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ABSTRACTS

Keynote Lectures

Wednesday, 1 September 2010, 11.45–12.30, Aula Duża

KL-01 New trends in breast pathology
Chairperson: T. Tot, Sweden

001
New trends in breast pathology
*M. van der Vijver**
*The Netherlands

Thursday, 2 September 2010, 11.45–12.30, Aula Duża

KL-02 Emerging mechanisms of tumor initiation and progression: lessons from the bladder cancer model
Chairperson: H. van Krieken, The Netherlands

001
Emerging mechanisms of tumor initiation and progression: lessons from the bladder cancer model
*B. Czerniak**
*The University of Texas, M D Anderson Cancer Center, Houston, USA

Bladder cancer has served as one of the most important sources of information about the events that underlie the development of human solid malignancies. Although "field effects" that alter the entire bladder mucosa appear to initiate disease, tumors develop along two distinct biological "tracks" referred to as papillary and non-papillary that present different challenges for clinical management. More recently, whole organ mapping combined with genomic platforms have identified a novel class of candidate tumor suppressors (forerunner genes) that localize near more familiar tumor suppressors but are disrupted at an earlier stage of cancer development. These studies suggested three steps for the involvement of the model tumor suppressor locus, RB1, in tumor development. In the first step, one allele of forerunner (FR) gene and RB1 is inactivated by deletions. In the second step, homozygous inactivation of the FR genes is accomplished by hypermethylation or mutations. The inactivation of FR genes is associated with the initial clonal expansion of preneoplastic urothelial cells. In the third step, the contiguous tumor suppressor, RB1, is inactivated by a mutation, which is associated with clonal evolution into carcinoma in situ progressing to invasive cancer. Furthermore, we have discovered that aggressive muscle-invasive tumors express molecular markers characteristic of a developmental process known as "epithelial-to-mesenchymal transition." Emerging evidence indicates that urothelial cancers contain subpopulations of tumor-initiating cells (cancer stem cells), but the phenotypes of these cells in different tumors may be heterogeneous, raising questions about whether or not the two major subtypes of cancer share a common precursor.

Friday, 3 September 2010, 11.45–12.30, Aula Duża

KL-03 Predictive pathology: fact or fancy?
Chairperson: F. Carneiro, Portugal

001
Predictive pathology: fact or fancy?
*F. Bosman**
*University Hospital Maastricht, Dept. of Pathology, The Netherlands

Pathology as a medical specialty has been around for about a century and a half, even though the interest in understanding disease, the focus of pathology as an academic discipline, has inspired physicians since the dawn of mankind. Understanding disease remains the primary focus of pathology. In the practice of diagnostic pathology, this knowledge, notably through its morphological expression, is applied to the diagnosis of disease through the examination of cells and tissues. Relatively new in this field is the notion of pathology as 'the science behind the cure'. This phrase, coined by the Pathological Society of Great Britain and Ireland, refers to a widening of the scope of pathology: 'Understanding disease' continues to be applied to diagnosis and classification, but now also to conceiving of new ways to treat and of new diagnostic tools assisting the physician in choosing the appropriate treatment. Targeted therapy, the target being a molecular pathway involved in the pathogenesis of the lesion, along with biomarkers that are predictive for response to treatment, are in the forefront of pathology. Targeted drugs that require 'companion' diagnostics, a combination also called 'theranostics', are reshaping the practice of pathology. It is this

paradigm shift in pathology that makes it one of the most exciting medical specialties of today. I would love to be a resident in Pathology again. The prospects for this mother of disciplines in modern medicine have never been as bright.

Is this entirely new? Certainly, the molecular revolution with 'high-throughput' technologies, array diagnostics such as 'MammaprintR' and 'deep sequencing' to name just a few technologies we have had to become familiar with, have had and will continue to have in the years to come a profound impact on the way we practice pathology. But guiding the surgeon's hand or the pen of the oncologist in prescribing drugs has been a primary responsibility for pathologists ever since examination of cells and tissues was being applied to the practice of medicine. Prognostic factors have interested pathologists long before the onset of the molecular revolution. Grading and staging of neoplastic disease based upon morphological characteristics remains the mainstay of stratification of patient groups for treatment optimization. And predictive value is not limited to molecular markers: Small cell carcinoma of the lung, to name just one example, will not be treated surgically, but chemotherapy is effective, even though as a rule not to the extent that the patient can be cured. We dispose of numerous morphological parameters that are prognostic and predictive.

