosed in out-patients as well as hospitalized patients, current knowledge on antimicrobial susceptibility pattern is essential for appropriate therapy and the aim of this study was to determine the distribution and antibiotic susceptibility patterns of bacterial strains isolated from patients with community acquired infections (OFs) at Guarulhos as well as identification of ESBL producers in the population of different oral pathogens throught exsudate isolates from symptomatic OFs cases attending to the Hospital Geral at Guarulhos were identified by conventional methods (2006-april 2007) and antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion method. Isolates resistant to third generation cephalosporin were tested for ESBL production by double disk synergy test method and the results points that of the 157 tested sample 71 samples showed growth of pathogens among which the most prevalent were *Streptococcus pyogenes* (78%)followed by *S.aureus* (35%) and the majority (70.25%) resistance was observed against ampicillin and co-trimoxazole. Most of the isolates were resistant to 3 or more number of antibiotics, the forty nine percent of isolates were detected to produce ESBL among which 41,21% were *Streptococcus* isolates.
This study revealed that *Streptococcus pyogenes* was the predominant bacterial pathogen of community acquired OFs in Guarulhos,it also demonstrated an increasing resistance to Co-trimoxazole and production of ESBL in OF pathogens in the community.

P-450

Immunohistochemical study of prognostic markers in Retinoblastoma

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Background-Prognosis of Retinoblastoma has tremendously improved due to improved modalities of treatment and early diagnosis. This study was designed to correlate expression of p53, apoptosis and ki-67 with histological differentiation.

Methods- Retinoblastoma cases were retrieved from our departmental records from 1996–2002. Immunohistochemistry was performed on 90 wax blocks of retinoblastoma for p53,apoptosis and ki-67.
Results - Total no. of unilateral and bilateral Retinoblastoma(RB) cases were 69 & 21 repectively with a M:F ratio of 61:29. Age ranged from 3 mths-132 mths. Total no. of cases which showed positive staining for p53, ki- 67 and apoptosis were 37(41%), 74(82%) and 37(41%) respectively. More no. of ki-67 positive cases were obtained in poorly differentiated retinoblastoma(PDRB) i.e 52 as compared to p53 in PDRB i.e 26. Similarly larger no. ki-67 positivity was found in Well differentiated Rb (22) than p53(11).
Conclusion - We thus conclude that ki-67 is an important risk factor/prognostic marker as compared to p53 and apoptosis.There was no statistically significant correlation between histological differentiation and immunohistochemical markers studied.

P-451
Morphological characteristics of tissue reaction to implantation of polypropylene meshes

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Introduction: Mesh polypropylene endoprostheses are most often used in herniology. We studied the morphology of tissue reaction to implantation of PROLEN (Ethicon), ESFIL (Linteks), Biomesh P1 Mesh (Cousin Biotech) mesh and unwoven polypropylene endoprostheses - Biomesh NK 2 Mesh (Cousin Biotech).
Methods: Experimental study was carried out on mice, implanted polypropylene prostheses. On days 3, 7, 14, and 28 after the intervention the animals were sacrificed and material was resected for morphological study.
Results: We found that after 3 implantation of Esfil and the unwoven endoprosthesis caused a more pronounced inflammatory reaction of tissues. Solitary fibroblasts and new vessels were detected around all implants. By day 7 formation of granulation tissue was observed. After 14 days numerous collagen fibers were seen in the presence of a moderate count of fibroblasts. The total count of fibroblasts in the zone of implantation of the unwoven prosthesis was lower than with the meshes. Implantation of the meshes was associated with an increase in the number and thickness of collagen fibers, some these fibers grown between the implant fibers. After implantation of unwoven endoprosthesis a fine capsule was seen at the implant periphery against the background of pronounced tissue edema and there were many loose collagen fibers and fibroblasts between the endoprosthesis elements, virtually each polypropylene fiber was surrounded by collagen fibrils. By day 28 cellular reaction was presented mainly by fibroblasts. The connective tissue capsule enveloping the unwoven prosthesis was much thinner and the connective tissue components totally grew into the implant.
Conclusion: Implantation of the unwoven endoprosthesis caused a more pronounced inflammatory reaction. The growth of connective tissue components between the implant fibers with the formation of a fine connective tissue capsule enveloping the prosthesis was observed during earlier period of experiment.

P-452
Severe infantile carnitine palmitoyltransferase II (CPT II) deficiency in 19-week fetal sibs

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Background: Long chain fatty acid oxidation in mitochondria is regulated by two carnitine palmitoyltransferases (CPT) and by carnitine:acylcarnitine translocase. In the inner mitochondrial membrane, CPT type II (CPT II) converts palmitoylcarnitine to carnitine and palmitoyl-CoA. CPT II deficiency typically manifests in adolescence with muscle pain and rhabdomyolysis following vigorous exercise. Presentation in infancy is less common. Antenatal presentation has been rarely reported.
Methods: Fetal autopsies and CPT gene sequencing were performed.
Results: Prenatal ultrasound at 19-weeks gestation showed a fetus with enlarged polycystic kidneys. A previous pregnancy five years earlier ended in termination for polycystic kidneys. The suspected clinical diagnosis was Meckel-Gruber syndrome. At autopsy, the renal parenchyma was largely replaced by cysts which appeared to increase in diameter toward the medulla. The liver showed diffuse microvesicular steatosis. A subsequent pregnancy was terminated for polycystic kidneys and Dandy Walker malformation. Fetopsy was not performed.
Fetopsy of the present case showed micrognathia, hypospadias, cystic renal dysplasia with cyst diameter increasing toward the medulla, and microvesicular hepatosteatosis. Fatty accumulation in the liver, renal tubular epithelium, myocardium, and skeletal muscle was demonstrated by Oil red O staining. Diffuse microvascular proliferative changes associated with small old and recent hemorrhages were

seen in the cerebral cortex. A diagnosis of glutaric aciduria or related beta-oxidative enzyme deficiency was suspected. Analysis of the CPT II gene showed a homozygous del1237AG truncating mutation in exon 4. This mutation has been previously reported in Ashkenazi Jewish patients. *Conclusions*: Since abnormal cerebral findings different from our case have only been reported once previously in a fetus with CPT II deficiency, CPT II deficiency should be included in the differential diagnosis in fetuses with cystic dysplastic kidneys, particularly with unusual cerebral findings, in Ashkenazi Jewish families.

P-453

FLEX Ready-to-Use antibodies – a high quality and standardized IHC solution

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Background Pathology laboratories face major challenges to deliver high quality results in a timely and cost efficient manner. An increasing number of cases are evaluated by means of immunohistochemistry (IHC), and the need for high quality and standardized IHC solutions is growing in order to ensure correct patient diagnosis and efficient work flow in the laboratories. A key prerequisite is high performance antibodies. In an international study supported by eight independent international experts, new ready-to-use (RTU) antibodies have been demonstrated to match world wide IHC performance acceptance criteria. When used with associated instrumentation, these will support standardization in laboratories across the world.
Method The study was designed with eight independent experts who evaluated the results in terms of diagnostic relevance. The antibodies were developed to match instruments with dedicated protocols so that the end result was easy to standardize. The final formulation of each antibody was defined to assure optimal sensitivity and specificity by using antibody specific "quality indicators". These were divided into high expression (HE) and low expression (LE) structures that typically were identified in normal tissues (control tissues). The HE structures were used to assure staining capacity, whereas the LE structures were considered as sensitivity markers of the IHC performance.
Results and conclusion A range of 60 clinical important RTU antibodies with high sensitivity and specificity has been studied. The series include key antibodies such as Ki67, CD79α, CK5/6 and Calretinin. Review of the resulting performance with the eight independent experts, demonstrated that it is possible to conduct testing in a standardized way using RTU antibodies and associated instruments and protocols that satisfy performance acceptance criteria world wide. The RTU antibodies are developed to provide the same IHC result world wide, thus contributing not only to obtaining standardized results, but also a diagnostically correct result.

P-454

Acute lung injury and histopathological findings in illicit drugs users with toxicologic corelation

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BACKGROUND:Illicit drugs and psychoactive substances affect all anatomical lung compartments to produce diverse morphological changes. Major classes of drugs wich cause respiratory manifestations are opiates,stimulants and cannabinoids.The intrapulmonary site of injury and the histological pattern of response depend not only on the pharmacological agents, but also on the dose,chronicity of use,route of delivery and presence of additives and adulterants. The pulmonary pathohistological findigs most common include oedema, pulmonary haemorrhage and haemosiderosis,panacinar emphysema,bronchiolitis obliterans, ineterstitial pneumonia or fibrosis.
MATERIALS AND METHODS:The investigation was included 48 judicial autopsies of heroin or other drug consumers who suddenly died from 1995–2007 year. Autopsies, postmortem toxicologic studies and serological research of anti-HIV/HBV/HCV antibodies were performed. Histological sections of all cases were reviewed and dominant pathomorphological changes are finded in the pulmonary tissue and liver.
RESULTS:Cases were mostly male 35/48(72,9%) and all white.The deceased patients ranged in age from 19 to 49 (mean,31 years). 22 patients had serological evidence of hepatitis C virus infection,9 had hepatitis B virus surface antigen and one was HIV positive.A postmortem toxicologic examination was performed on all of the deceased patients,and drugs in the fatal range were identified in only four of them(8,33%),in the toxic range in six(12,5%) and in minimal concetrations in nine of the deceased patients (18,7%).Drugs are identified in the fatal, toxic or minimal range included heroin, morphine,methadone,diazepam,cocaine and amphetamine.
Pulmonary oedema was the cause of death in 42 patients (87,5%)of the 48 drug users.Of the remaining patients as cause of death,four had acute myocardial infarction and two had massive intracranial bleeding.Under light microscopy, the histopathological analysis of lung tissue from the deceased patients indicated oedema,diffuse alveolar damage, emphysema, alveolar haemorrhage and haemosidero-

sis,interstitial fibrosis,bronchiolitis obliterans, arterial medial hypertrophy and inhaled particulates.The most common histological features observed in drug users were acute alveolar haemorrhage 37/48 (77,1%), haemosiderosis40/48 (83,3%) and emphysematous changes 39/48(81,3%).
CONCLUSION:The pulmonary disease is a most common cause of death in drug users.

P-455
MULTILOCULAR CYSTIC THYMOMA:A CASE REPORT
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BACKGROUND:Primary anterior mediastinal neoplasms comprise a diverse group of tumors and account for 50% of all.Thymic cysts are rare and represent approximately 1 to 3% of all anterior mediastinal masses.Their etiology is contraversal:may be congenital or acquired,either due to inflammation or in association with an inflammatory neoplasm such as Hodgkin`s lymphoma,seminoma or thymoma.
CASE PRESENTATION:A rare case of multilocular cystic thymoma is presented.The patient is a 49 year-old woman who came to consult with a persistent dyspnea and cough. The chest computed tomographic scan revealed a solitary, lobulated and nonenhancing mass,11 cm in maximum diametar,near the right atrium and aorta ascending.Physical examination and laboratory work-up were within normal limits.Total tumorectomy was performed via medial sternotomy.Histopathological examination on the resected specimen revealed a thymoma stage I,with cystic appearance,according to the classification of Masaoka.The postoperative course was uncomplicated.
CONCLUSION:Encapsulation and resectability of thymoma is associated with good prognosis. Complete surgical resection is the cornestone of curative therapy.

P-456
Utility of Atomic Force Microscope (AFM) in identification of membranne characteristics of different neoplasms
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The atomic force microscope (AFM) is a very high-resolution type of scanning probe microscope, with demonstrated resolution of fractions of nanometre. Samples viewed by AFM do not require any special treatment. It can work perfectly well in liquid environment without any vacuum system. The AFM works in the same way as ours fingers which touch and probe the environment when we cannot see it. Initial applications in Biology are coming but structural analysis and manipulation of cellular membranes and applications of antibodies on probing tip to localise determinates antigens or receptors of signal vies are interesting challenges.
Method – AFM at mode contact in aqueous media was used to study cultivated cells from malignant mesotelioma and mammary carcinoma. As a first stage, differences between both types of cells and correlation with optical microscope dates of same cells were made to analyse utility of AFM in classify morphological dates for differentiate characteristic types of cells.
Results -AFM provides a true three-dimensional surface profile. Typical patrons of malignant mesotelio and carcinoma cells can be obtained to differentiate between both types of cancer. A more irregular surface and larger protrusion of nucleus were typical of malignant mesotelioma cells, and correlation with optical microscope image was apparent.
Conclusion-AFM is a unique imaging technique that allows high resolution studies of cells in a native state with a topographic map of the membrane surface. This surface can be characteristic of cellular type and to change dynamically in response to external and internal stimuli.

P-457
In vitro and in vivo testing of biocompatibilized polyurethane materials for medical use
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Background. Polymeric biomaterials fulfill several medical uses as artificial organs, prostheses, in dentistry, bone repair, drug delivery systems and regenerative tissue engineering. In order to be safely used in patients, their biocompatibility has to be tested on *in vitro* and *in vivo* models. The aim of our study was to investigate the *in vitro* biocompatibility and *in vivo* tissue response for two new polymeric biomaterials, prepared by compounding polyurethane (PU) with collagen (COL) and with a collagen-elastin (COL-EL) mixture, respectively, compared to PU material.
Methods. *In vitro* biological evaluation of the biomaterials in direct contact with human dermal fibroblasts (HDF) was

performed. Cell viability and proliferation were assessed by MTT test and cell adhesion and morphology were observed by light microscopy. Material biodegradation was measured by the collagenase degrading test. *In vivo* biocompatibility of polymeric biomaterials was evaluated following subcutaneously implantation in rats, for periods of up to 6 weeks. The biomaterials together with surrounding tissue were fixed, processed and paraffin-embedded for histopathological evaluation.

Results. MTT test results indicated the highest HDF viability for PU-COL composite. Light micrographs showed that cells adhered, spread and proliferated onto the surface of all polymeric biomaterial variants. It was also observed that HDF retained their normal phenotype. The degradation of PU-COL-EL composite was slower than that of PU-COL material. Histological and immunohistochemical analysis of polymeric implants showed that they induced a focal but chronic inflammatory reaction, which was limited to a small area around the materials. A moderate foreign body reaction was seen around the implant surface.

Conclusion. Both cell culture studies and *in vivo* experiments showed that the two composites PU-COL and PU-COL-EL exhibit a better biocompatibility than PU alone. They could be used as bioactive scaffolds for tissue engineering and medical device fabrication.

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P-458
Withdrawn

P-459
AGE-ASSOCIATED LOSS OF NEURONS IN BRAIN STEM STRUCTURES.

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Background. Age-associated loss of neurons in central nervous system was an axiom in biology for a long time. Stereological techniques have failed to confirm these earlier findings, there is evidence of focal neural death and synaptic or receptor loss. Focal damages in vital nervous centres may play a significant role in aging and age-related pathology.

Methods. The research was undertaken on autopsy material. We studied the state of nucleus dorsalis n. vagi,nucleus sensorius superior n. trigemini.

Autopsy material has undergone standard histological processing. Serial cross-sections of medulla oblongata were prepared. The calculation of neurons in the right and left nucleus dorsalis n. vagi and nucleus sensorius superior n. trigemini was undertaken with mean number identification.

Results. 70 autopsies were studied. The age varied from 22 to 80 years. The study revealed a 22% decrease of mean number of neurons in n. dorsalis n. vagi in age group of 71–80 years comparing with age group of 21–30 years. A strict correlation between the alterations in n. vagus dorsal nuclei and persistence of chronic atrophic pyloroantral gastritis and chronic atrophic duodenitis was also revealed. This correlation plays also an important role in cases of gastric and duodenal ulcer persistence when the decrease of neurons takes place in age groups of 31–40, 41–50 and 51–60 years. The revealed decrease of neurons in nucleus sensorius superior n. trigemini in the same age groups was 34,4%.

Discussion. Age-related focal neuronal loss is constant feature as neuronal regeneration is resrticted by low amount of neural stem cells in adult organism. It may result in persistence of a number of pathologic processes and a decrease of life duration.

P-460
Efficiency of fetoplacental unit study in fetal death: Review of a series of 339 cases.

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Background: Fetoplacental unit study in fetal death is important, to try to determine its cause and to improve the follow-up of next gestations.

Objective: To determine how often can be determined the cause of fetal death based on the fetoplacental study, clinical data and cytogenetic study of fetal tissue, and the prevalence of the different causes.

Material: 339 consecutive cases of fetal death between 12 and 41 weeks of gestation, in wich fetal autopsy and histopathologic study of the placenta have been done, happened between 1997 and 2006.

Method: Review of clinical, cytogenetic, autopsy and histopathologic reports, and review of autopsy and histopathologic material in selected cases.

Results: Alterations highly suspicious to be the cause of death could be determined in 265 cases (78%), being placental pathology (96), amniotic fluid infection (87), funicular pathology (40) and malformations (18) the more prevalent. Other less common causes were oligohydramnios (7), immaturity (10), cervical incompetence (5), maternal death (1) and abortive drug (1).