That pathological parameters have predictive value is therefore nothing new. This has become more so with the discovery that steroid hormone receptor expression predicts the response to anti-hormonal treatment and that HER2/neu amplification predicts the response to HER2/neu antibodies in breast cancer. It has become clear that the type of c-Kit mutation in a GIST predicts whether or not the patient will respond to a tyrosine-kinase inhibitor (imatinib), which dose of the drug would be appropriate or which second-generation inhibitor might be used. K-RAS mutations will tell the oncologist that the patient will not respond to EGFR inhibitors in the form of small molecules (gefatinib) or monoclonal antibodies (cituximab). The latter is also a striking example of the limitations of many of the tools that we dispose of: The K-RAS mutation status will tell us who will not respond to the therapy, but not who will. Based upon detailed knowledge of the molecular characteristics of many types of cancer, pharmaceutical companies are now developing a host of new drugs and more often than not are teaming up with pathologists to develop diagnostic tests that might predict which patient groups might profit from the new drug.

This should not become a hype, the type of buzz that went around the H1N1 pandemic which in hindsight was not justified by what happened in the field. Let us remain modest. First of all, the number of diseases for which the combination of new drug and predictive molecular test exists remains limited. Secondly, the impact of this new and promising approach to final outcome should keep us modest. For colorectal cancer, new therapies have prolonged survival, but not cured more patients. Thirdly, expectations that we will be able to predict at the level of the individual patient what the best therapy would be and how the disease will respond or progress is an illusion. 'Individualized' medicine is still based upon studies on large patient groups with as outcome parameters that with a certain level of probability will indicate what might happen. A lot remains to be studied and developed. This implies also a new role for pathology. We should team up with clinical trials, using this context to conduct biomarker studies that can be implemented in daily practice. We should collaborate with drug developers to get to the desired 'companion' diagnostic tools. For pathology, exciting times indeed.

Working Group Sessions

Wednesday, 1 September 2010, 08.00–11.00, Aula Duża A

WGS-01 Diagnostic challenges in modern breast pathology

Chairpersons: I. Ellis, United Kingdom
W. Domagala, Poland

001

Novel variants of lobular intraepithelial neoplasia

*F. Tavassoli**
*USA

002

Immunophenotypic molecular subtypes of breast cancer in patients with constitutional mutations

*W. Domagala**
*Pomeranian Medical University, Dept. of Pathology, Szczecin, Poland

Objective: The following topics will be outlined: (1) Evolution of the histologic classification of breast cancer; (2) The influence of immunohistochemistry on the histologic classification of breast cancer; (3) The molecular classification of breast cancer; (4) Attempts to agree on the immunophenotypic molecular classification of breast cancer; and (5) The influence of patient's constitutional molecular background on the immunophenotypic molecular subtypes of breast cancer: prognostic significance—therapeutic implications.

Method: This lecture is to be said in Session: Diagnostic Challenges in Modern Breast Pathology, Sept 1st 08:00–11:00. The entire abstract is above in the first box. I am sending this abstract also to Dr. Rys to Krakow. Please note that the title is slightly changed as compared to the primary version.

003

Breast lesions mimicking malignancy

*Z. Varga**
*Switzerland

Cell types arising normally in the breast can occasionally exhibit peculiar overgrowth or nuclear features mimicking malignant cells and thus representing potential diagnostic pitfalls. Myoepithelial cell hyperplasia can reach an extensive mass and imitate lobular neoplasia or ductal carcinoma in situ with pagetoid spread. Ductal epithelial cells with apocrine phenotype can appear, with highly worrisome nuclear features making the distinction between a high-grade ductal carcinoma in situ and a banal apocrine change at times quite challenging. The evidence of a preserved myoepithelial layer is a commonly accepted morphological criterion for noninvasive processes in the breast. The distinction between adenosis, highly differentiated ductal carcinomas and cancerisation of the lobules through in situ ductal carcinoma can be easily done by decorating myoepithelial cells in immunohistochemical reactions. An important exception to this rule is, however, the benign microglandular adenosis, which completely lacks the outer myoepithelial layer. Intraductal epithelial proliferations can be quite florid in their extension. The question whether they represent atypical hyperplasia resp. ductal carcinoma in situ or appear simply within the scope of a florid intraductal hyperplasia occurs often in daily diagnostic service. The use of a distinct and technically reliable diagnostic panel (E-cadherin, basal cytokeratins, hormone receptors, Her2 immunohistochemistry and in situ hybridisation, collagen IV reaction, Ki-67, S-100 and myoepithelial markers) can clear the situation in most of these difficult cases and thus can lead to the correct diagnosis.

004

Immunohistochemical differentiation of intraductal breast lesions

*W. P. Olszewski**
*Center of Oncology, Dept. of Pathology, Warsaw, Poland

Objective: Immunopathological methods, particularly immunohistochemistry (IHC), are now routinely used in all invasive breast carcinoma cases as part of the evaluation of predictive factors (ER, PgR, and HER2). Other IHC-detected antigens are used for the determination of invasiveness (e.g. p63 and SMA), searching of primary (e.g. GCDFP15 and mammaglobin) or in scientific field as surrogates of genetic subtyping (ER, PgR, HER2, EGFR, LMWCK, HMWCK). Increasing breast cancer screening on one hand and advanced surgical methods of biopsy (e.g. core biopsy, vacuum-assisted core biopsy) on the other produce a growing number of pathology material in which pathologists must diagnose on the basis of scarce tissue. Most of these cases are noninvasive. However, the spectrum of possible therapies for noninvasive lesions includes observation on one side and mastectomy with radiotherapy on the other.
Method: Therefore, determination of the exact nature of intraductal breast lesions has to be precise and sure. It generates the use of IHC antibodies in intraductal breast lesions.
Results: In some cases, results of such approaches create new entities. An example of such situation is differential diagnosis between CLIS and DCIS. In a small number of cases, the interpretation of obtained stains (E-cadherin and CK34BE12) adds entities like positive hybrid lesion and negative hybrid lesion.
Conclusion: The aim of the lecture was the presentation of increasingly used IHC antibodies in intraductal breast lesions and its correlation with histological features of usual ductal hyperplasia, atypical ductal hyperplasia, lobular neoplasia, ductal carcinoma in situ, and columnar lesions (CCC, CCC+Atypia, CCH, CCH+Atypia). The other topic is IHC expression of surrogates of genetic subtyping in carcinoma in situ.

005

Low-grade and high-grade breast cancer pathways

*I. Ellis**
*United Kingdom

006

TNM7

*G. Cserni**
*Hungary

Background: The TNM, i.e. the tumour node metastasis classification of malignant tumours, is about 70 years old and was last updated in 2009. Although the oncology and pathology communities are expected to use this modified version from 2010, the Breast Working Group and the Organizing Committee has considered including a brief review of the topic in keeping with the educational aspects of the Intercongress Meetings of the European Society of Pathology.
Method: The lecture will briefly summarize the basic concepts of the TNM classification in general and will focus on the new aspects which are of interest to pathologists dealing with breast cancer specimens. Some former proposals to modify the TNM will also be discussed, including the European Institute of Oncology proposal to abolish the present categories and replace them with coded prognostic data reporting and the replacement

of the nodal categories based on the number of involved lymph nodes by categories based on the lymph node ratio (proportion of involved and examined nodes).
Results: NA.
Conclusion: The TNM has been criticized for not providing enough prognostic information to guide treatment. However, it must be kept in mind that the TNM was not devised for making treatment decisions solely on the basis of the information it provides but for reporting the anatomic extent of disease in a categorical format. TNM7 still fulfils this aim. No system is perfect, and TNM7 is not an exception to this. Should we use it, let us use it as correctly and uniformly as possible.