There were more cases between 12 and 24 gestation weeks (227/339). The proportion of unknown cause was slightly higher in this period also, so there were more cases between 12 and 24 gestation weeks (55/74) in with no cause was found.
Amniotic fluid infection, malformations and other less common causes had higher incidence before week 24, while placental and funicular causes had higher incidence after weeks 24 and 31 respectively.
Conclusion: In our series of 339 cases of fetoplacental study in fetal death, cause could be determined in 78% of cases, with varying percentages of the different causes depending on the weeks of gestation.

P-461

Calcitonin targets extracellular signal-regulated kinase signaling pathway in cancers

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[Backgrounds] The mitogen-activated protein kinase (MAPKs) signaling pathway is a potential target in cancer therapy. Constitutive phosphorylated extracellular signal-regulated kinase (ERK1/2), which is one of the MAPKs has been detected in a variety of tumors. Calcitonin (CT) is a polypeptide hormone secreted by the thyroid gland and has been used to treat the osteoporosis and humoral hypercalcemia of malignancy. The purpose of this study was to determine the mechanism of the effects of CT on ERK1/2 phosphorylation in breast cancer cell line and to further investigate the therapeutic usefulness of CT targeting ERK1/2. [Methods] Fourteen human cell lines were used to detect ERK status using Western blotting. To investigate the effect of CT for tumor growth, Female athymic BALB/c nu/nu mice were used. All protocols for in vivo studies were approved by the Institutional Animal Care and Use Committee of Wakayama Medical University. [Results] CT decreased ERK1/2 phosphorylation in cancer cells, MDA-MB-231, MDA-MB-435, T24, DU145, CaR-1 and VMC-RCW, showing constitutive phosphorylated ERK1/2. However CT had no effect on cancer cells, MCF-7, T47D, MRK-nu-1, TT, FTC-133, KP-1NL, KP-3, and CCK81, showing non-constitutive phosphorylated ERK1/2. In MDA-MB-231 cells, a breast cancer cell line, CT phosphorylated c-Raf at Ser^{259} via the PKA pathway, resulting in suppression of ERK1/2 phosphorylation. CT significantly reduced the tumor volume of MDA-MB-231 cells compared with saline buffer *in vivo*. However, CT did not exert any significant effects on the proliferation of MCF-7 cells, a breast cancer cell line. [Conclusion] CT decreased constitutive ERK phosphorylation in cancer cell lines. In addition, CT suppresses the *in vivo* proliferation of cancer cells with constitutive ERK phosphorylation. CT would be a candidate for the treatment of cancer patients with accompanying osteoporosis, and could also be used in combination with a complex hormone therapy.

P-462

Low-passage tumour cell lines: A challenge for biorepositories

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Low-passage tumour cell lines are those generated from tumoral tissue with the least possible number of passes, by which means it is aimed to preserve the characteristics of the original tumour as best as possible. These lines represent an excellent in vitro model for the study of human tumours and they are the tool of choice for transcriptional modification experiments using RNAi, and for drug-sensitivity studies, etc. Despite their usefulness, there exist no standard or guides for its characterisation.
The most critical problems are related to:

- Exhaustive morphological and phenotypical characterisation, and their study in the context of the molecular profile of the original tumour.
- Identification of the degree of transformation of the cells in culture, their replicative capacity and their ability to avoid the process of senescence.

Two of the objectives of the Andalusian Tumour Repository Network are to generate cell lines of this type and to distribute them to researchers. Characterisation and distribution must be carried out employing some minimun quality standards. We are working towards obtaining good markers that will improve the characterisation of these lines.

- The characterisation panel must be developed for each type of cell line. The most common features is the loss of markers typical of the lineage. For example, carcinomas may have a defect in their cytokeratin expression.
- The most widely accepted sign of cellular transformation is the presence of “foci transformation”. These are structures of cells distributed in layers. Our analysis shows that they have a cell cycle arrest profile that is different from all other cells in culture. The cells in these foci have a low proliferation ratio, and exhibit expression of p16 and p27 and a loss of expression of cyclin D1.

P-463

Age related morphological changes in the seminiferous tubule wall

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Background. Possible correlations between ageing and morphologic changes in lamina propria (LP) of the seminiferous tubules (ST) were assessed.

Method. The studied material consisted of testicular tissue samples from 28 cases with orchiectomy for prostate adenocarcinoma. Tissue samples were processed by the classical histological technique and stained, on serial slides, with Hematoxilin-Eosin, Goldner trichrome and immunomarked for smooth muscle Actin (SMA) and collagen IV (CIV).

Images were acquired and measurements were performed with an image analysis software, after previous calibration. The assessed parameters were: LP thickness (LP-Th) and Hyalin "Collar" thickness (HyC-Th). 30 tubules were randomly selected, with X40 objective, for each case and 5 random determinations for each tubule and for each parameter were performed. Mean thickness (M) for each ST (M-x-Th/ST), case (M-x-Th/C) and age group (M-x-Th/A) were calculated (x = assessed parameter). Regression line (RL), Slope (m) and Significance test for Slope ("p") were calculated to assess the correlation of each parameter with ageing.

Results. M-LP-Th showed a discrete decreasing trend with ageing, but without an obvious statistical correlation (SC). The internal LP layer, apposed to BM (usually formed of a loose, reticular, fibrillary network), revealed, not rarely, areas of collagen focal denseness which often showed foci of hyaline degeneration. Sometimes, these foci had circumferential, "collar"-like disposal around the tubule. M-HyC-Th had a discrete decreasing trend with ageing but without an obvious SC. The percentage occupied by HyC in LP had also a mild decreasing trend but without SC with ageing.

Conclusion. LP of the seminiferous tubule undergoes, with ageing, a wide variety of degenerative changes, with "mosaic", focal distribution and no tendency to advance with ageing as demonstrated by the great variety of morphologic measurements (ranging from total absence to surrounding the entire circumference of the tubule) and statistical determinations.

P-464

Tuberous sclerosis complex in a fetus of 25 weeks gestation:autopsy findings

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Background: Tuberous Sclerosis Complex (TSC) is an autosomal dominant multisystem disorder in which CNS, eyes, kidneys,skin and heart are most commonly affected by malformative,hamartomatous or neoplastic lesions.We present a case of TSC in a 25 wk-gestation fetus.

Method & Results: Prenatal ultrasound at 24 weeks had shown rhabdomyoma of the left atrium and cystic dysplasia of the left kidney.Fetal autopsy revealed multiple neocortical tubers and subependymal nodules (SENs) of the fixed brain, atrial rhabdomyoma and generalized myocadial rhabdomyomatosis, a multicystic left kidney with histological features pathognomonic of TSC and hamartomatous lesions of the lungs.

Conclusion: Typical pathological features of TSC are already present at 25 weeks of gestation.Ultrasonographical detection of cardiac rhabdomyoma should rise suspicion of TSC, especially when combined with renal lesions.Typical brain lesions evade prenatal ultrasound detection at this age.

P-465

Thresholding challenges in quantification of immunohistochemical cytoplasmic stain

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Immunohistochemical stain is widely used in clinical and research laboratories to obtain measurements of neoplastic biomarkers. The protein level was shown to be correlated to immunohistochemical stainings and this fact has led to the development of computer-assisted analysis of immunostains. But image segmentation/thresholding that works well in other fields is not as good when it comes to quantify the cytoplasmic expression of various proteins.

We propose the use of no-counterstained images to allow automated and accurate identification of diaminobenzidene (DAB)-labelled antigens. For this study, we have used a set of sixteen images. Initially, the slides were DAB stained only, images were captured, then the slides were counterstained with hematoxylin and images were captured again.

DAB chromogen develops a brown coloration; the brown color is a mixture of red, blue, and yellow color. The hematoxylin which is normally used for counterstaining, has a blue color. So, when a hematoxylin counterstained image is thresholded for DAB, the resulting mask will always include nonspecific areas due to the blue component that is part of the DAB brown coloration. A statistical analysis has shown differences between thresholding the no-counterstained and the counterstained images.
Many computerized applications are inconsistent in quantifying DAB staining due to the nonspecific background, but our method was consistent for all staining levels.

P-466

Survivin expression in posterior uveal melanoma: an immunohistochemical study.

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PURPOSE: Survivin, an inhibitor of apoptosis (IAP) protein, acting in the G2/M phase of the cell cycle, is expressed in several human cancers. The aim of this work was to study the survivin expression in primary posterior uveal melanomas treated with enucleation without prior radiotherapy. **METHODS:** It was performed immunohistochemical staining for survivin (DAKO clone 12C4, with specificity for an epitope present in the first 34 N-terminal amino acids of the survivin protein), with melanin bleaching, in formalin-fixed, paraffin-embedded blocks from thirty two uveal posterior melanomas. Specimens were divided according to size, cell type and extracellular matrix deposition. The intensity of survivin immunostaining was scored for both, cytoplasmic and nuclear staining independently, as follows: 0, absent; 1+, weak; 2+, moderate; and 3+, intense. **RESULTS:** There was a high expression of survivin in uveal posterior melanoma. Twenty three cases (69,6%) of 32 uveal melanomas expressed survivin protein, among them, 13 cases positively expressed in the cytoplasm (7 intense, 4 moderate, 2 weak), 3 cases expressed predominantly and intensely in the nuclei, and 7 cases expressed moderately in both nuclei and cytoplasm. The intranuclear staining had a particular dotted pattern. The survivin was also weakly positive in the extra cellular compartment that is in the aqueous humor of the eye of 15 cases. The Pearson chi-square analysis demonstrated a significant correlation (p=0,0034) of survivin expression to epithelioid or mixed cell type being absent mostly in the spindle cell tumors. Similarly, the nuclear expression of survivin correlated to epithelioid cell types (p=0,0008), the isolated nuclear expression being associated exclusively to epithelioid tumors with extensive necrosis. It also strongly correlated with the largest basal dimension and the loops or network pattern of extracellular matrix deposition. **CONCLUSION:** This study suggests the possible role of nuclear survivin in the progression of uveal melanoma, the survivin being associated to the known risk factors of uveal melanomas which are the epithelioid cell type, extracellular matrix deposition in looping patterns and monosomy 3. The extracellular presence of the survivin in the aqueous humor of the eyes may reflect also the possible role of survivin in the secondary processes unleashed by the tumor that is neoangiogenesis and immunomodulation, which both need antiapoptotic mediators.

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P-467

UNUSUAL SITES OF METASTASIS: PRESENTATION OF THREE CASES

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Introduction: The improved survival of patients with carcinoma together with aging of population has led to a higher incidence of metastasis in unusual sites. We report herein the cases of three patients with cancer in the past who presented metastatic disease in non-common sites. **Material and methods:** The first case corresponds to a 73-year-old man presenting a solitary duodenal mass seven years after a nephrectomy for a Renal Cell Carcinoma. The second one relates to a 55–year-old woman with a history of meningeal hemangiopericytoma nine years earlier, who presented with ictericia and abdominal pain. The third case corresponds to a 57-year-old man with lung cancer and multiple disseminated metastases. **Results:** The histopathology of the resected specimen on the first case confirmed a metastatic renal cell carcinoma in duodenum. A pancreatic mass was found on the second case which microscopically was diagnosed as being a hemangiopericytoma. Ten years after the initial diagnosis, distant metastasis to liver were also found. An autopsy was requested on the third case when he passed away and a lung adenocarcinoma metastasizing into a non-previously diagnosed 7-cm-renal cell carcinoma was described. **Discussion:** In patients with a previous history of cancer and newly diagnosed symptoms, the possibility of local recurrences or metastasis should be kept in mind. We would like to highlight the importance of long-term follow up in order to provide accurate diagnosis and exclude the presence of possible metastasis.

P-468

Vascular endothelial growth factor A expression in uveal melanoma.

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AIMS. Immunohistochemical investigations of the VEGF A expression in uveal melanomas revealed variable results. The aim of this study was to evaluate the expression of vascular endothelial growth factor A (VEGF A) in the anterior and posterior uveal melanomas and his relationship with the extracellular matrix deposition pattern and other parameters of high risk significance. **METHODS.** Thirty two posterior uveal melanomas and 3 cilliary body melanomas were investigated, using melanin bleaching and immunohistochemistry for VEGF A (Dako). The extracellular matrix deposition was assessed using PAS without haematoxylin counterstaining. No eyes had received prior treatment. The intensity of VEGF A was scored as the following: (0) <5%; (1) 5–25%; (2) 25–50%; (3) 50–75%; and (4) >75%. The mitotic index was assessed on each tumor on 10 field delimited by a Weibel reticle of 0,658 mm square, eliminating the necrotic areas. **RESULTS.** Tumor cell type, location, necrosis, mitotic index and extracellular matrix were analyzed by using light microscopy. Histopathologically, the tumor cell types were spindle cells in 12 (34,3%) of the cases, mixed cells in 16 (45,7%), and epithelioid cells in 7 (20%) tumors. Positive reaction for VEGF-A was present in 80% of the tumors. The intensity of VEGF A was weak (1) in 15/35 (43%), moderate (2) in 12/35 (34%) and high in 8/35 (23%) cases. The VEGF A was also present in all positive cases in the extra cellular compartment of the eye, in the aqueous humor. The mitotic index varied between 3 and 35 mitosis on 10 field occupied by tumor cells. Microvascular loops/networks were seen in 15 (42,8%) of the tumors, in 9 (25,7%) the arc pattern sometimes with branches, in 6 (17,2%) parallel pattern with or without crosslinking, the rest of 5 (14,3%) displaying the straight pattern. The intensity of the VEGF A was correlated with the other prognostic factors. On multivariate analysis, the correlation between VEGF-A expression and the epithelioid cell type especially with necrosis, associated with high mitotic rate and the loop/network patterns, was found to be statistically significant (p=0,0037). It displayed any relationship with

the tumor location, tumor size and age. **CONCLUSIONS.** Our data suggest that increasing VEGF-A expression can be used as a dependent prognostic factor for high risk posterior uveal melanoma beside the other well known parameters as the epithelioid cell type, extracellular matrix deposition in looping/network patterns and monosomy 3.

P-469

Balloon cell nevus of the conjunctiva (case report)

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Background Balloon cell nevus (BCN) is an uncommon variation of melanocytic nevi characterized histologically by a preponderance of peculiarly large nevomelanocytes with clear foamy or finely vacuolated cytoplasm(1) Balloon cells are altered melanocytes with clear vacuolated cytoplasm caused by a defect in the process of melanogenesis. Although rare, balloon cell change has been observed in a variety of melanocytic proliferations, particularly intradermal melanocytic nevi and melanoma. When present, such features may lead to difficulties in diagnosis, particularly with other clear cell neoplasms. The diagnosis should be made when more than 50% of the tumour shows balloon cell change.

The balloon cell nevus is a rare variant of the conjunctival nevi.

Case report A 22-year old asymptomatic man was admitted to our hospital with a pigmented lesion of the right eye. He reported that the lesion had become noticeable 6 weeks earlier. His ophthalmologic history wasn't any remarkable symptom. On examination, a slightly elevated, movable, tan-yellow nodule with a deep brown area was observed on the bulbar conjunctiva of the right eye. No other bulbar pigmentation was noted. An excisional biopsy was performed. A $0.7 \times 0.4 \times 0.2$ cm. conjunctival specimen was white-yellow and contained a slightly central dark brown pigmented lesion.

Histologic examination: The tumor mass involved the papillary and superfisial dermis and consisted of large cells with deeply basophilic, small, centrally-placed nuclei and abundant, slightly eosinophilic vacuolated or granular cytoplasms with microvesicles. Some cytoplasms has moderate amounts of intracelluler melanin. Some multinucleate cells were also noted. Some areas showed small aggregates of small lymphocytes between balloon cells. There was no mitotic figure. Diagnosis was made as a balloon cell nevus of the conjuctiva.

Cardiovascular Pathology

P-470

HYPERTROPHIC CARDIOMYOPATHIES OF ISCHEMIC ETHIOLOGY

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Aim: The multidisciplinary analysis of particularly necropsic aspects related to arterial hypertension of myocardial ischemia. **Material and methods:** 200 autopsies were made sampling fragments from myocardial aries injuried as well as from neighbouring territories. Fragments from miscellaneous organs were gathered too. Histological techniques used were: H.E., V.G., Weigert, Lee, PAS, PAS-Alcian, Gomori, achieving photo images from zones with ischemic lesions. **Results:** The correlations among pathological versus clinical and paraclinical data are tested according to the chronological recording of their appearance, starting with the risk factors, going to the collection of symptoms presented in admised moment, then to the actual clinical signs and paraclinical investigations registered during the hospitalization stage and submitted as graphical representations (ECG, PHONO) or iconic drawings (ECHO, CT). The first table shows the risk factors as: arterial hypertension (HTA), hypercholesterolemia (colesterol), smoking (fumat), diabetes mellitus (diabet zaharat or DZ), overweight (obezitate) and dislipidemia described in terms of standard deviations on age groups for male patients. **Conclusions:** 1. Compensated systemic HHD may be asymptomatic an suspected only in the appropriate clinical setting by ECG or echocardiographic indications of left ventricular enlargement. 2. Other causes for such hypertrophy must be excluded. 3. In many patients, systemic HHD comes to attention by onset of atrial fibrillation (owing to left atrial enlargement) or CHF with cardiac dilation, or both. 4. Depending on the severity, duration and the underlying basis of the hypertension and on the adequacy of therapeutic control, the patient may: (1) enjoy normal longevity and die of unrelated causes, (2) develop progresive IHD owing to the effects of hypertension in potentiating coronary atherosclerosis, (3) suffer progressive renal damage or cerebrovascular stroke, (4) experience progressive heart failure. 5. The risk of sudden cardiac death is also icreased. 6. The electronmicroscopy can thoroughly describe the bio-morphology of extracellular matrix, the modification of it being important in the

evolution of the ischemic cardiopaty. 7. The abundance of data given by the necropsy examinations, followed by hystopathology studies supplies the area of paraclinic investigations being needed the sampling of myocardial biopsies in view of microscopy diagnosis of patitents during life, as well as the necropsy of all cases of ischemic cardiopaties, including all known and multi-disciplinary investigated cases.

P-471

Cardiac 'MICE' What is it really?

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Background: Mesothelial/Monocytic Incidental Cardiac Excrescence (Cardiac MICE) is an entity first described by Rosai et al in 1979. The authors present two cases and put forward their view concerning its still controversial histogenesis. **Material and Methods:** One of the cases refers to a 66-year-old woman with floppy mitral valve and coronary atherosclerosis that was submitted to diagnostic coronary angiography and afterwards to mitral valve surgery and aortocoronary bypass; and whose lesion was detected inside the left atrium during surgery, and macroscopically accepted as a thrombus. The other case concerns a 76-year-old man with a pacemaker due to complete atrioventricular block, that after having undergone surgical revision as a consequence of electrode exteriorization one year after its insertion, had the electrode removed the next year, in the context of infection resistent to antibiotherapy. The surgical specimen, obtained from the right side of the heart, was attached to the electrode and considered as a vegetation. **Results:** Histological evaluation of both samples disclosed a lesion made up of fragments of mesothelium and macrophages/histiocytes supported by a fibrin meshwork. On the second case, it was associated to real organizing thrombus and "pannus" enveloping the electrode. **Conclusion:** Cardiac MICE is a rare begnin tumoral lesion (1). It may be located in any of the four heart chambers depending upon each clinical setting (2). Finally, the common element shared by both cases - cardiac manoeuvres - emphasizes the intracardiac shifting of pericardial mesothelium fragments during surgical/interventional cardiac procedures as the mechanism of origin (3) and consolidates its classification as an Iatrogenic Disease.

P-472

TGF-BETA PATHWAY IS ACTIVATED IN LEAFLETS OF NON SYNDROMIC MITRAL VALVE PROLAPSE

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BACKGROUND Mitral valve prolapse (MVP) is the leading cause of mitral valve incompetence in western countries. Although its etiopathogenesis remains uncertain, a genetic defect has been recently identified in some syndromic (ie, Marfan syndrome) and non syndromic cases. The finding that TGF-beta dysregulation may play an important role in the development of MVP prompted us to evaluate whether the Smad receptor-mediated intracellular TGF-beta pathway is activated in isolated MVP as well.
METHODS Myxomatous mitral valve specimens were obtained at surgery from patients (13 cases, 11 males and 2 females, mean age 55.5±12.7 years) who underwent valve repair or replacement due to MVP. Other connective tissue disorders or familial valve disease were excluded. Age and sex-matched control samples of mitral valve were obtained from homograft Tissue Bank (5 cases, mean age 49± 9 years). Valve morphology, mucoid substance, elastic fibers and mitral valve thickness were assessed on routinely stained histology sections. Specific primary antibodies recognizing active phosphorylated form of Smad2 (P-Smad2, Dako) in valvular myofibroblasts (vimentin and smooth muscle myosin positive) were used. Picro-Sirius staining was applied to examine the amount of collagen I and III.
RESULTS MVP leaflets exhibited alterations in architecture with fourfold increase in thickness (2,5±0,8 vs 0,6± 0,3 mm, $p<0.0001$) and increased cell density in the spongiosa (110,9±64,6 vs 51,8±27,5 cells per high power field, $p=0.04$) compared to the normal valves. A higher density of nuclear staining for P-Smad2 (38% vs 12%, $p< 0.0001$) was also observed, indicating an increased activation of TGF-beta pathway. Collagen I and III have higher expression in MVP leaflets than in controls.
CONCLUSION Our data support the hypothesis that an increased activation of the intracellular TGF-beta response pathway may contribute to the pathogenesis of non syndromic MVP.

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The evaluation of angiologic distortions (involutive-structural) in the appearing of cerebral stroke

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Background and aims: We have studied the involutive-structural element and the stroke. The cerebral stroke in the practice of emergency medicine frequently causes the death of patients. Actually, its appearing is noted in subjects of "professionally active" age of the modern society (30 - 40 - 45 years). **Method:** We have analyzed the macro- and microscopic morphologic panorama of the altered region in 300 deceased patients. **Results:** We have noted frequently in the complex of distortions of the cerebrovascular circulation the acceleration of involutive structural modifications in the angio-phlebolic system. Among the components of the constituents which predispose to cerebral stroke, well known and described in detail up to date, attention is attracted towards the phenomenon of "elastolysis": the behaviour of elastic structures, the biomolecular, physico-chemical state of the elastic tissue. **Conclusion:** This state might influence substantially the advancement of pathomorphologic processes, which result in serious consequences (stroke). The premature appearing of involutive factors results in precocious senescence of the subject. The phenomenon of "elastolysis" becomes an associated factor in the appearing of cerebral stroke.

P-474

Myocardium fluorescence in human myocardial infarction

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Background and aims: The study aimed towards finding new criteria forthe determination of the duration in time of myocardial infarction (MI).

Method: Fifty-two hearts from persons (PRS) deceased from MI, in which MI beginning was documented clinically and by ECG, were selected. Thirty hearts of PRS deceased almost instantaneously from traumas served as controls. In each case we did 1000 measurements of myocardial primary fluorescence (MPF) at the wavelength of 360 nm, both in the necrotic (ischaemic) zone (NZ) and in extrainfarctic zones (EZ).

Results: During 8–12 h from MI beginning, the MPF decreased concordantly in both zones, being 89.6±2.3% of controls. At 24 h after MI onset MPF in NZ was 78.8±4.3%, in EZ 84.4±3.1% of controls. Minimal NZ MPF was on Days 4–5 and 9–10 (66.3±2.5% and 62.6±2.1% of controls, $p<0.001$). EZ MPF on Days 4–5 was 77.4±3.5%, on Days 9–10 82.3±2.2% and on Days 14–15, 85.7±2.8%, remaining even up to Days 30–35 of MI 7.3±0.8% below controls. MPF variations in time were approximated by splines.

Conclusion: By solving a system of equations, which included formulae for EZ and NZ MPFs, it was possible to determine the time elapsed from the MI onset, with an accuracy of 9.6 hours.

P-475

Mediastinal nonleukemic granulocytic sarcoma with cardiac infiltration: case report and literature review

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Introduction: Granulocytic Sarcoma (GS) is a rare extramedullary tumor composed of immature myeloid cells. It is usually associated with leukemia or other myeloproliferative disorders but can also occur without overt hematologic diseases. The involvement of heart is very rare and usually diagnosed at autopsy. We reported the clinical and pathological features of an isolated mediastinal GS with cardiac infiltration with no evidence of leukemia involving bone marrow or peripheral blood, and reviewed the literature. Method and results: Patient male, 31 years old, was admitted with history of dry cough and progressive dyspnea for five months. He had done previously transthoracic-eco, which revealed mass occupying approximately 65% of the left atrium. The mass invates da cavity of the left ventricle in diastole through the mitral valve, which caused great restriction on the filling. The previous morphological and immunohistochemistry studies were angiossarcoma, paraganglioma and small cell carcinoma. The chemotherapy was started with etoposide and cisplatin, no good clinical response. Three months later, the thorax computed tomographic scan showed the mediastinal expansive mass, with right pleural effusion and pericardial spill. The case was reviewed showing positive for myeloperoxidase and CD99, favoring the diagnosis of GS. Treatment was directed to GS with daunorrubicina and cytarabine, with clinical remission. Meanwhile patient presented febrile neutropenia, envolving to death ten months after start of symptons. Conclusion: Previous literature showed that almost half of the patients with heart primary GS was misdiagnosed initially. GS most often represent relapse or initial presentation of AML. We reported a case of isolated heart GS without a history or subsequent development of AML

at last follow-up. Immunohistochemistry is extremely helpful for recognizing isolated myeloid sarcoma. The research in literature disclosed the presence of only five of GS unrelated to the leukemia to exclusively mediastinal presentation and only one with cardiac infiltration.

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ANATOMOCLINICAL CORRELATIONS IN COMPLICATED CONGENITAL LEFT ATRIAL CARDIOVASCULAR DISEASE

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Aim: The presentation of a rare case of a left cardiac malformation hart with non surgical resolving.
Material and methods: During year 2000 there have been done necropsies on 19 years old women known with coarctation of aorta and endoluminal isthmoplastia with balloon and mitral supravalvular diaphragm through incomplete identified mechanism and pulmonary hypertension releaved by the presence of media hypertrophy and vasoconstriction between focal siderophagia on histological cups of both lang certified by ordinary and specific stains: HE, VG, Weigert, PAS.
Results: On the secondary irreversible pulmonary hypertension background through necrotized arteritis and plexiform lesions have appeared bronchopneumonic centers with hyaline intraalveolar membranes and interruptions of basal kidney glomerular membrane.
Discussion: The complicated histopathological aspect of pulmonary lesions leads to etiology of final episode about presence of immune complexes of circulatory anti basal membrane auto-antibodies against of non-collagen domain of α-3 chain of collagen what is on the basis of pathogenesis of Goodpasture syndrome to explain the final pulmonary and renal inflammatory lesions.
Conclusions: The correctly and in time diagnosis of patients with congenital left cardiac lesions that may be classified in Shone syndrome characterized by four obstructive cardiac lesions situated in left heart: supravalvular mitral ring, deformation on mitral valve in parachute, supraaortic stenosis and aortic coarctation is the first step to the heavy surgical treatment of these cases.

P-477
LEFT ATRIAL MYXOCHONDROSARCOMA RELAPSE

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CASE PRESENTATION
Ten months before the examination, the 76-year old female patient had suffered from periodical dyspnea and from congestive cardiac insufficiency symptoms. The echocardiographic test detected the presence of a tumoral mass in the left atrium, on the interatrial septum. The tumoral mass was extirpated by surgery (dr Vasile R.'s team) and white-grey fragments displaying firm areas, of medium consistency, and measuring between 3 cm and 0.5 cm, were submitted to histological exams, alongside other, hard fragments. The histological aspects detected through HE, VG, PAS-Alcian, Gomori colorations varied in the different examined territories, as follows: subendocardially there appeared myxoid images showing round, fusiform and faintly starred cells, images of chondroid and osteoid metaplasia, alcianophile matrixes alongside fascicled areas with numerous larger, hyperchromatic, bi-nucleate atypical cells. The reappearance, ten months later, of the initial clinical symptoms called for reexamining the patient, especially echocardiographically, which made it possible to detect the relapse of the left atrial tumor. Surgery was performed and a cauliflower-like tumor measuring 5/4/3 cm, of firm consistency and hard at the implantation basis was removed. Numerous histological sections as well as the same colorations, including immunohistochemical ones, were performed at the Victor Babes Institute, which showed images similar to those detected in the primary tumor. However, there prevailed the chondrosarcomatose aspects with osteoid differences and cells having atypical aspects.

P-478
The Clinical Role of Recurrently Elevated Macro Creatine Kinase Type 1

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The typical creatine kinase (CK) isoenzymes include CK-BB, CK-MB and CK-MM. Macro CK type 1, one of the atypical CK enzymes, has been identified in human serum, but the clinical significance still remains uncertain. In our laboratory, 105 patients who expressed serum macro CK isoenzyme type 1 were identified from March 2004 to May 2007. We found macro CK type 1 recurred after a least one month in 16 patients.
Clinical diagnoses were myopathy in 15 patients, and one had acute coronary syndrome. The averages of serum total CK, macro CK type 1 were 10,784 and 2,249 (U/L), respectively. The linear regression analysis between macro CK and total CK revealed a good correlation ($y=3.5054x+2381.3$, $R^2=0.7822$, $P<0.001$). Three patients had critical

illness, including one respiratory failure and two mortalities. Good linear correlation is documented between total CK and macro CK type 1. In conclusion, the macro CK type I isoenzyme recurred in patients with myopathy mostly.

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Arrhytmogenic right ventricular dysplasia cases in forensic autopsies year 2004 in Barcelona

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SUB-HEADING. Arrhytmogenic right ventricular dysplasia in forensic autopsies.
BACKGROUND. Arrhytmogenic right ventricular dysplasia (ARVD) is a progressive heart muscle disease often familial characterized by structural and functional abnormalities of the right ventricle due to replacement of the muyocardium by fatty and fibrous tissue extending in some cases to the left one (a factor considered of bad prognosis). Clinical presentation consists of arrythmias of RV origen that include, premature ventricular beast, ventricular tachycardia, ventricular fibrilation and sudden death. Most cases are related to young people performing exercise.
METHOD. Retrospective review of cases about (ARVD) after performing complete forensic autopsies an histologic examinations at the INT-CF Barcelona in 2004.
RESULTS. Out of a total 875 studies performed at the National Institute of Toxicology in Barcelona (2004), 47.3% (n=414) were due to sudden death and only one case diagnosed as (ARDV).
CLINICAL CASE: A 46 y. old caucassian man were studied at the cardiology department because of syncope episodes. Electrophysiologic studies were normal and he was allowed to performed exercise. While he was playing his first tennis match he underwent a loss of consciousness and sudden death. A complete judicial autospy was performed with toxicologic screening.
Macroscopic Findings (Gross): A 405 g heart, showed epicardial fat thickened with focally dilated right ventricle. Minimal atherosclerotic changes of about 30% stenosis at main and left coronary artery. Neither aneurysmal changes nor wall thinning were related.
Histopathology: Transmural fibrofatty infiltration of the right ventricle with foamy/bubbly myocardiocystes wth left ventricle involvement (biventricular). No inflammatory changes were related.
Vitreous humor test were negative. Toxicologic analysis showed paracetamol and metamizol in blood and bile.
DISCUSSION. About 0.24% of all sudden death cases studied at the histopathology service of the INTCF (Barcelona) in 2004 were classified as ARVD.
ARDV is a well-known disease related to sudden death as result of ventricular fibrilation during exercise and the diangosis is of paramount importance in terms of genetic counseling to other family members.

P-480

Role of myocardial endothelial permeability in the development of heart failure following open-heart surgery

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Introduction During open heart surgery some damages of cardiomyocytes and myocardial capillary endothelium occure, with the consequences to the structure and function of cardiomyocytes as well.
This lesions are the result of the usage of the cold multicomponent cardioplegic (CPL) solution, long term hypoxia and reperfusion.
Aim The aim of the study is to assess the influence of intraoperative changes of myocardial capillaries on the severity of heart failure (HF) in the postoperative course.
Methods Two groups were analysed. Group A, consisting of 13 patients who underwent open-heart surgery and with signs and symptoms of mild HF in the postoperative course. Group B – 7 patients who died from second to tenth postoperative day due to HF. The ischemic period was 77,6±4,2 min in gr.A, and 94,2±5,2 min in gr. B. Myocardial intraoperative biopsies from the right ventricle, taken during different time during the ischemic period and reperfusion, were analysed by electron microscope using quantitative stereological analyses.
Results Myocardial ultrastructural analysis showed increase in permeability of the capillary wall, edema and degenerative changes of cardiomyocytes that were similar in both groups. Stereological analysis of endothelial cells organelles revealed decrease of 48% in volume fraction of basic pinocyte vesicles in gr.B after release of the cross-clamp as compared to immediate preoperative level, suggesting that the elimination of metabolic products from interstitium to microcirculation is altered.
Conclusion Ischemic changes of myocardial capillary endothelium during the open-heart surgery, as assessed by stereological analysis, are more profound in patients who died due to HF, suggesting that the disturbance of trans-endothelial transport may play certain role in the development of HF.

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Detection of apoptosis in myocardial infraction with the use of a single-stranded DNA (ss-DNA) monoclonal antibody.

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Background: Cardiac myocyte death during ischemic injury has been thought to occur exclusively by necrosis, but recently several studies have demonstrated that large numbers of myocytes undergo apoptosis in response to ischemic disorders. Apoptotic cells are usually identified by terminal deoxynucleotide transferase-mediated dUTP nick end-labeling (TUNEL), but recently, a single-stranded DNA (ss-DNA) monoclonal antibody has been reported to be sufficiently sensitive to detect apoptosis. The aim of this study was to investigate and quantify the presence of apoptosis in acute and chronic ischemic cardiac disorders.
Method: We studied myocardial samples of hearts with histologic findings of acute myocardial infraction (group A, n=20), old myocardial infraction (group B, n=20), and chronic ischemic heart disease (group C, n=20). Myocardial samples of normal heart were also included in this study (control group, n=10). An immunohistochemical method was performed with the use of anti-ss-DNA monoclonal antibody (mAb), in order to investigate the presence of apoptosis in ischemic cardiac disorders.
Results: In all cases of acute myocardial infraction (group A), the infracted area included extensive presence of both apoptosis and necrosis. In the tissue bordering on and away from the obviously infarcted areas, positive nuclei were intermingled with non stained normal myocytes. The number of positive nuclei decreased with the distance from the infraction foci. Stained nuclei were dispersed with intermingled normal cardiomyocytes, in salvaged areas at the old stage of infraction and in chronic ischemic disease. Absence of ss-DNA-positive cells was confirmed in normal control myocardium.
Conclusions: The results suggest that cardiomyocyte apoptosis in the border zone is responsible for cellular loss in the acute stage of myocardial infraction and may play a role in the evolution of the chronic heart failure in patients with old myocardial infraction and chronic ischemic disease.