Wednesday, 1 September 2010, 08.00–11.00, Aula Duża B

WGS-02 Gastrointestinal stromal tumours

Chairpersons: M. Miettinen, USA
J. Lasota, USA

001
Prognostication and differential diagnosis of GIST: an update

*M. Miettinen**
*Armed Forces Inst of Pathology, Soft Tissue and Orthop Pathol., Washington, DC, USA

Objective: The precise application of modern targeted treatment of gastrointestinal stromal tumors (GISTs) requires accurate GIST diagnosis and prognostication.
Method: This review is based on existing literature on GISTs and the author's personal experience.
Results: GIST is defined as KIT or PDGFRA-driven mesenchymal tumor of the GI-tract, with a characteristic set of histologic features (spindle cell or epithelioid), and generally by immunohistochemical positivity for KIT (CD117). DOG1/Ano1 is a helpful additional marker for the positive identification of GIST. Current prognostication is based on mitotic activity, expressed as mitotic figures per 50 high-power fields in an area of 5 mm^2 and on tumor size. Different criteria are applied for gastric and intestinal GISTs because the latter, even with the same parameters, have a more malignant behavior. In the differential diagnosis of GIST, one has to consider the following facts: wide histologic variation (especially in gastric GISTs), the fact that GISTs are the most common mesenchymal tumor in the GI tract and whole abdomen, and occurrence of GISTs virtually anywhere in the abdomen and rarely in distant peripheral metastatic sites. Tumors that have to be considered in the differential diagnosis especially include true smooth muscle tumors, glomus tumor, gastrointestinal schwannoma, clear cell sarcoma, synovial sarcoma, desmoid, inflammatory fibroid polyp, inflammatory myofibroblastic tumor, and the newly described plexiform fibromyxoma and gastroblastoma.
Conclusion: Detailed histologic examination supported by immunohistochemistry and KIT/PDGFRA mutation analysis will yield optimal diagnostic accuracy.

002
Clinical significance of KIT and PDGFRA mutations in gastrointestinal stromal tumours, inflammatory fibroid polyps and mucosal malignant melanomas

*J. Lasota**
*USA

GISTs are the most common mesenchymal neoplasms of the gastrointestinal tract and represent a morphological and biological continuum from small, microscopically detected benign tumors to large sarcomas. KIT or platelet-derived growth factor receptor α (PDGFRA) oncogenic mutations are a typical molecular feature of GIST. KIT and PDGFRA genes map to chromosome 4q12 and encode highly homologous transmembrane glycoproteins. Activation of KIT regulates important cell functions, including proliferation, apoptosis, chemotaxis and adhesion. Imatinib mesylate that specifically inhibits ABL, KIT, and PDGFRA receptor tyrosine kinases has been successfully used in the treatment of clinically advanced, unresectable, and metastatic GISTs. Majority of KIT exon 11 mutants respond well to imatinib, while KIT exon 9 mutants are less sensitive to imatinib and require more aggressive treatment. Also, tumors with the PDGFRA Asp842Val substitution, which corresponds to imatinib-resistant KIT Asp816Val mutation reported in human mastocytosis, are resistant to imatinib. Although many patients benefit from imatinib treatment, resistance often develops due to secondary KIT or PDGFRA mutations or KIT locus amplification. Sunitinib mesylate, a multi-targeted inhibitor of KIT, PDGFRs, VEGFRs, FLT3, and RET receptor tyrosine kinases and other kinase inhibitors have been used for the treatment of imatinib-resistant GISTs. The type of secondary KIT mutation can indicate response to sunitinib treatment. KIT mutations were identified in mucosal MMs and PDGFRA mutations in gastric and intestinal IFPs. Although the presence of KIT mutation does not necessarily correlate with KIT expression in MM, an inhibition of KIT receptor tyrosine kinase might still be beneficial in the treatment of selected cases.

Wednesday, 1 September 2010, 08.00–11.00, Aula Średnia A

WGS-03 Novelties in inflammatory skin diseases

Chairpersons: D. Massi, Italy
W. Biernat, Poland

001

Infectious dermatites

*J. E. Calonje**
*Harrow, United Kingdom

002

Histopathology of alopecia: Getting to the root of the problem

*C. Stefanato**
*St. John's Institute for Dermatopathology, London, United Kingdom

The histopathology of primary non-scarring and scarring alopecias may be complex. The reason for this is that often, the scalp biopsy provided is inadequate, and the clinical history and pattern of the alopecia are not provided to the dermatopathologist. Increased diagnostic yield may be achieved by the combined use of transverse and vertical sections. This requires good communication between the dermatologist performing the biopsies, the dermatopathology laboratory and the dermatopathologist. Common forms of scarring alopecias include the lymphocytic, (discoid lupus erythematosus, lichen planopilaris, central centrifugal cicatricial alopecia, pseudopelade of Brocq), the neutrophilic, (folliculitis decalvans, dissecting folliculitis), and the mixed, (acne keloidalis) entities. Among the non-scarring alopecias are androgenic alopecia, telogen effluvium, alopecia areata, trichotillomania and traction alopecia. In all cases of primary alopecia, a multi-team approach involving the dermatologist for adequate tissue sampling, the laboratory for appropriate laboratory processing and the dermatopathologist, together with pertinent clinical information affords the best opportunity of arriving at the correct diagnosis.