P-482

Cardiac myxoma: new biomolecular insights for a classificatory re-consideration

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Background and methods. Cardiac myxoma is the most common primary cardiac tumour but its classification remains difficult, being retained of unknown origin. We used biomolecular investigation in conjunction with ancillary methods to a large series (n=30) of these tumours.
Results. Mixoma tissue was constituted from abundant hypocellular myxoid and oedematous matrix, resembling primordial cardiac jelly. Immunohistochemical investigation revealed that mixoma cells express -smooth muscle actin, whereas alpha-cardiac and alpha-skeletal actin isoforms were almost absent. Co-expression of CD34 and α-smooth muscle actin by double immunohistochemisty and confocal microscopy suggested loss of stem endothelial markers and the acquirement of myocytic antigens Early cardiac differentiation markers were also investigated by RT-PCR. We documented the presence of transcripts for Sox9 (100%), Notch1 (87.5%), NFATc1 (37.5%), Smad6, metalloproteinases 1 and 2 alone or in variable combinations and the absence of ErbB3 and WT1. Myxoma cells maintained phenotypic heterogeneity in vitro, including the expression of alpha-SMA and the presence of stress fibres.
Conclusions. These results strongly suggest the cardia myxoma cell exhibit a phenotype resembling embryonic endothelial-to-mesenchymal transformation preceding terminal differentiation of endocardial cushions.

Skin Pathology

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Basal Cell Carcinoma of Vulva

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Background. The basal cell carcinoma is the malignant neoplasm more prevalent in the world; nevertheless, its location in vulva is infrequent. **Objective.** To describe a case of basal cell carcinoma of the vulva from the Hospital Universitario de Santander. **Clinical case.** Female patient of 62 years old, that consults to display a lesion in mayor labia of 3 years of evolution. In the physical examination an ulcerated lesion of 2.5×1.2 cm, poorly delimited, with bleeding and necrosis was observed. A made skin ellipse excision, in the pathological study was recognized a basal cell carcinoma of vulva. The patient at the moment is asintomatic without displaying signs of recurrence of the disease. **Conclusions.** The basal cell carcinoma of vulva is a cancer little frequent, rare time is metastasizing but sometimes it's locally aggressive. One has been associated to numerous factors; nevertheless its etiologic is not

completely known. Its clinical presentation is varied and it does not specify, which makes difficult diagnose of precocious way. The election treatment is the surgical split with clean margins. The basal cell carcinoma presents foretell good but due to his rate of recurrence it is necessary to carry out periodic monitoring after the treatment

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ANNULAR ACQUIRED ELASTOTIC HEMANGIOMA TREATED WITH IMIQUIMOD.

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BACKGROUND Acquired Elastotic Hemangioma is a distinctive clinicopathological variant of hemangioma described by Requena et al in 2002. It seems to be an underrecognised entity. Because of the shortage of published cases, some dermatoscopic, clinical features or therapies of AEH are to be described. We describe an annular clinical presentation of AEH in a man treated with imiquimod. The dermatoscopic pattern of another case of AEH is presented.

METHOD Three cases of acquired elastotic hemangioma are described. The histological, clinical, and inmunohistochemical features are studied. Topical use of imiquimod 5% cream was used for the treatment of one case of AEH.

RESULTS Acquired elastotic hemangioma is a vascular proliferation that appears on sun-damaged skin of middle aged or elderly people. For the first time, to our knowledge, we describe a case in a man, and an annular clinical presentation of AEH. Dermatoscopy pattern of the other case located on the forehead of a woman shows generalised erythema, linear irregular vessels and white patches maybe due to fibrotic or elastolytic changes. We don't see typical vascular lagoons that we commonly see in vascular lesions as angiomas. One of the cases was treated with topical Imiquimod and the other two cases were followed up with no other treatment.

CONCLUSIONS Acquired elastotic hemangioma is a distinctive clinicopathological variant of acquired hemangioma. AEH is presented in the sun exposed skin of patients in the adulthood, not exclusively in women. The typical clinical presentation is an erythematous plaque. The first case of annular clinical presentation of AEH is described. The clinical differential diagnosis of annular lesions must include AEH. The dermatoscopic pattern of one case of AEH is described as generalised erythema, linear irregular vessels and white patches. Topical imiquimod could avoid surgical excisions after a punch biopsy of AEH.

P-485

Cutaneous Gamma-Delta T-cell Lymphoma arising in the setting of Behcet's disease

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Cutaneous Gamma-Delta T-cell lymphoma is an uncommon lymphoma composed of a clonal proliferation of mature activated gamma-delta T-cells expressing a cytotoxic phenotype. Malignant lymphoma is rarely associated with Behcet's disease as only 12 cases have been reported in literature including a case of cutaneous t-cell lymphoma. We report a new case of cutaneous gamma-delta T-cell lymphoma emerging in the course of Behcet's disease in a 40-year-old man. Diagnosis of cutaneous gamma-delta T-cell lymphoma was established based on the combination of clinical, histological, immunophenotypical and molecular findings. Through a review of the current literature, we analyze the unique clinicopathological, molecular and immunohistochemical features of this rare cutaneous lymphoma.

P-486

An inaugural association in skin tumours:melanoma and trichoblastoma: A new entity.

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INTRODUCTION: The coexistence of two neoplasms within a single cutaneous lesion is rather uncommon. The combination of malignant melanoma and basal cell carcinoma within a single tumour is an unusal finding. We report herein, the association of malignant melanoma and trichoblastoma that had never been reported yet at our knowledge.

CASE REPORT: we report a case of a melanoma colliding with a trichoblastoma on the head of a 69 year old north african man, the patient had a prior history of multiple trichoblastomas on the scalp. He presented with 3/2/1 cm purple, black firm exophytic and eroded mass on the scalp. He had surgical excision. Histologically, the tumour presented a dual pattern of trichoblastoma intermingled with typical features of malignant melanoma..Immunohistochemical studies for cytokeratin highlited the epithelial componement and studies for S100 protein and HMB45 confirmed the melanocytic componement.

To our knowledge, this is the first description of melanoma with non pigmented trichoblastoma. The diffrential diagnosis, pathogenesis and prognosis of this collision tumour are discussed.

P-487

Nodular form of Kaposi sarcoma.

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Hereby we report a case of a 67 years old female. Examined skin biopsy from upper thigh of patient with negative anamnesis. The lesion had ovoid-nodular borders growth patern, without encapsulation (4×3 mm) formed with spindle elements, with high mitotic and proliferation aktivity.

Kaposi's sarcoma (KS) is an aggressive localized tumor of endothelial cells (including blood and lymph vessels) that is characterised by the multifocal skin lesions. Mucous membrane, lymphatic glands and internal organs might be affected as well. While KS is classified as tumorigenic, it is not very well understood whether it occurs as a monoclonal proliferation of a single malignant cell or whether it is of a polyclonal, hyperplastic-reactive origin. The results of several studies indicate that at the onset of the disease is an altered hyperplastic-recative cell that further proliferate. The disease is typically described in association with HHV-8 (human herpes virus-8) infection and several genetic abnormalities including gain of 8q and 1q, loss of 3p and 7q22, 8p11, 13q11 and 19q13 rearrangements. However, the data obtained from either earlier or later stages utilizing comparative genomic hybridization remain controversial. Here, we show in our case monosomy of chromosome 17 utilizing FISH method on paraffin sections. Interestingly, we did not observe the previously reported abberations of gain of 1q and 3q associated with diagnosed KS.

P-488

A SERIES OF 10 CASES OF PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE, & THEIR RELATION TO EBV

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Background: In general, primary cutaneous B-cell lymphomas present an indolent course, but a subtype having a worse prognosis and specific immunophenotypical characteristics has been recognized by the EORTC. This subtype is known as diffuse large b-cell lymphoma, leg type, and it predominantly affects the inferior extremities of the elderly. It is made up of sheets of large lymphoid cells with rounded nuclei and prominent nucleoli, similar to centroblasts or immunoblasts. In an intense manner, cells express Bcl-2, Bcl-6 and Mum-1. They do not express CD30. We present here a peculiar case of this type of lymphoma in which the expression of CD30 was observed to coincide with infection by EBV. We carried out a study on a series of 10 cases. In the literature, very few similar cases have been cited, and it is unknown if a relationship between EBV and the physiopathology of this entity exists or if this is only a chance discovery.

Material and methods: We selected 10 cases with a diagnosis of primary cutaneous diffuse large B-cell lymphoma, leg type, which were reviewed from morphological, immunohistochemical, molecular and cytogenetic perspectives by two independent pathologists. After conducting a study using *in-situ* hybridization, we found the expression of CD30, along with the presence of RNA for EBV, in this series.

Results and Conclusions: We observed the coincidence of the expression of CD30 and the presence of RNA for EBV in the tumoral cells of only one of the 10 cases studied. The similarity of this case, in particular, with the case described in the Literature leads us to suspect that a relationship, not yet studied, exists between both variables. In order to prove this, perhaps it would be necessary to increase the number of cases in the series for study.

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P-489

ROLE OF STEROID RECEPTORS IN BIOLOGICAL BEHAVIOUR OF CUTANEOUS MELANOMA

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Background: In the last two decades, the incidence of skin cancers and, in particular of cutaneous malignant melanoma (CMM), has shown a progressive increase. Recent improvement of diagnostic techniques have lead us to diagnose most CMM at an early stage: nevertheless, the death rates from CMM have not yet significantly decrease. The traditional clinical and pathological parameters are unable to provide a precise outcome prevision. For this reason, attempts continue to be made to find new molecular prognostic markers for this tumour. Experimental and clinical evidence indicate that sex hormones have a pivotal role in the growth of several type of human cancers.

The data existing about the relationship between the biological behaviour of CMM and sex hormones are conflicting. Epidemiological data indicate that melanomas metastasize with higher frequency in male than in female, and that the survival after metastasis is longer in female than in male patients.

The aim of this study was to analyze whether sex hormones (oestrogen, progesterone and androgens) could be have a significant role in determining CMM growth.

Methods: The study was performed by immunohistochemistry: oestrogen receptor (ER)-alpha, ER-beta, progesterone receptor and androgen receptor (AR) expression was evaluated on a selected series of CMM (formalin-fixed, paraffin-embedded tissue).

Results: The results were correlated with the clinical and pathological data and with patient's outcome. In addition, the findings were correlated with the age-group category of patients (young patients: 0–44 years versus older patients) and, for female, with pre-menopausal, post-menopausal, or pregnancy state.

Conclusion: The significance of the different expression of steroid receptors in our series of CMM was discussed. Moreover, consideration about the use of steroids as therapeutic targets in the treatment of CMM was also made.

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GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GAPDH) AND GROWTH ARREST PROTEIN 7 (GAS7): TWO NOVEL CANDIDATE GENES INVOLVED IN THE PROGRESSION OF CUTANEOUS MALIGNANT MELANOMAS

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Background: The glycolitic function of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been widely reported in nearly all cell types. However, the non-glycolitic role of this molecule has been recently described with prognostic importance in diverse tumor models such as breast and renal cancer, and, interestingly, also in melanoma patients. Otherwise, the biological role of growth arrest protein 7 (GAS7) as a tumor suppressor gene or as an immunomodulator has been only elucidated in central nervous system neoplasms and in malignant cutaneous melanomas.

Methods: A molecular and immunohistochemical study was carried out in a series of 74 melanoma patients including primary (< 1 mm, 15 patients; and ≥ 1 mm lesions, 16 patients) and metastatic conditions (in-transit, lymph node and organ metastases; 17, 13 and 13 patients, respectively). GAPDH and GAS7 gene expression was evaluated by means of immunohistochemistry (protein) and quantified by Real Time PCR (mRNA). For the immunohistochemical study, a tissue microarray was constructed and each case was analyzed by triplicate. Additionally, the presence of point mutations in specific exons within these two genes (codon 175 in GAPDH and codon 225 in GAS7) was analyzed by using a c-DNA sequenciation methodology. To determine the statistical correlation between variables and the distinct melanoma groups, univariate statistical tests were utilized (chi-square, ANOVA and Fisher's tests).

Results: In univariate statistical studies, a remarkable loss of GAPDH and GAS7 m-RNA expression levels demonstrated the strongest statistical differences between primary and metastatic melanomas ($p<0.05$). This finding was also corroborated at the immunohistochemical level. Unfortunately, point mutations in specific exons were not encountered.

Conclusion: Differences in GAPDH and GAS7 gene expression levels in human malignant cutaneous melanomas may act as the trigger changes leading tumor cells through the final phases of the disease.

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Podoplanin expression in tumor cells of canine mastocytoma. A case report

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Background. Mastocytoma is a frequent tumor in dogs and rare in human. In the dog, mastocytoma represents between 9 and 15% from all tumors of the skin. The histochemical and immunohistochemical features are highly specific for the diagnosis, but the molecular profile is not completely characterized. Podoplanin is a well known marker of the lymphatic endothelium, but is not entirely specific. Its expression was found in some malignant human tumors and it was not reported in animal tumors. The present case report shows a multiple mastocytoma with tumor cells with expression of podoplanin. Case report. A 9 years old dog was admitted with four tumors, located in the skin of the nose and abdomen. One ulcerated tumor from the nose and one non-ukcerated from the abdomen were removed and specimens were fixed and embedded in paraffin. Sections were stained with haematoxylin-eosin, toluidine blue, alcian blue-safranin pH1.42, mast cell tryptase, CD117, and podoplanin (clone 18H5).Results. A diffuse proliferation with medium sze polygonal shaped cells was found in the dermis, extended to the subcutaneous tissue. Nuclear atypia were moderate and rare mitotic figures were found. All tumor cells were positive for toluidine blue and alcian blue-safranin. The diagnosis was confirmed by immunohistochemistry with mast cell tryptase and CD117. All tumor cells were positive with strong of moderate intensity for podoplanin, with diffuse cytoplasmic.pattern. There were not found differences in the expression of the markers used in the study between the two specimens.Conclusion. It is reported a case of a multiple canine mastocytoma with tumor cells expressing podoplanin. To the best of our knowledge, it is the first report on podoplanin expression in mastocytoma tumor cells.

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Syringocystadenoma papilliferum with features of proliferating trichilemmal tumor

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Syringocystadenoma papilliferum is an uncommon benign tumor of an apocrine type appendages. It usually arises upon nevus sebaseus. This case is an unusual one with dense keratinization of trichilemmal type and inwards of squamous epithelium looking as nests of squamous cells simulating squamous cell carcinoma.

P-493

Masson's Hemangioma: A Lesion Often Mistaken for Angiosarcoma

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Intravascular papillary endothelial hyperplasia (Masson's hemangioma) is an unusual benign, non-neoplastic, vascular lesion characterized histologically by papillary fronds lined by proliferating endothelium. The main significance of intravascular papillary endothelial hyperplasia is its clinical and histological resemblance to soft-tissue sarcoma and possible misinterpretation as such. An otherwise healthy 52-year-old white man had nodular lesion of the foot. Pathologic analysis demonstrated the mass to be a Masson's hemangioma, a papillary proliferation of thin-walled capillaries intimately associated with thrombus. Considered a benign intravascular lesion, the treatment of choice is complete excision. The lesion has a propensity to occur in the head, neck, fingers, and trunk. There is only one case in the literature, described this lesion on the foot. In 1923, Masson regarded this disease as a neoplasm inducing endothelial proliferation, however, now it is considered to be a reactive vascular proliferation following traumatic vascular stasis. The recognition of the morphologic features of this lesion and its inclusion in the differential diagnosis of vascular mammary tumors will reduce the likelihood of its misdiagnosis as an angiosarcoma and avoid unnecessary and aggressive therapy.

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Histopathological spectrum of Grover's disease: a review of 52 cases

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Background: Grover's disease ("transient acantholytic dermatosis") is a dermatosis of unknown cause, characterized microscopically by acantholisis that frequently is associated with dyskeratosis of individual keratinocytes. Histopathologically, the lesions tend to adopt features

similar to pemphigus (P), Darier's disease (D), Hailey-Hailey's disease (H-H) and spongiotic (E). A superficial lymphohistiocytic infiltrate is usually present and eosinophils are a common finding.
Method: Microscopical evaluation of 52 cases of Grover's disease.
Results: In 28 cases, the lesions involved ≥2 mm of the epidermal suface. The patterns observed where D: 25, P:24, E:12 and H-H:7. In 14 cases there was coexistence of more than one pattern. In two cases the infiltrate extended deeply into the dermis and in most biopsy specimens (43) eosinophils where found in significant amounts. Neutrophils where also common at the base of the lesions or forming subcorneal pustules (42) and in 35 cases vasculopathic changes such as endothelial tumefaction, erythrocyte extravasation or iron deposition where identified.
Conclusions: In our study the lesions tended to involve wider areas of the epidermal surface than is stated in the literature and we frequently found hints of vascular damage and neutrophilic infiltrate. These findings that have been overlooked in previous studies.

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Superficial acral fibromyxoma. A review of five cases

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BACKROUND: Superficial acral fibromyxoma (SAFM)is a rare soft tissue tumour which has recently been described by Fetsch, as aproliferation of spindle/stellate cells embedded in a predominantly myxoidmatrix with accentuated microvasculature. METHOD: Five resected cases of SAFMwere reviewed from our files (2003 to 2007). Collected clinicopathologic datawere: sex, age, location, visit's reason, cellular type, atypia, mitosis count,stroma, other histological findings, immunoreactivitiy (CD34, vimentin, CD99,EMA, S-100 protein and muscle specific actin) and surgical borders. RESULTS: There were four men and one woman (age range:28 to 73 years). Toe was common affected site. Reasons for visit were nodulararappearance in three patients, cystic lesion and posttraumatic onychogryphosisin others. All cases had proliferation of spindle cells, increase number ofblood vessels and mast cells. Three showed myxoid stroma and two myxoid andcollagenous matrix. Tumour cells had slight nuclear atypia in two cases andfocally moderate in one case. Mitotic figures were infrequent or inexistent.All cases showed immunoreactivity for CD34 and vimentin, and negativity forS-100 protein and muscle specific actin (HHF35); three stained for CD99 and twostained weak focal for EMA. Surgical borders were invaluable in two, affectedin other two and free in last one. No tumour recurrence appeared (follow-up 53to 2 months). CONCLUSION: SAFM represents a new entity in spectrum of softtissue tumours, including myxoid and mesenchymal neoplasms. Moreover, the widerange of appearances required making a differential diagnosis with fibroushistiocytoma, dermatofibrosarcoma protuberans, superficial angiomyxoma,acquired fibrokeratoma, sclerosing perineurioma, acral myxoinflammatoryfibroblastic sarcoma, digital myxoma, ossifying fibromyxoid tumour, low-grade myxofibrosarcoma,myxoid neurotheketoma, myxoid neurofibroma and solitary fibrous tumour.

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TYROSINASE A USEFUL MARKER FOR IDENTIFYING PAGETOID INVASION IN MALIGNANT MELANOMA

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Background: In most of the cases, malignant melanoma (MM) is diagnosed based on hematoxilin-eozin (H&E) appearance, supplemental investigations being performed in a minority of cases. We address our study to differentiation between particular types of nevocellular naevi (predominantly junctional, lentiginous or displastic) and MM. In these cases, one of the clues of differentiation is the presence of melanoma cells within the superficial layers of the epidermis (pagetoid growth). This feature is not always easy to interpret on H&E, immunohistochemical stains being necessary.
Method: We studied 23 cases of thin MM (nontumorigenic MM with Breslow score less than 1 mm). We analyzed the presence of pagetoid growth in consecutive slides stained with H&E, S100 protein, HMB45, tyrosinase and TRP2. The number of the invading melanoma cells was counted in each high power field, the highest figure being attributed as the score for each slide. The scores recorded in immunohistochemical stains were compared with those recorded in H&E for the same case. Statistical analysis was performed using EXCEL and EPIINFO programs (statistical significance $P<0.05$).

Results: The presence of epidermal invasive melanoma cells was noticed in all but 3 cases in H&E stains. The extent of pagetoid invasion revealed by S100 protein and H&E was similar. HMB45 was intense positive in all the cases with slightly more numerous intraepidermal invasive cells comparing with H&E (statistically unsignificant). TRP2 showed slightly more numerous cells in pagetoid growth than HMB45 but not significant comparing with H&E. Tyrosinase highlighted most numerous pagetoid spreading cells, the differences being statistically significant comparing both with HMB45 and H&E.
Conclusion: Tyrosinase is a useful marker for pagetoid invasion comparing with other immunohistochemical markers or H&E, helping in differentiating MM form naevi with prominent intraepidermal compartment.
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Multicentric reticulohistiocytosis. Report of a case presenting with clinical features of dermatomyositis.

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Background: Multicentric reticulohistiocytosis (MRH) is rare disease of unknown etiology and probable immunologic basis, characterized by a skin papulonodular eruption. This disorder often courses with destructive polyarthritis, and can be associated with other diseases and malignancy. The clinical presentation of MRH mimicking dermatomyositis has been reported previously.
Case Report: A 72 year man presented with muscular weakness and skin lesions for six month. The skin lesions were confluent papules and nodules in the extension areas of both hands, fingers, elbows and knees. Moreover, small periungual nodules (coral beads) and in both ears were seen. Muscular biopsy was normal. The biopsies of two of these skin lesions showed a superficial and deep dermal infiltration of histiocytes, some of them multinucleated, with large eosinophilic, granular cytoplasm with ground-glass appearance. These histologic characteristics were diagnostic of MRH. The patient had also arthritis in both knees. Screening for neoplasms was negative.
Conclusion: MRH can present with clinical features of dermatomyositis. Our case emphasizes the usefulness of histologic study of the skin lesions to differentiate both conditions and perform a correct diagnosis and treatment.

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Clinico-pathological findings in a series of cutaneous sea-urchin granuloma

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Background:Injuries cause by sea-urchin spines are only rarely reported in the medical literature. They occur usually in the hands and feet and tend to heal after forced or spontaneous extrusion of most of the foreign material. Rarely, papules or small nodules appear after some weeks in the sites of injury, and tend to persist unless treatment, usually surgery, is applied. Histologically, they show granulomatous inflammation, classically described as sarcoid-type.Results:We have retrospectively evaluated 23 skin biopsies from 20 patients (16 males, 4 females, ages ranging from 23 to 68 years) with the diagnosis of sea-urchin granuloma and definite antecedent of sea urchin injury. Most lesions were solitary and located in the dorsum of the hands (8 cases), fingers (7 cases) and forearm (4 cases). Intervals from puncture ranged from 2 weeks to three years, and it was usually comprised between 1 and 6 months (9 cases). The lesions were most commonly described as nodules (13 cases).Histopathological examination revealed the presence of granulomas in all but one case. In 14 out of the 23 biopsies, the inflammation was predominantly granulomatous. Sarcoid granulomas were the predominant inflammation pattern (11 cases) but suppurative and necrobiotic granulomas were also common. In many cases, more than one type of granuloma could be seen. In 4 cases, acute or chronic, non-granulomatous, inflammation was the predominant finding. Surgery was the elective treatment in all cases and antibiotics were added in four.Conclusion:Sea-urchin granuloma encompasses a wide morphological spectrum of granulomatous reaction involving the dermis and the subcutaneous fat tissue. Histopathological findings are not specific and mixed patterns of inflammation are common. Documentation of previous sea-urchin injury and clinico-pathological correlation are necessary for the diagnosis.

P-499

Malignant proliferating trichilemmal tumour of the scalp: Report of a case and review of the literature.

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Bakckground: Malignant proliferating trichilemmal tumour is a rare skin tumour that originates from the root sheath of the hair. It mimicks poorly differential squamous cell carcinoma ant its biological behaviour is unpredictable, because rarely can produce distant metastases.
Methods
Results: we report on a case of a malignant proliferating trichilemmal tumour of the scalp in a 76-year-old man and we discuss the clinicopathologic features of this entity.
Results: Our patient proceeded with a cystic lesion in the scalp measuring 8 cm. The lesion was removed with wide excision. The histological examination of the lesion revealed a malignant proliferating trichilemmal tumour. Eleven months after the excison, the patients is free of disease.
Conclusion: Malignant proliferating trichilemmal tumours are rare malignant lesions that affect mainly older women. The appropriate treatment includes wide resection and dose postoperative follow-up of the patient to facilitate the early diagnosis of distant metastases.

P-500

intravascular lobular capillary hemangioma: a rare intravascular tumor (report of two cases)

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BACKGROUND Lobular capillary hemangioma (LCH), also known as pyogenic granuloma is a frecuent benign vascular tumor. Intravascular lobular capillary hemangioma (ILCH) is a rare form of LCH in which the angiomatous proliferation is confined within a lumen of a vein.
METHOD We report 2 cases of ILCH involving the periophthalmic region in two male patiets (25 and 65 years old).
RESULTS The two lesions are similar and consist of an intraluminal polyp attached to the vein wall and composed of lobules of capillaries lined by flattened or rounded endothelial cells and separated by a fibromyxoid stroma. Angiomatous lobules usually can be demostrable in the adjacent venous media.
CONCLUSION ILCH was described by Cooper et al. in 1979. They described 18 cases, all of which arose in veins of the superficial soft tissues in the neck or upper limbs of young or middle aged adults. Since then only 4 cases have been described in the periocular adnexa region. Distinction from other primary intravascular neoplasm like intravascular papillary endothelial hyperplasia (Masson's tumor), malignant endovascular papillary angioendothelioma (Dabska tumor) and epithelioid hemangioendothelioma is straightforward.
ILCH is a benign lesion that is usually cured by local excision.

P-501

CXCR2 Expression and Progression of Cutaneous Melanoma

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BACKGROUND: Incidence of cutaneous malignant melanoma (MM) has been increasing worldwide in the last decades. There are evidences supporting a role for chemokines and their receptors in MM progression and metastasis. In this regard, expression of CXCR1 and CXCR2 receptors in MM has been suggested to be involved in vessel density, aggressiveness and metastatic potential.
METHODS: We examined the expression of CXCR2 in human MM by inmunohistochemical analysis of 71 tumors on tissue arrays from formalin-fixed, parafin-embebed specimens including 30 primary cutaneous MM: 14 thin, and 16 thick; and 41 metastatic MM: 16 in transit metastases, 12 metastases in regional lymph nodes, ans 13 distant metastases. A histoscore was established to evaluate the intensity of the immunostaining and the percentage of positive tumor cells.
RESULTS: Comparative analysis of the expression between the different groups of tumors yielded the following results:

- CXCR2 expression was lower in thin than in thick MM.
- The expression of this receptor was higher in "in transit" metastases than in thin primary tumors.
- A lower expression of CXCR2 was found in lymph node and distant metastases compared to primary MM and "in transit" metastases.

CONCLUSIONS: Differences on CXCR2 expression between thin and thick primary MM may reflect differences in metastatic potential between both groups. Our results support the implication of CXCR2 overexpression in cutaneous "in transit" metastases of MM, but not in lymph node and distant metastases.

P-502

Nevoid basal cell carcinoma syndrome- the importance of early diagnosis and proper treatment

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Background: Nevoid basal cell carcinoma syndrome (NBCCS) is a rare autosomal dominant disorder, caused by mutation of a tumour supressor gene (PTCH) on chromosome 9q22.3-q31. It is characterized by multiple basal cell carcinoma (BCC) of the skin, odontogenic keratocysts, skeletal anomalies, ectopic calcification and a predisposition of additional neoplastic processes.

Methods: We are presenting 30-year-old male, mentally retarted, with NBCCS, who had undergone resection of multiple BCC of his forehead 6 years earlier. Also facial dismorphisms, as flat occipital area, broad forehead, deep set eyeballs, hypertelorism, epicanthus, broad nasal root and tip, thin upper lip and tooth anomalies, had been observed. Three years later BCC of the forehead and a new tumour mass at the left mandibular angle had occured. CT scan had revealed destruction of the frontal bone with tumour invasion into the frontal lobe of the brain. Irradiation therapy had achieved significant improvement. After two years he had presented with metastases of BCC, confirmed by fine-needle biopsy, in the right submandibular and preauricular area, that had been successfully treated by irradiation therapy. At current examination, he was hospitalized due to massive haemoptysis and died the same day after admission.

Results: Autopsy macroscopically revealed extensive destruction of frontal and ethmonasal bones by tumorous mass, that was observed also in the right submandibular region. As well, lamellar calcification of the falx cerebri and palmo-plantar pits were detected. On the trunk and extremities numerous exophytic masses were presented. Histology showed numerous BCC metastases in the lungs, mediastinal lymph nodes, liver and spleen. The observed lesions of the skin were confirmed as BCC or epidermoid cysts.

Conclusions: BCC occurence in young person other than in NBCCS is exceptional. The diagnosis, carefull monitoring and proper treatment, such as surgical wide excision is mandatory. As well, avoiding sun exposure and radiation is essential.

P-503

Mesenchymal tumour in dermis

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Background: Dermatomyofibroma is a distinct clinicpathological entity into the group of mesenchymal cutaneous neoplasias with myofibroblastic differentiation. It is frequently found in young women, in the region of the shoulder and also in the upper trunk.

Case report: We present the case of a 13 year-old female with an acquired lesion in presternal skin, with no traumatic antecedents. A sample of skin is received,which has a superficial, lightly raised, greyish lesion of 5×4 mm in its great dimension.

Results: Histologically, a proliferation in upper dermis is observed, formed by cells with ill-defined citoplasm and oval nucleus with no atipia; these cells are characteristically arranged in bundles and fascicles parallel to the skin surface. Immunohistochemically, cells express vimentin and smooth muscle actin and are negative for desmin, CD 34, S100 and epithelial markers. Diagnosis is dermatomyofibroma.

Comments: Dermatomyofibromas are benign mesenchymal cutaneous tumours with myofibroblastic differentiation, with a typical arrangement of fascicles parallel to skin surface. This entity was first described by Kamino et al. in 1992 with this name and previously in 1991 by Hügel as "dermic fibromatosis plaque-like". Less than 60 cases of dermatomyofibroma have been reported, mostly in adult women. Differential diagnosis includes hypertrophic scar, dermatofibroma, pilar leiomioma, neurofibroma, adult myofibromatosis, extraabdominal fibromatosis and dermatofibrosarcoma protuberans plaque-stage. For the latter, the immunohistochemical pattern helps; our case is positive for smooth muscle actin and vimentin and negative for factor XIIIa, CD 34 and S100. As for behaviour, they are benign tumours that usually do not recur.

P-504

Metastatic renal cell carcinoma: an able mimicker of a wide range of skin lesions

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Background: Cutaneous and mucosal metastases from renal cell carcinoma are uncommon, although in some cases they

can be the first manifestation of the disease, causing serious problems of differential diagnosis due to their similitude to certain dermatological neoplasms.
Method: The clinicopathological characteristics of four patients with skin metastasis at the time of presentation are reviewed.
Results: All patients were males and their ages ranged from 43 to 74 years. One case presented with multiple papules on the trunk with microscopical appearance similar to a sweat gland carcinoma. The other three lesions were solitary and located in the tongue, thorax and foot. The lingual lesion was composed of nests of spidle and epithelioid cells that showed focal epitheliotropism simulating a melanoma. The other two ones simulated clear cell sweat gland tumors.
Conclusion: Cutanous metastatic renal cell carcinoma is an able mimicker of a wide range of primary skin neoplasms. It is important to keep in mind this option in the differential diagnosis of clear cell, spindle cell and ductal tumors. In suspicious cases, a panel of immunohistochemical markers and ultrastructural studies can be helpful to rule out or confirm this possibility.

P-505

MERKEL CELL CARCINOMA OF THE SKIN STAINING PATTERN

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Background Merkel cell carcinoma (MCC) is an unusual and aggressive primary neuroendocrine carcinoma of the skin, that mainly affects sun-exposed areas of elderly people. They recurre locally in 22–46% of patients, have regional metastases in up to 75% of patients, and have a five year survival between 30 and 64%. The aim is to determine an immunohistochemical pannel to allow distinguishing MMCs from other small round blue cell tumours and to study plausible of the prognostic markers.
Method Six cases and 2 recurrences of MCCs were identified over the last 8 years in a retrospective analysis of the cutaneous tumour registry from our hospital. Paraffin sections were stained for chromogranine, CK20, BCL-2, TTF-1, C-KIT, P53, P16, Ki67, EGFR and CD99.
Results Reactivity with chromogranine, BCL-2 and P16 was demostrated in all cases, as well as CK20. The staining patterns for CK20 ranged from punctate (perinuclear) to localized or diffuse. No one expressed nor TTF-1 neither CD99. C-KIT and EGFR were focally positive in 2 cases. P53 was positive in 3 cases in more than 80% of neoplastic cells. Ki67 was expressed in more than 35% of cells in 4 cases and in less than 10% in 2 cases.
Conclusion We note that the classically described perinuclear dotlike keratin staining pattern is the predominant but not the only one. We differ from the literature that nor the majority neither one third of MMCs express C-KIT and CD99 respectively. Universal staining for P16 can be used for diagnostic purpose and it raises the possibility of an HPV relation. Positive staining for EGFR could predict sensitivity for specific treatment. Both the positive and negative elements of the staining profile of MCCs provide additional useful diagnostic information for the differential diagnosis between MCC and other carcinomas that may simulate it.