003

Graft versus host disease: an update

*D. Massi**
*University of Florence, Dept. of Critical Care Medicine, Italy

Our current understanding of histopathological features of cutaneous graft versus host disease (GvHD) is largely based on experience gained during decades of ablative HSCT, in which day 100 was regarded as the temporal cutoff for the separation of acute vs. chronic GvHD, and traditionally, acute and chronic GvHD were considered to be distinct histopathological entities. Acute GVHD in the skin has been generally described as an interface dermatitis, with vacuolar basal alteration, dyskeratotic (apoptotic) keratinocytes in the epidermis and/or adnexal structures, scant dermal lymphoid infiltrate, and lymphocytic satellitosis. In contrast, chronic GvHD has commonly been reported as either lichenoid or sclerodermoid GVHD, characterized by homogenization of dermal collagen bundles, with little or no epidermal involvement. At variance with this dichotomic picture, histology of cutaneous GvHD following reduced intensity conditioning regimens is protean, displaying a variable constellation of histopathological alterations. These changes frequently blur the distinction between clear-cut clinical GvHD categories, as known in the classical ablative HSCT setting. Sampling errors and other technical issues may occur and may significantly affect the number of false negative cases. The utility of skin biopsies varies according to the pathologist's experience and to the prevalence of GvHD. Clinicians' overall perception is that undertreatment of GvHD is much worse than overtreatment of patients without GvHD. Therefore, early immunosuppressive therapy in patients with a skin eruption often precedes the results of the histopathological analysis. The pathogenesis and pathology of GVHD will be illustrated by elucidating the selective epithelial targets in the afferent stage and cellular response in the efferent stages.

004

Panniculitis: a practical diagnosis approach

*M. Fernandez-Figuera**
*Spain

005

Pathology of cutaneous drug eruptions with focus on the novel effects of targeted therapy for cancer

*A. Lazar**
*USA

Background: The untoward effects of pharmaceutical agents on the skin are an important source of morbiditary and can complicate the ideal selection of agents for our patients.
Method: Our practice in a large cancer center sees drug eruptions in the context of cancer treatment and must often be evaluated within a differential diagnosis with graft versus host disease on our bone marrow transplant service.

Results: With the advent of specific, targeted therapy for cancer, a new spectrum of interesting and unusual drug eruptions has emerged related to the mechanism of action of these agents.
Conclusion: This presentation will focus on the unique cutaneous effects arising in association with targeted therapy for cancer.

Wednesday, 1 September 2010, 08.00–11.00, Aula Średnia B

WGS-04 Native kidney disease: kidney and tumours
Chairpersons: M. Picken, USA
M. Wagrowska-Danilewicz, Poland

001
Nephrotic syndrome and neoplasia
M. Wagrowska-Danilewicz, M. Danilewicz*
*Medical University of Lodz, Dept. of Nephropathology, Poland

Background: The adult onset of nephrotic syndrome has been reported to be associated with underlying cancer in about 10% of cases. It is believed that the glomerular injury in many instances is due to the deposition of immune complexes in the glomerular capillaries. On the other hand, the course of illness in many cancer patients is complicated by infections, which may also be involved in the pathogenesis of glomerular injury. The malignancy is usually found simultaneously with, shortly before, or within a short time after the diagnosis of glomerulopathy. The glomerular lesion of paraneoplastic nephrotic syndrome usually presents as membranous nephropathy in patients with solid tumors, particularly adenocarcinomas of the lung and gastrointestinal tract. Minimal change disease is strongly associated with Hodgkin's lymphoma, and the most common lesions observed in patients with chronic lymphocytic leukemia are membranoproliferative glomerulopathy and membranous nephropathy. Extracapillary crescentic glomerulopathy, IgA nephropathy and focal segmental glomerulosclerosis are rarely associated with neoplasia.
Method: We present six cases of nephropathies associated with malignancies: lung, gastric and colon cancer, as well as Hodgkin's lymphoma and chronic lymphocytic leukemia. All patients were over the age of 55 and were presenting nephrotic syndrome.
Results: Membranous glomerulopathy, minimal change disease and membranoproliferative glomerulopathy were established on the basis of light microscopy, immunofluorescence and electron microscopy. The renal lesions in paraneoplastic nephropathies did not differ from idiopathic forms of glomerulopathies.
Conclusion: The search for malignancy is warranted in all patients over the age of 55 presenting with nephrotic syndrome, particularly in cases of membranous nephropathy. (This work was supported by grant of Polish Ministry of Science and Higher Education NN402 088735).