P-506

Combined malignant melanoma and basal cell carcinoma tumor of the intermingled type: case report and rewiew of the literature

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Background: The coexistence of two different malignant skin tumors in the same histologic specimen is an unusual finding.
Case Report: A 76-year-old man with a nodular tumor on the front showing a combination of malignant melanoma (MM) and basal cell carcinoma (BCC).
Results: A 1,2 cm. nodular non-pigmented lesion on the front was considered clinically as BCC or epidermal appendages tumor. Histologic examination showed a nodular tumor consisting of a BCC nodulocystic variant and a nodular MM located adjacent as well as admixed within the epitelium of the BCC. The MM showed no junctional component and measured 6 mm in thickness.The diagnosis of the two neoplasms were confirmed by immunohistochemical studies: BCC cells showed a keratinocytic phenotype (cytokeratins, p63) and tumor cells of the MM a melanocytic phenotype (HMB-45, Melan-A, S100 protein).
Conclusion: The combination of MM and BCC in a single tumor is extremely rare and a chance occurrence. These tumors were described as combined, colliding, basomelanocytic, biphasic, contiguous or even parasitic or metastasic. The few cases reported were located on the upper part of the body, mainly in the trunk/back and are more frequent in male being the mean age of 61 years. The clinical picture is rather variable and does not point to the diagnosis. There are two variants: a collision type in which components of each cell type are clearly demarcated and an intermingled type in which both cell types grow intimately together almost on a single-cell level.

P-507

Cutaneous reactive intravascular histiocytosis

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BACKGROUND Reactive intravascular histiocytosis is a rare entity, of unclear pathogenesis. It has a broad clinicopathologic spectrum, and some cases have been associated with rheumatoid arthritis and other systemic diseases. It has been related to early stages of reactive angioendotheliomatosis.

CASE REPORT A 60 year-old man presented with asymptomatic thoracic cutaneous lesions. They were erythematous and reticulated papules, some of them with purpuric appearance, clinically suggestive of a vascular lesion. A complete clinical study ruled out any associated disease. Two biopsies of these lesions were performed and both showed similar histological characteristics. In the papillary and reticular dermis irregular dilated vascular structures were seen. In the lumina of these vessels there were mononuclear cells with abundant cytoplasm, of histiocytic appearance, without atypical cytologic features. These cells were positive for CD68 and CD31. Other tested markers (CD1a, S-100, CD45, CD20, CD79a, CD3 and CD34) were negative.

CONCLUSION The cutaneous reactive intravascular histiocytosis is a benign process that can present without any other condition, and probably represents an initial phase of reactive angioendotheliomatosis. The differential diagnoses include benign and malignant vascular lesions, and intravascular large B-cell lymphoma, which has an aggresive clinical course.

P-508

Prognostic relevance of mitotic rate in patients with melanoma

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Background Interest in the prognostic clinical relevance of biological variables has focused on a small number of factors, and among the different markers, cell proliferation can reasonably be supposed to be related to tumor aggressiveness. Recently, a renewed interest was witnessed in one of the proliferation markers first used, the mitotic rate, which is the fraction of cells in mitosis at any given time. The aim of this study was to evaluate the prognostic value of mitotic activity and the relationship with overall survival.

Method The prognostic relevance of mitotic rate was analyzed in a series of 49 patients with histologically confirmed cutaneous melanoma. Mitotic figures were counted in areas selected on the basis of the following criteria: presence of good cellularity and high density of mitotic figures.

Results The mitotic rate was categorized in 3 groups: $1/mm^2$, $1–4/mm^2$, and $>4/mm^2$ and was significantly related to 5-year overall survival (95%, 91% and 74%). In conclusion, a high number of mitotic figures is associated with a higher probability of developing distant metastases and a shorter survival. Our study shows that mitotic activity is an independent prognostic factor, possibly even more important than other biomarkers known.

P-509

Hypertrophic scars arising at the biopsy site of melanocytic lesions

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Background: Hypertrophic scar (HS) results from an abnormal response to wounding and has been noted after a variety of local injuries. Hypertrophic scars have not been reported after biopsy of melanocytic lesions, one possible explanation being that such lesions are not biopsied in routine clinical practice. Herein, we report two cases of HS arising after biopsy of melanocytic lesions, located at the epidermis. The aim of this study was to investigate their histopathological features and pathogenesis.

Method: The patients, one male, 25-years-old, and one female, 61-years-old, underwent a 4 mm punch biopsy. The diagnoses were dysplastic junctional naevus, and lentigo maligna, respectively. After approximately two months the lesions were excised. The specimens were fixed in buffered formalin and processed for routine histopathological study. Immunostaining for vimentin, α-SMA, desmin, CD68, CD3, CD1-α, tryptase, S-100 and Ki-67 (MIB-1 clone) was also performed.

Results: In both lesions, at the area of previous biopsy, histopathological features consistent with HS were noted. These included flattening and slight acanthosis of the overlying epidermis, scarring of the papillary dermis, prominent vertically oriented blood vessels and nodular aggregates of collagen with fibroblasts. The scar remained within the boundaries of the previous biopsy. The lesional cells expressed vimentin and focally α-SMA, consistent

with their fibroblastic/myofibroblastic nature. There was no increase in the number of macrophages and T-lymphocytes, while an increase in Langerhans and mast cells was noted. Interestingly, the number of basal keratinocytes within the cell cycle (as indicated by MIB-1 immunostaining) was increased.

Conclusion: Hypertrophic scars can develop after biopsy of melanocytic lesions that do not involve the dermis, indicating that the pathogenetic mechanism is more complex than isolated dermal phenomena. The persistence of activated keratinocytes in HS epidermis indicates that epidermal processes may stimulate dermal changes. Langerhans and mast cells might be mediators in this process.

P-510

THE CORRELATION OF P53 AND P63 EXPRESSIONS WITH HISTOPATHOLOGIC SUBTYPE AND NUCLEAR MORPHOMETRIC FEATURES IN BASAL CELL CARCINOMAS

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Background: Basal cell carcinomas usually have low recurrence rate and very low metastatic potential. However, tumors with aggressive course may cause tissue destruction as well as high recurrence and metastatic rate, eventually leading to death. Since histopathological subtyping can be inadequate in determining tumor aggressiveness, immunohistochemical and morphometric parameters have also been considered. The aim of this study is to establish histopathological subtypes of basal cell carcinoma, immunohistochemical expressions of p53 and p63, and morphometric features of tumor cell nuclei by computer-assisted analysis system and eventually investigate the correlations between these parameters.

Method: 100 tumors from 92 cases, which had been diagnosed as primary basal cell carcinoma from 2001–2007 in the Pathology Department of Zonguldak Karaelmas University School of Medicine were included. The study group consisted of 92 patients, 47 male and 45 female, with an age range between 38 and 88 (average 64.34). The aggressiveness and histopathological subtypes of tumors were ascertained and p53 and p63 immunohistochemical stains were performed. About 100 nuclei with sharply demarcated contours were also included for morphometric analysis in all cases. Statistical analysis was performed; however since most tumors were nodular type and numbers of the cases with other subtypes were inadequate, correlation between histopathological subtypes and the other parameters could not be investigated by statistical analysis.

Results: Correlation between p53 and p63 staining intensity and the nuclear morphometric parameters as well as tumor aggressiveness were not statistically significant. There was a significant correlation between age and p53 staining intensity ($F=3.54$, $p=0.017$).

Conclusion: Larger series are to be studied to establish the precise correlations, if any, between the stated parameters. Nevertheless, the correlation between age and p53 staining intensity probably denoting the pathogenesis of basal cell carcinoma is noteworthy.

Key words: Basal cell carcinoma, immunohistochemistry, p53, p63, nuclear morphometry

P-511

Cutaneous manifestations of cowden syndrome

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Background: Cowden disease, also known as multiple hamartoma syndrome, is a rare autosomal dominant genodermatosis, with variable expression, that results from a mutation in the PTEN tumour suppressor gene. It is associated with multiple cutaneous lesions and neoplasias or hamartomas in internal organs, which especially develop in gastro-intestinal tract, thyroid and breast.

Case report: A 40 year-old male presented with small cutaneous papules in the face and upper trunk. These lesions had a smooth surface or were verrucoid, as well. In palms and soles showed traslucent punctate keratoses. The patient had a past history of testicular seminoma at the age of 20 and thyroid goiter. Few months ago also presented several polyps in esophagus, stomach and colon.

The histological study of four skin lesions were perfomed. They were trichilemmoma, an inverted follicular keratosis, a seborreic keratosis and a sclerotic fibroma.

Conclusion: In Cowden syndrome the cutaneous signs may be diverse but allow early recognition of the disease. It is important to suspect the syndrome in order to rule-out internal malignancies and perform a familial screening.

P-512

Howitt and pelisse syndrome with stenosis of vagin: A case report

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Hewitt and pelisse syndrome with stenosis of vagin: A case report
Vulvovaginal-gingival lichen planus is a distinctive erosive form of lichen planus, it is clinically very similar to mucous membran pemphigoid.
Vulvovginal ginginal lichen plans principally affect the inner aspect of labia minore, vestibula and gingival,servix may also be involved and oral lichen plan can occur. The condition is chronic and painful. Vaginal synechia and adhesion develops and leading in some cases to vaginal stenosis The manifestation of this syndrome do not necessarily all occur synchronously.
We report tow cases that had this syndrome with stenosis of vagin and oral lichen plan and gingivitis.

P-513

Langerhans cell histiocytosis of the adult presenting as a localized cutaneous lesion. A report of six cases

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Background: Most frequently Langerhans cell histiocytosis (LCH) presents as a multisystemic disorder of young patients. Since the introduction of immunohistochemistry atypical clinicopathologic forms of LCH have been described. Among them, we would like to highlight its presentation as a single cutaneous lesion in adults. In these cases there is no clinical suspicion and the diagnosis is based on the pathologic study.
Methods: We reviewed pathologic and clinical data of six patients with LCH that presented as a single cutaneous lesion with no evidence of extracutaneous involvement.
Results: Patients age varied from 24 to 67 years. Lesions were located in the arm (3), trunk (2) and genitalia (1). Clinically they were regarded as epitheliomas (2) or non-specific tumoral lesions. Histology was similar in five cases and consisted of a polymorphous dermal infiltrate of eosinophils, lymphoid and histiocytic cells. The latter showed eosinophilic cytoplasm and a characteristic irregular nuclei with grooves. Multinucleation was seen in four cases. Eosinophils were abundant and intimately mixed with the histiocytic population. No emperipolesis or granulomas were seen. The sixth case showed a neoplastic proliferation of poligonal to spindle cells with few lymphocytes. Nuclear atypia and mitotic figures were common. In all cases histiocytes strongly expressed S-100 protein and CD1a. Isolated cells were positive for CD68. There was no expression of HMB45 or Melan A. In the follow up of these patients, no other cutaneous or extracutaneous lesions related to LCH were detected.
Conclusions: LCH may present as a localized cutaneous disease affecting adults. The most common form resembles eosinophilic granuloma located elsewhere. Immunohistochemistry is needed to confirm the diagnosis and to exclude arthropod bites. More rarely, it may present as a monomorphous neoplastic proliferation of atypical cells. These cases should be considered when faced with an atypical cutaneous tumor that strongly expresses S-100 protein.

P-514

APOCRINE HIDRADENOCARCINOMA: AN INFRECUENT SWEAT GLAND NEOPLASIA.

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BACKGROUND The diagnosis of hidradenocarcinoma is very difficult because nomenglature has been inconsistent, it's a rare neoplasia and variable morphology of cells composing the neoplasm.Face, hands and feet are the most usual localitations. Inmunochemistry of this tumor has been reported in only one case report.
METHODS We present the case of a 81 year-old female, with no significant personal history, to whom an asyntomatic eye nodule is detected. The diagnose before surgery was epidermoid carcinoma.
RESULTS After gross (a white, rounded nodule of 10 mm in its greatest dimension) and microscopic examination, a diagnose of apocrine hidradenocarcinoma is made.
COMMENTS Sweat glands tumours are rare. In our literature review we have found 71 published cases, and only 47 of these cases, there are clinical information. The patients are 50 year-old and these tumours are more frequents in males. These neoplasias are considered as malignant potencial tumours with capacity for lymphatic invasion and for metastasize. No case de vascular o perineural invasion. All cases have scattered atypical cells and mitosis.
Inmunohistochemical profile, ki-67 and p53, may be helpful in the diagnosis of difficult lesions.Others inmunohistochemical markers such as CEA, S-100, EMA, have not clarified the question of apocrine differentiation.

P-515

Significance of immunohistochemical expression of cyclooxygenase-2 in actinic keratosis of the skin.

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BACKGROUND Cyclooxygenase-2 (COX-2) is a catalyzing enzyme in the conversion of arachidonic acid to prostaglandins; COX-2 is not usually detectable in normal tissues but is induced in inflammation and carcinogenesis. Aktinic keratosis is a common intraepidermal neoplasm of sun damaged skin characterized by various atypia of keratinocytes.
MATERIAL AND METHODS Our aim was to investigate the expression of COX-2 by means of immunohistochemistry in a series of 30 cutaneous actinic keratoses of the atrophic (n=20) and hypertrophic type (n=10). The cut-off point of COX-2 immunopositivity was set at 10 , only strong staining intensity of the keratinocytes was regarded as positive.
RESULTS COX-2 was positively expressed in 12 samples (40) and the percentage of immunoreactive keratinocytes rarely exceeded 20 . Positive immunostaining demonstrated a cytoplasmic pattern. COX-2 immunopositive cells were mainly located close to the dermoepidermal junction, away from areas of hyperkeratosis or parakeratosis. In half of the COX-2 immunopositive specimens, a heavy inflammatory infiltrate of the papillary dermis was noticeable.
CONCLUSION COX-2 appears to be expressed in a considerable number of actinic keratoses, though at rather low percentages of keratinocytes. The observation of COX-2 positive keratinocytes away from areas of keratinization may be related to COX-2 role as an anti-apoptotic growth promoter since keratinization is known to be strongly correlated with the apoptotic potential of keratinocytes.

Kidney Pathology

P-516
Costimulatory molecule b7-h3 is overexpressed in renal angiomyolipoma

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BACKGROUND. Renal angiomyolipoma (AML) is a highly vascular renal tumor, the pathogenesis of which has not been well established. We recently demonstrated high expression of the T cell coregulatory molecule, B7-H3, in the tumor vasculature of renal cell carcinoma. Here, we investigated expression of the coregulatory molecule B7-H3 in surgically resected renal AML.
METHOD. We evaluated tumor specimens from 110 patients who underwent partial or radical nephrectomy for renal AML at our institution between 1970 and 2004. Immunohistochemistry was performed using anti-B7-H3 antibodies to stain paraffin-embedded surgical tumor sections.
RESULTS. B7-H3 expression was found in the AMLs from all 110 patients. Diffuse expression was noted on the membrane and within the cytoplasm of the smooth muscle, vascular and lipoid components of the tumors. Seven patients demonstrated 20%-30% of tumor cells staining for B7-H3, 14 patients had 30%-60% of cells positive, and 91 patients had greater than 60% of cells expressing B7-H3, including 61 patients with greater than 90% expression. Also, 52 patient specimens contained normal renal tissue adjacent to the AML in which no B7-H3 expression was observed. Levels of B7-H3 expression, as determined by percentage of cells within the AMLs that demonstrated immunoreactivity, did not correlate with patient age ($p=0.43$), gender ($p=0.27$), tumor size ($p=0.21$), or symptomatic presentation ($p=0.35$).
CONCLUSION. B7-H3 is uniformly expressed by renal AMLs. These results support potential use of B7-H3 as a marker of renal AML. Further evaluation is necessary to ascertain the role of B7-H3 in AML pathogenesis or treatment of these tumors.

P-517
AMYLOIDOSIS – MORPHOLOGICAL FINDINGS IN THE KIDNEY

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Although amyloidosis is a well-known disease with typical histochemical and electronmicroscopic characteristics, which give enough reasons for diagnosing the illness, it could sometimes cause differentialdiagnostic difficulties. We had the chance to observe 3 cases; during the lightmicroscopic investigation in two of them, we found some interesting changes in the podocytes; in the third case we applied an ultrastructural investigation and observed different osmiophilic depositions in the glomerules. We could diagnose amyloidosis in the cases with podocyte changes in the second punctural renal biopsy, which was carried out after a year, mainly with Congo-rot stain in polarized light; in the third case we used the same method and diagnosed amyloidosis in the first biopsy.
We conducted an amyloidosis typification which proved the presence of type AL deposition. We observed depositions in the cytoplasm of the podocytes and in the glomerul endothelial cells, which probably represent some of the precursors of the amyloid fibrils. In the three cases we could not prove a disease, which could cause amyloidosis.

P-518

High grade transitional cell carcinoma of the renal pelvis with divergent differentiation mimicking a renal abscess

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We describe an unusual case of high-grade transitional cell carcinoma of the renal pelvis with sarcomatoid and squamous differentiation that presented as a left renal abscess. The patient had originally been treated for minimally invasive transitional cell carcinoma of the urinary bladder and ureteral orifices five years prior. Computerized tomography scan findings were consistent with an abscess of the left kidney. Percutaneous nephrostomy with drainage afforded no clinical improvement. Nephrectomy was performed and tissue was removed piecemeal because of the diagnosis of an abscess. Macroscopically the tissue was fragmented and necrotic with patches of gray-tan abscess. Microscopic sections revealed a biphasic neoplasm with squamous and sarcomatous elements that were co-existent with evident morphologic transition. There was also evidence of residual papillary transitional cell carcinoma in the renal pelvis. The sarcomatoid component was immunoreactive for cytokeratin and vimentin. A malignant process must always be considered as an underlying cause when patients present with an abscess especially when there is a prior history of malignancy.

P-519

BRIDGING PODOCYTES IN HUMAN CRESCENTIC GLOMERULONEPHRITIS

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The concepts concerning the cellular composition of extracapillary proliferation in crescentic nephropathies have evolved during the last decade. Epithelial cells, both parietal and visceral, and macrophages have been found to participate in crescents formation. Several years ago Le Hir M. & coll. (2001) found, in an experimental crescentic glomerulonephritis (GN), podocytes adhering to both glomerular basement membrane and parietal basement membrane, and thus, forming bridges between the glomerular capillary tuft and the Bowman's capsule. Consequently, he proposed that the spreading of podocytes on the capsular membrane initiates the crescentic proliferation.
Twenty-eight renal biopsies from patients with extracapillary proliferative GN have been examined both in light and electron microscopy. From these cases we have selected five patients (two anti-GBM GN, one post-infectious GN and two ANCA associated vasculitis) showing only, or mostly cellular crescents. The EM investigation was targeted to identify some ultrastructural markers of the cells making up the crescents.
The two cases of ANCA associated vasculitis showed very large cellular crescents. Among their cellular components we found elongated cells having one end applied in close contact, on the glomerular basement membrane, and the opposite end stretched toward the Bowman capsule in between the neighboring cells. These elongated cells, identified as podocytes with modified phenotype, showed a much developed rER and some electron-dense vesicles. The majority of the large neighboring cells had an euchromatic nucleus and cytoplasmic glycogen granules. Since the glycogen rosettes are a cytoplasmic particularity of parietal epithelial cells, we considered them as such.
These facts endorse the le Hir & coll. theory, and also suggest the validity of the podocyte bridges formation in the human crescentic nephropathies.

P-520

ROLE OF NF-KB IN CELL APOPTOSIS AND PROLIFERATION IN RAT RENAL TUBULES AFTER ACUTE EXERCISE

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It is widely accepted that an acute physical exercise can induce pathological changes in untrained organism. Our previous studies have demonstrated that exercise-induced apoptosis was confined to the renal distal tubular cells. Under the normal condition, in response to the tubular cell's injury occurs the process of regeneration via neighboring cells proliferation. NF-κB is a transcription factor that plays a pivotal role in the expression of various target genes regulating both, cell proliferation and apoptosis.
The aim of this study was to examine if NF-κB could control exercise-induced apoptosis and cell proliferation in renal proximal and distal tubules.
Material and methods: Twenty male Wistar rats formed groups of running (n=10) and control animals (n=10). The animals from the exercise group were subjected to running on a treadmill at 1.0 km/h until exhaustion. The control animals remained in their cages throughout the experiment. Apoptosis was detected in paraffin sections by the TUNEL technique. Expression of Ki-67 and NF-κB was examined on paraffin sections by immunohistochemistry, using

mouse monoclonal antibody and rabbit polyclonal antibody, respectively.

Results: Apoptosis was detected only in cells of distal tubules. Expression of nuclear proliferation marker Ki-67 was more prominent in distal renal tubular cells. Expression of NF-κB was different in both renal tubules; in proximal tubular cells was confined to the nucleus while in distal tubular cells to the cytoplasm.

Conclusion: The obtained results could suggest that apoptosis of distal tubular cells is followed by cell proliferation what could allow the regeneration of damaged cells. The nuclear translocation of NF-κB could be involved with the protection of proximal tubular cells against apoptosis while the cytoplasmic location could suggest that apoptosis inhibition in distal tubular cells does not exist.

P-521

Kidney carcinoma associated with TFE3 gene fusion coexistent with ureteral transitional cell carcinoma-case report-

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Background. Renal carcinomas associated with TFE3 gene fusions, included in the new WHO classification of kidney tumors (2004), have interesting morphological and biological features. In addition, the coexistence with ureteral carcinoma represents a challenge for the pathologist.

Material and method. The aim of this study is to analyse the morphological and immunohistochemical profile of a case: 55 y.o. male patient, Turkish ethnic, with both renal and ureteral carcinoma (Constantza has a Turkish minority representing 6,4% of population).

Results. Macroscopically: nephroureterectomy specimen of 10/8/6 cm with polycyclic outline; at the upper pole, a 3/3/2,5 cm intraparenchimatous nodular lesion, well circumscribed, yellowish, soft, with hemorrhage and necrosis; upper third of the ureter with a soft, grayish 1,5/0,5 cm mass with papillary surface. Histopathologically, renal tumor exhibits papillary growth patterns of polygonal cells, eosinophilic granular cytoplasm, hyaline inclusions, pseudostratified nuclei of Fuhrmann grade 3; foamy macrophages in papillary cores, necrosis; immunolabeling: CK7+, RCC-Ma+, EMA+, Ulex europaeus-, WT1-. This morphological features determined us to establish, initially, the diagnosis of papillary renal carcinoma, excluding other renal tumors that share similarities with it (classic renal cell carcinoma with papillary architecture, collecting duct carcinoma, metanephric adenoma). Surprisingly, our diagnosis was invalidated by the evidence of TFE3 protein overexpression, assessed by immunohistochemistry (strong nuclear reaction). This finding allows us to certify that the renal tumor belongs to the new class of kidney carcinoma associated with TFE3 gene fusion. Ureteral tumor was a high grade transitional cell carcinoma (G3), with positive immunolabeling for p63, that excludes the ureteral invasion by the renal malignant lesion.

Conclusions. This report emphasizes the utility of immunomarkers in cases with overlapping features. Also, it reveals distinct morphogenetic characteristics of the tumoral association in the Turkish patient: TFE3 gene fusion and p63 overexpression, creating a distinct molecular profile - target for further research.

P-522

Experimental substantiation of nephropathies because of the food dye consumption (E 102)

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Background: Last decades the quantity of various nephropaties at children increasing. Probably, to this process concern not only negative ecological factors, but also some food factors. For an experimental research yellow food dye tartrasine (Å 102) has been selected. It is used in fruit-drops, cakes, wafers, fruit water manufacturing.

Methods: Female rats Wistar received a tartrasine solution daily in an adequate doze (at extrapolation on the person 0.5 liters of fruit water) during 1 month before pregnancy, then during the period of time of pregnancy and feedings of posterity. Kidneys of rats before pregnancy, after pregnancy and feeding, and kidneys of one-month rat posterity were investigated morphologically with the using of immunomorphological and morphometric techniques.

Results: At adult female rats the glomerulas are reduced in sizes. In mesangium the number of macrophages (CD16+) is increased, in basal membrane of capillaries IgG is found in places. Epithelium of proximal canalicules is injured: PAS-positive apical parts of epitheliocytes are frequently absent, accelerated apoptosis is observed.

The consuming of tartrasine during 4 months adult rats have kidneys with microscopic signs of proceeding mainly mesangeal-proliferative glomerulonephritis with sclerosis of separate glomerulas and interstitial-tubularis nephritis. A plenty of apoptosed changed nucleus of canalicularis epithelium is combined with reduction of their average size. At one-month rats which intrauterinally had influence with tartrasine and consumed it with parent milk, dysplastic glomerulas presence, large cortex areas without glomerulas

are observed. Mesangium of glomerulas contains the increased number of macrophages, IgG presences, accumulation of collagen I type is marked.
Conclusion: The food dye tartrasine (Å 102) is dangerous for kidneys because of toxic and xenobiotic actions.

P-523

An atypical case of huge type 1 papillary renal cell `carcinoma with like-type 2 aggressive behaivor

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Background: Type 1 papillary renal cell carcinoma is associated with favorable outcome, while the type 2 tumor is associated with higher metastatic progression and larger tumor size. However prognosis of the papillary renal cell carcinoma remains debatable in the literature. In Venezuela, papillary renal cell carcinoma is particularly unusual. **Case Presentation:** Authors report clinicopathological features of an unusual case of papillary renal cell carcinoma (type 1) with vascular invasion in a 70 years old male with hematuria of two month of evolution. The tumor was located on the upper lobe of the right kidney, and his size was 10×9 cm. Total nephrectomy was done. Grossly, well-circumscribed tumor with considerable hemorrhage and necrosis areas were seen. Microscopically, the tumor displayed a papillary architecture, papillae covered with a single layer of small cuboids cells with scanty cytoplasm and nucleoli, foam cells in stroma, tumoral necrosis and hemorrhage, renal vein was involved. Immunohistochemically was intensely reactive for CK7 and CD10. **Conclusions:** Contradictorily, to the generally reported in type 1 papillary renal cell carcinoma, this case presented threatening characteristics, such as; vascular invasion, tumor size (>7 cm), and extensive necrosis, these elements are more linked to type 2 papillary renal cell carcinoma. The fact that papillary tumors represent a heterogeneous group of different entities whose identification at cytogenetic and molecular level is currently being studied, possibly explain the more aggressive behavior in this patient.

P-524

The correlation of pathological findings in kidney biopsy and first clinical and laboratory manifestations of Primary focal and segmental glomerulosclerosis

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Background: The first clinical presentations and morphologic features of FSGS are various among patients and they are important predictor of prognosis in them. The aim of this study was to analyze the correlation of clinical, laboratory and pathological features at presentation of FSGS.
Methods: In this cross-sectional study, pathological findings of 64 cases of primary FSGS were reviewed by single renal pathologist without knowledge of patient identity or outcome. Clinical and laboratory data will be obtained on each patient at the time of biopsy. The data include systolic and diastolic blood pressure, the level of plasma creatinine, BUN, glomerular filtration rate (GFR), serum albumin, and the level of proteinuria. Then compare histopathological findings with clinical and laboratory data at the time of biopsy.
Results: The mean of Serum creatinin level was significantly higher in the patients with the presence of synechiae with the Bowman's capsule, and the presence of interstitial fibrosis and the presence of the global scars in their biopsies(p value<0.05), and also there were significant different in the mean level of GFR in the patients with the presence of interstitial fibrosis in their biopsies in the comparison with the patients without the interstitial fibrosis (p value<0.05).Also we discovered positive correlation between the level of plasma creatinin and global sclerosis (r=2,21,p-value=0.04),and between the level of GFR and global sclerosis(r=2.01,p-value=0.02). All the patients with renal insufficiency had intrestial fibrosis in their biopsies in comparison of only the 24 patients (48%) of the group without renal insufficiency (p value<0.05). There were no significant difference between patients with and without hypertension and nephrotic- ranged proteinuria.
Conclusion: in our studies we found that in addition to the interstitial fibrosis, global scars and the synechiae of Bowman's capsule are correlated with serum creatinin level and the level of plasma albumin and GFR.Although the prognostic role of these laboratories and the pathological features have not been proved until now, but because the prognostic role of the laboratory findings such as Pcr which is correlated with these pathological features, has been proved,there is a hypothesis that these two pathological features could have prognostic roles in FSGS patients.
Key words: Focal and Segmental Glomerulosclerosis (FSGS), creatinin, Albumin, BUN, interstitial fibrosis, global scar, synechiae with the Bowman's capsule.

P-525

Useful Markers for Differential Diagnosis of Oncocytoma, Chromophobe Renal Cell Carcinoma and Conventional Renal Cell Carcinoma

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ABSTRACT: Renal oncocytoma, conventional RCC (with granular cell type), and chromophobe RCC have different prognosis. Some times differentiation between them is difficult in H & E slides. In study of 128 renal tumors during 5 years, we selected 76 cases (30 conventional RCC (CRCC),16 papillary RCC, 21 chromophobe RCC (ChRCC), 8 oncocytoma, 1 collecting duct carcinoma and staining with Hale's colloidal iron, CK7, CK8, CK18, CK19, CK20, Vimentin, EMA, CD10 and RCC marker were done. No significant difference was seen between renal tumor subtypes with CK8, CK19, CK18, CK20 and EMA. The most useful markers were vimentin, CK7, CD10, RCC marker and Hale's colloidal iron. Hale's colloid iron staining with diffuse reticular fine cytoplasmic pattern was present in ChRCCs, but was absent in other subtypes and oncocytomas.Vimentin, CK7, CD10, RCC marker and Hale's colloidal iron can be used for the differential diagnosis of the problematic epithelial tumors of kidney (CRCC, ChRCC and oncocytoma), i.e. ChRCC: Vimentin, CD10 and RCC marker negative, CK7 positive and diffuse fine reticular cytoplasmic pattern of Hale's colloidal iron. Oncocytoma: Vimentin, CK7, RCC marker and CD10 negative, Hale's colloidal iron negative. CRCC: CK7 negative, Vimentin, CD 10 and RCC marker positive, and Hale's colloidal iron negative.

P-526

Pathological changes in kidney biopsies from patient with drug-induced nephropathy

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Background: The influence of variety drugs on the kidney first of all associate with an allergic acute tubulointerstitial nephritis. But durable excessive usage nonsteroidal anti-inflammatory drugs (NSAID) or analgesics often results in chronic renal failure with decrease of function. We aimed to examine clinical and pathomorphological correlations in such chronic secondary drug-induced nephropathy.

Methods: Our study included 21 kidney biopsies from patients with tubulo-interstitial nephritis without any other renal diseases (after careful clinical and morphological examination), with long history of chronic usage of NSAID (1–22 years). The mean age of the group 39,5 years, 12 men, 9 women.

Results: Diffuse interstitial fibrosis and diffuse weak nonspecific inflammatory infiltration of interstitium by various cellular elements with admixing of eosinophilic granulocytes were observed in 90,5% of patients. Marked tubular changes (atrophy, subatrohy) were showed in 47,6% cases, not numerous casts in 67,7%. The focal changes of glomerular capillaries (total or segmental gialinosis, sclerosis of basement membrane, weak mesangial hypercellularity) had 57% of patients. Electron microscopy examination of glomeruli showed marked changes of podocytes - focal fusion and even desquamation of foot processes, microvillous transformation of the cell membrane in 76% of cases. Analysis of clinical features and its correlations to morphological changes showed interrelation between the level of proteinuria and the level of interstitial and glomerular fibrosis/gialinosis.

Conclusion: Analyses of clinical and pathological features let us to develop the score system for the calculation the risk of the probable morphological changes progressing in the kidney under the influence of NSAID.

P-527

FIBRILLARY GLOMERULONEPHRITIS: A REPORT OF TWO CASES

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Background: Fibrillary glomerulonephritis (FGN), is identified in about 1% of patients who undergo native kidney biopsy. FGN is defined by electron microscopic finding of randomly arranged, straight nonbranching fibrils ranging in diameter from 10 to 30 nm. The deposits do not stain with Congo Red. We have reported two cases of FGN.

Method-Results

Case 1: The kidney biopsy performed from a-15 year-old boy presented with hematuria with a serum creatinine level above 7.0 mg/dL. Microscopically while 8 of the 18 glomeruli were globally sclerosed 4–5 glomeruli showed cellular crescents. The other glomeruli demonstrated mesangial hypercellularity. Congo Red Stain was negative for amyloid. Minimal interstitial inflammation and focal atrophy of the tubules were observed. The histomorphology of the extraglomerular vessels were normal. Ultrastructurally extensive 15–20 nm fibrils deposition within the

glomeruli were noted. The final diagnosis of the case was FGN.
Case 2: A 36 year old man presented with nephrotic syndrome. The kidney biopsy consisted of the renal cortex with 16 glomeruli; 4 of these were globally sclerosed and mesangial hypercellularity were seen in the other glomeruli. The basement membranes of the peripheral capillary walls were markedly and regularly thickened. While the tubules showed focal atrophy, the vessel walls showed mild sclerosis. Congo Red stain was negative for amyloid in the glomeruli, interstitium and vessels. The immunofluorescence microscopy showed fine granular deposition for polyclonal IgG, IgM, IgA, and C3 along the glomerular capillary walls. One of the glomerulus was examined ultrastructurally and numerous fibrillary deposits (30 nm diameter) in the expanded mesangium was seen. All of these findings suggested the diagnosis of FGN.
Conclusion: The finding of Congo red negative organized deposits on renal biopsy should prompt a careful search for FGN by ultrastructural analysis.