002
Plasma cell dyscrasia and renal amyloidosis: what can we learn from basic science?
*P. Westermark**
*Sweden

003
Plasma cell dyscrasia: amyloidosis, LCDD and cast nephropathy
*M. Picken**
*USA

The clinical and morphologic spectrum of plasma cell dyscrasia continues to expand. While in the most advanced stage of this disease patients are diagnosed with overt multiple myeloma, at the opposite end of the spectrum, MGUS is more typical. Not infrequently, the underlying disease is clinically silent or can be associated with renal symptoms that prompt further investigation and thus underscore the role of renal pathologists in the early detection of the underlying process. While light chain cast nephropathy affecting distal tubules and light chain amyloid or non-amyloidotic LCDD are well recognized, in recent years, attention has focussed on the pathology affecting the proximal tubules and interstitium. Proximal tubulopathy associated with an underlying plasma cell dyscrasia may be very subtle and associated with the formation of crystals or only with light chain restriction detectable in the lysomes within proximal tubules. Interstitial nephritis may be associated with a rather nonspecific morphology, and only an enhanced index of suspicion may lead to the discovery of an associated light chain restriction. Thus, renal pathologists are in an advantageous position to diagnose the underlying process at an early stage. Therefore, it is critical to perform a full investigation of the kidney biopsy, including immunofluorescence studies that employ testing for immunoglobulin light chains, as well as electron microscopy. Renal pathologists should be alerted particularly by biopsies that yield seemingly nonspecific or non-diagnostic results. Investigations with Congo red stain, careful evaluation of immunofluorescence studies, and electron microscopic analysis of organized and related deposits are necessary.

004

Non-Hodgkin lymphomas and PTLD in renal biopsies

*A. C. Feller**
*Germany

005

Cytostatic drug and radiation-associated renal lesions

*H. Regele**
*Austria

Malignant tumors might impair renal function in multiple ways. In addition to a direct impact of the tumor or its derivatives like light chains in the case of multiple myeloma, renal injury might also be a consequence of therapeutic interventions. Certain cytostatic drugs (especially platin-based compounds), but also irradiation, are long known to cause renal tissue injury. The damage might affect both the tubulointerstitial space and the vasculature. In addition, it was shown that recently introduced biological agents might lead to additional patterns of drug-induced injury. Blocking the action of VEGF in order to interfere with tumor-induced neoangiogenesis might for example cause renal thrombotic microangiopathy. In my talk, I will discuss the histological patterns of injury caused by the different treatment modalities and the underlying pathogenic mechanisms. The knowledge of these mechanisms might help prevent renal damage.

Wednesday, 1 September 2010, 08.00–11.00,
Sala Wystawowa A

WGS-05 Pitfalls in non-neoplastic lung pathology

Chairpersons: L. Carvalho, Portugal
R. Langfort, Poland

001

Pitfalls in infectious lung pathology

*K. Grunberg**
*The Netherlands

002

Pitfalls in pediatric lung pathology

*W. Timens**
*University Medical Center, Dept. of Pathology, Groningen, The Netherlands