P-528
COLLAGENOFIBROTIC GLOMERULOPATHY: A DESCRIPTION OF TWO CASES

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Collagenofibrotic glomerulopathy (CG) is a recently discovered entity characterized by massive accumulation of spiraled and frayed collagen fibrils in mesangial and subendothelial areas: clinically it presents with nephritic syndrome. The reported cases are still rare, principally in the West counties, as it is the knowledge in the diagnosis of this disease. Case 1: female patient, 21 years old, white; patient was previously healthy. No familiar history of renal diseases. She showed for routine exams and in the urine analyses she had proteinuria (1,6 g/24 h) and microscopic haematuria with one year of evolution. Case 2: female patient, 15 years old, white, began the disease with edema and hypertension for two months; urine analyses showed proteinuria of 2,49 g/24 h and dyslipidemia (total colesterol: 426 mg/dl and triglycerides: 264 mg/dl), without other alterations; she presented partial answer to the use of corticosteroids. Renal biopsy: in both cases, at light microscopy, the only alteration was discrete mesangial hipercelularity; the immunofluorescence was negative. The electronic microscopy found massive accumulation of spiraled and frayed collagen fibrils in mesangial and subendothelial areas compatible with collagen type III. At picrosírius method under polarized light, the fibers displayed birefringence and greenish staining, highly suggestive of type III collagen. These two cases of CG are probably the first ones to be reported in Brazil and in South America. Even being rare the CG deserves distinction as a differential diagnosis in the cases of nephritic syndrome, mainly in adolescents/young adults. The CG illustrates the importance of electronic microscopy for diagnosis and best understanding of glomerulopathies.
Grant: CNPq, Fapemig, Funepu, UFTM

P-529
HEREDITARY PROTEINURIA: DIFFUSE MESANGIAL SCLEROSIS AND FINNISH TYPE NEPHROPATHY

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The nephrotic syndrome in the childhood (NSC), congenital or within the first years, is a rare condition which has an elevated rate of mortality and an evolution to an end-stage renal disease (ESRD). With the genetic studies and recent discoveries on the molecular structure of the glomerular filtration barrier (diaphragm slit proteins, like nephrin and podocin), there was an increase in the knowledge of these pathologies, but there is still a small amount of cases reported. Between the main pathologies that have features of NSC are the diffuse mesangial sclerosis (isolated or associated with Denys-Drash syndrome) and the Finnish type nephropathy (FN). Case 1: six-month-old African-American female, born at term; at five months old, began with diarrhea which fast evolution to oliguria, anasarca and hipertension. Exams showed high proteinuria and loss of renal function. Renal biopsy diagnostic was of DMS. Case 2: 13 years-old white boy; routine exams showed creatinine (Cr)=2,1 mg/dl and proteinuria 5,2 g/24 h. In the past history (two years old) he went to a nephrectomy (Wilms' tumor). He has bilateral cataracts and cryptorchidism. Renal biopsy showed the diagnosis of DMS, probably as a part of the Denys-Drash syndrome. Case 3: white female patient, 24-days-old, born at 36 weeks due to a placenta abruption (weights 2080 g). She presented intense edema and severe proteinuria. Renal biopsy showed severe tubular dilatations (cysts) and the electronic microscopy showed absence of the slit diaphragm between the foot processes, which was

highly suggestive of FN. Although new studies had been published in the past few years in this area, the discussion about the diagnosis between clinicians and pathologists was still the best way to deal with the NSC.
Grant: CNPq, Fapemig, Funepu, UFTM

P-530

Renal Carcinoma of Collecting Duct of Bellini.

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Background: Collecting Duct Carcinoma of Bellini (CoDCB) is a very rare renal malignancy (less than 1%) with poor prognosis since most of the cases are metastatic at presentation. Although the diagnosis should be considered in patients presenting with marked deterioration of the general status and/or the presence of a very large invasive renal tumour on abdominal CT scan.
Methods: From January 2003 up to the end of December 2007 two cases of CoDCB (2.9%) were diagnosed among 68 renal malignancies in our department. The first case was about a 50-y.o. male patient with gross hematuria. The patient underwent a radical right nephrectomy. On dissection a whitish tumor, measuring 11.5×9×8 cm, was found on the posterior surface of the kidney, infiltrating the perinephric fat. The second case was about a 52 year old female with a renal tumor (m.d. 8 cm) that invaded the renal fat but also the renal vein.
Results: The tumors fulfilled the major and minor criteria of Sringly et al. More specifically our cases showed the typical irregularity of tubular architecture with high nuclear grade (hobnail cells, Fuhrman 3&4). Small papillary architecture and desmoplastic stroma with numerous inflammatory granulocytes were noted. In the second case although the tumor was smaller than the first, invasion of the renal vein was found because of its central. The immunohistochemical staining supported the diagnosis (soy bean agglutinin, CK8, 18, high molecular weight Keratin, EMA, vimentin).
Conclusion: Our cases, besides the other typical morphological characteristics, showed the characteristic positive immunohistochemical profile and the positive staining for soy bean agglutinin, a marker of the distal nephron. CoDCB presents with a very poor prognosis and our cases were staged pT3a and pT3b respectively.

P-531

Immunohistochemical profile of Chromophobe renal cell carcinoma. A four year review.

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Background: We re-evaluated 62 cases of Renal Cell Carcinoma (RCC) which were diagnosed in our department from January 2003 to December 2007. According to the strict morphologic criteria of WHO classification, we estimated the immunohistochemical diagnostic utility of various antigens, for the assessment of Chromophobe (Chr) subtype renal cell carcinoma.
Methods: Seven carcinomas from the total 62 cases were classified as Chr (11.3%). According to the Furrhmann grading system 1 case was grade II, 5 cases were grade III and 1 case grade IV. No case was grade I. The following antibodies were applied to all the cases: CK7, EMA, CD10, CD117 and Vimentin (Vim).
Results: The immunohistochemical phenotype of Chr showed strong positivity for CK7, CD 117 and EMA while all the Chr RCCs were negative for Vim and CD10.
Conclusion: We conclude that whenever the differential diagnosis included eosinophilic variant of RCC the most sensitive markers were CD117and CK7 in combination with the negativity of CD 10, and Vim.

P-532

Malignant Fibrous Histiocytoma of the kidney. A rare case.

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Background: Various types of sarcomas have been reported so far in the adult kidney and among them Malignant Fibrous Histiocytoma (MFH) is a very rare neoplasm in that special location. It was first described by O'Brian in 1964. Usually MFH of the kidney arise from the renal parenchyma or from the renal capsule. Also retroperitoneal soft tissues often develop MFH.
Methods: A 59-year old patient presented with hematouria and general malaise and underwent a left nephrectomy. Part of the descending colon was also excised. We received the left kidney measuring 19×12×10 cm along with the perinephric fat. The left colon was attached to the kidney

and its length was 19 cm. Grossly the tumor had whitish-yellow color, hard-elastic consistency, while on sectioning it had solid, partly lobular and fasciculating appearance and was measuring 12×12×8 cm. The tumor was found to occupy the lower pole approaching to the middle of the kidney, infiltrating part of the renal pelvis and part of the ureter which was also removed. It was also infiltrating the perinephric fat up to the serosal layer of the excised left colon. Renal artery and vein appeared to be free of tumor infiltration.

Results: Histological examination the tumor demonstrated spindle shaped fibroblast-like cells arranged in a storiform pattern with fibrous stroma and clusters or sheets of rounded histiocyte-like cells. Pleomorphic giant cells with bizarre nuclei (Tuton cells) were also observed. Our diagnosis was also supported immunohistochemically with and the tumor was immunoreactive for Cytokeratin, CD10 and CD117.

Conclusion: MFH is unusual urological malignancy and differential diagnosis includes leimyosarcoma, sarcoma and sarcomatoid carcinoma. Their distinction is based mainly on immunohistochemistry. Histogenesis of MFH is linked to mesenchymal cells from renal capsule. On conclusion MFH has strong predilection for local recurrence and its prognosis is poor, therefore close and life-long follow-up is advised.

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WITHDRAWN

P-534
SARCOMATOID CARCINOMA OF THE RENAL PELVIS AND URETER: A CASE REPORT WITH IMMUNOHISTOCHEMICAL FINDINGS

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Background: Sarcomatoid carcinoma is a rare tumor of the urothelium. In this case report we present a sarcomatoid carcinoma of the renal pelvis and ureter and discussed its morphological and immunohistochemical aspects.

Case: A 69-year-old male presented with gross hematuria and abdominal pain. Clinical imaging work up revealed severe hydronephrosis with dilated right renal pelvis and extensively large right kidney. Suspicious of renal cell carcinoma, right nephrectomy and periton biopsy were performed. Histopathologically, the tumor was composed of epithelial and sarcomatous areas. The epithelial component was a high-grade papillary urothelial carcinoma in renal pelvis and ureter. The sarcomatous component had spindle, chondroid and rhabdomyoid appearance. Immunohistochemically, the epithelial component was reactive for epithelial membrane antigen, pankeratin, cytokeratin 7 and 20. The sarcomatous elements were positive for vimentin, smooth muscle actin and focally desmin and epithelial membrane antigen but negative for S-100, myoglobin, pankeratin, cytokeratin 7 and 20. Only the sarcomatous component of the tumor infiltrated through the renal parenchyma and extended to perirenal fat tissue. Similarly, periton biopsy revealed only sarcomatous elements. The patient died a day after the nephrectomy.

Conclusion: Sarcomatoid carcinoma of the renal pelvis and ureter is a high-grade malignant neoplasm with poor prognosis. Demonstration of epithelial characteristics in sarcomatous component by means of immunohistochemistry confirms the diagnosis.

Key words: Renal Pelvis, Ureter, Sarcomatoid carcinoma, Immunohistochemistry

P-535
Multilocular cystic renal cell carcinoma.

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Background: Multilocular Cystic Renal Cell Carcinoma (MCRCC) is a rare variant of the Clear Cell Renal Carcinoma (CCRC) with a favorable outcome and still not well-known clinical significance and biological behavior.

Method: A 47-year old patient presented to our hospital with dysuric problems. On ultrasound and MRI scan a space occupying cystic mass was found. Similar findings were also observed on CT scan. The patient underwent a radical kidney excision and on dissection a cystic, whitish tumor measuring 5 cm in its greater diameter was found.

Results: Microscopically the tumor appeared partly solid with neoplastic cells organized in islands or tubular structures being separated by each other by thin, slender fibrovascular septa. The neoplastic cells were cuboidal and in some places polygonal with a central nucleus, without nucleoli and clear cytoplasm (grade 1 according to Furhman grading system). In other areas the tumor appeared cystic composed mainly of thin-walled cysts of varying size and shape. The neoplastic cells that were investing the wall of these cystic spaces were epithelial clear cells. These cysts were filled with eosinophilic amorphous material. The tumor was examined immunohistochemically and reacted

for Pankeratin (AE1/AE3), Vimentin, EMA, CD10 and NSE. Negativity was observed for the following antigens: Cytokeratin 7, Cytokeratin 20, p53 and MIB-1.

Conclusion: MCRCC has a benign clinical course and many authors suggest nephron-sparing surgical treatment. Preoperative diagnosis is difficult and has to be differentiated from other space-occupying cystic renal lesions. Our case appeared to be limited to the kidney with no invasion of the perinephric fat or the Gerota's fascia. The renal vessels and the ureter were also free of direct invasion by the neoplasm. According to the 1997 criteria of tumor-node-metastasis (TNM) classification our case was stage pT1b.

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Hepatocyte growth factor (HGF) and transforming growth factor (TGF-β1) expression in 42 renal biopsy from lupus nephritis patients. Role of the HGF/TGFB1 ratio as a prognostic factor.

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Background. The identification and validation of molecular markers of prognosis are the future for several pathologies. Until now in lupus nephritis (LN) the prognostic value of different clinical and histological factors remains debated. Several animal models demonstrate that HGF and TGF-β_1 are implicated in renal damage.

Objective. To evaluate HGF and TGF-β_1 expression in renal specimens of LN patients and their role to predict the renal outcome after six months of immunosuppressant therapy.

Methods. 42 consecutive patients with newly diagnosed LN were included in the study. Renal biopsy specimens were classified according to the WHO criteria. Immunohistochemical expression of HGF and TGF-β_1 was evaluated. We divided patients in two groups on the basis of the response to therapy at 6th month in responders (R) and non responders (NR).

Results. Immunohistochemistry confirmed that HGF and TGF-β_1 are expressed in the tubuli but not in the glomeruli. The TGFβ_1 extension score directly correlated with the chronicity index ($r=0.40$, $p=0.03$). We did not find any correlations with clinical parameters of renal involvement (proteinuria, creatinine clearance) as well as serum complement levels and antiDNA antibodies. The 29 R differs from the 13 NR only for the HGF extension score ($p<0.001$) and intensity score ($p<0.001$) and HGF/TGF ratio either for extension ($p<0.001$) and intensity score ($p=0.001$). A cut-off value >1 for the ratio HGF/TGFb1 is predictive of remission with a PPV of 95% and with an OR of 15.2 (95% IC 2.5–92, $p<0.0001$).

Conclusions. Consistent with these findings, our studies provide strong evidence supporting the biological significance and clinical relevance of HGF/TGFb1 ratio at baseline for the identification of patients with a good response to standard SLE therapy. Immunohistochemical assessment of HGF/TGFb1 ratio as a prognostic factor could be an efficient approach in the routine study of LN patients.

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WITHDRAWN

P-538

Nephrotoxicity by cyclosporine-a: the role of TGF-β1, apoptosis and intersticial fibrosis

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Background: Acute and chronic Cyclosporine-A (CyA) nephrotoxicity prevalence varies from 15% to 30% in different series. Chronic toxicity is the more frequent of both two and is characterized by acelular interstitial fibrosis and arteriolopathy. Interstitial fibrosis could be the result of and important profibrotic growing factor and apoptosis inductor i.e., TGF-β1.

Objective: To demonstrate that there is an increase expression of TFG-β1 and apoptosis in renal allograft biopsies in patients with histological data of CyA-toxicity.

Methods: Renal biopsies from 40 transplanted patients of living related donor were retrospectively reviewed. All patients have had a renal biopsy performed at the time of the transplant (immediate). Second biopsies were taken by protocol of early CyA withdrawal, at least after 6 months of treatment. Biopsies were reviewed looking for vascular or interstitial fibrosis as well as the expression of TGF-β1, immunomarkers of apoptosis and TUNEL. Vascular damage was scored as mild, moderate and severe. Expression of immunomarkers was measured by morphometry using image analyzer software. Pearson correlation was performed between the percentage of interstitial fibrosis and

TGF-β1 expression, immunomarkers of apoptosis and TUNEL.
Results: There was a clear correlation between expression of TGF-β1 and interstitial fibrosis in the first biopsy; however this correlation was highly significant in the second biopsy under the treatment with CyA. There was also a direct correlation between interstitial fibrosis and apoptosis (i.e., Bcl-6). TUNEL results showed an increase in apoptosis in the interstitium in the second biopsies compared to those biopsies taken immediately. However, this showed an affected cortex surface maximal increase in all those second biopsies with fibrosis scored grade 1 and 2, while there was a diminution in those biopsies scored as grade 3.
Conclusions: Data supports that CyA chronic toxicity in the renal allograft is mediated by TGF-β1 and the apoptosis induction.

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MIXED EPITHELIAL AND STROMAL TUMOR OF THE KİDNEY: A CASE REPORT

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Background: Mixed epithelial and stromal tumors of kidney are characterized by a mixture of epithelial and stromal elements that form solid and cystic growth patterns. Cystic areas composed of large cysts, microcyst and tubules. Solid areas consist of proliferations of spindle cells admixed focally gland-like structures. The stromal component ranges from hypocellular and fibrotic to more cellular with fibroblastic and myofibroblastic foci to cellular ovarian-like stroma. Smooth muscle cells may be prominent.These tumors were reported previously in the literature under various names, including adult mesoblastic nephroma. All have been adults and the mean age is perimenopausal.
Case: The case is a 44 years old woman with chronic hepatitis B. Abdominal ultrasonography showed a multi-lobulated mass with solid and cystic areas at the middle-lower pole of the left kidney and a hemangioma of the liver. Radical nephrectomy was performed. Macroscopic examination revealed a well- circumscribed 6×5×5 cm mass with solid and cystic components of the mid-lower poles of the left kidney.Microscopically the tumor was composed of a mixture of a spindle cell proliferation of variable cellularity and microcysts, tubules and large cysts are lined by columnar and cuboidal epithelium. Immunhistochemically spindle cells reacted with antibody to smooth muscle actin, desmin, vimentin, and estrogen, progesterone receptors. Epithelial elements of the tumor were positive for cytokeratin and vimentin.
Conclusion: We present a case of mixed epithelial and stromal tumor of the kidney by reviewing the literature.

P-540

Primary renal leiomyosarcoma, case report

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Background: Primary renal leiomyosarcoma is a very rare adult malignant neoplasm constituting about 1% of all primary malignant kidney tumors and 60% of all renal sarcomas. It carries a poor prognosis and fails most treatment modalities.
Method: The patient was 43 years old male who presented with two-month history of right flank pain without hematuria. Radiologic studies revealed a large right kidney mass compressing the inferior vena cava. Radical nephrectomy was performed. Subsequently, the patient developed multiple lung and liver metastases and local tumor recurrence. He did not response to multiple chemo and radiation treatment regimens and died one year after surgery.
Results: The right kidney was largely involved by a large tumor measuring 23 cm with nodular, lobulated, solid and fleshy cut surface and focal hemorrhage and necrosis. Microscopically, the tumor consisted of cellular growth of spindly cell with elongated plump moderately pleomorphic and hyperchromatic nuclei. Increased mitotic activity is noted (11/10 hpf). Some highly atypical cells are present. Areas of necrosis, hemorrhage, hyalinization and myxoid changes are present. The tumor cell arranged in short intersecting fascicles. The tumor cells were strongly positive for vimentin, actin and desmin, weakly positive for CD117 and negative for CD34, F-VIII, EMA and cytokeratin.
Conclusion: Although primary renal leiomyosarcoma is very rare, it should be considered in the differential diagnosis of spindle cell proliferation because of the poor prognosis and bad outcome despite aggressive therapy. It should be differentiated from other sarcomas and from sarcomatoid renal cell and urothelial carcinomas which have different treatment strategies and outcome. The distinction is based on the morphologic and immunohistochemical findings. The tumor may arise from renal capsule, wall of renal pelvis, hilar blood vessels or intrarenal small blood vessels. No specific cytogenetic abnormalities have been found.

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