Background: The morphological presentation of lung problems in infants and children is not always pathognomonic for a specific diagnosis. Basic patterns are not always easily recognized and may be different from the adult situation, even with the same classifying diagnosis.
Method: As lung neoplasms are seldom seen in children, this overview will focus on non-neoplastic lung disease.
Results: A main category is interstitial lung disease. The main adult patterns of interstitial disease can also be observed in children, although UIP, DIP and NSIP are rather rare and not always with similar aetiology (e.g. desquamative interstitial pneumonia, associated with smoking in adults). The distribution of the interstitial changes is in those cases similar to adults, but morphological presentation may be different because of a different lung structure. Some interstitial patterns are specific for childhood: for example, chronic pneumonitis of infancy, pulmonary interstitial glycogenosis (formerly cellular interstitial pneumonitis) and neuroendocrine cell hyperplasia of infancy, but even so, these are not always easy to discern form the other patterns. If a (wedge) lung biopsy is taken at late stage or only from the most severe abnormalities, a characteristic pattern may be obscured by nonspecific common final pathway changes like fibrosis.
Conclusion: All diffuse lung diseases in infancy can be caused by many etiologic factors. This means that although there are several hallmarks that can aid in making a proper pathological diagnosis, similar as in adults, proper clinical and radiological information is essential.

003

Pitfalls in vasculitis/vascular lung pathology

*F. Calabrese**
*Italy

004

Pitfalls in smoking-related lung diseases

*L. Carvalho**
*Faculdade de Medicina Coimbra, Dept. de Anatomia Patologica, Portugal

Objective: Tobacco and cigarettes are implicated in multisystemic carcinogenesis due to carcinogenic components that induce maintained inflammation, endocrine deregulation and genetic alterations in continuum. As ambience influence acts through a multifactorial behaviour gathering smoking, obesity and dietary mistakes, being sedentary, alcoholism, sexual promiscuity, toxic addition and general pollution, the isolated influence of smoking is reinforced. Smoking-related non-tumoral lung diseases comprise COPD, RB-ILD, DIP, ACIF/IBIP, HX (Langerhans cell histiocytosis), sarcoidosis (not consistent), eosinophilic lung, constrictive and lymphocytic bronchiolitis, emphysema together with sub-pleural and interstitial fibrosis UIP-

like or NSIP-like, as well as possible combinations of all these diseases and patterns. Smoking also increases or exacerbates infections (TP and other bacterial pneumonias, varicella pneumonia and influenza), pulmonary hemorrhage and pneumothorax and vascular walls fibrosis.

Conclusion: As smoking-related interstitial lung disease (SRILD) has been proposed to encompass clinical and radiological coordination, it means that molecular pathology achieves its role to define therapy as it is being tried in cancer. Despite observed morphology, it is possible to localize the defence response along the bronchial–bronchiolar–alveolar pathway as epithelial and mesenchymal cells develop particular ways of adaptation, either by EMT and MET. As inflammation stands through fibroblasts, cellular mTOR and macrophage activation, also epithelial hyperplasia and metaplasia, install both at bronchial and pulmonary stem cells pool. Smoking-related lung diseases are not consistent with a spectrum but rather with expertise to report delicate inflammatory changes in morphology that diverge to interstitial (and vascular) incapacities and/or to pre-neoplastic epithelial lesions and bronchial-pulmonary carcinoma.

005

Pitfalls in interstitial pneumonias

*H. Popper**
*Medical University of Graz, Institute of Pathology, Austria

Interstitial pneumonias can be classified into UIP, DIP, NSIP, OP, and DAD. Whereas DAD as an acute interstitial pneumonia is easily separated and diagnosed, due to the presence of alveolar damage and hyaline membranes, the other entities might cause problems in diagnosing. The most important distinction is between UIP and the other entities because UIP is a rapid progressive and deteriorating disease with a high mortality rate.

Usual interstitial pneumonia is histologically characterized by the presence of fibroblastic foci, cystic changes called honeycombing, areas of fibrosis and scars, and a temporal heterogeneity, which results in areas of early inflammatory changes, late changes, and also areas of uninvolved lung parenchyma. The disease usually starts from the lung periphery in a patchy distribution pattern. The lower lobes are usually more affected than the upper lobes.

The disease starts with tiny lesions where the surface epithelium is destroyed. Underneath myofibroblasts are proliferating and the stroma is changed into a myxoid stroma composed of acidic mucopolysaccharides. These lesions are called fibroblastic focus. This repair process is accompanied by a regeneration of pneumocytes, which usually undergo metaplastic changes (bronchiolization, cuboidal metaplasia, stem cell proliferation). Finally, this process results in fibrosis. Several fibrotic areas might finally confluent into a scar tissue. Fibroblastic foci are not only seen in alveolar septa but also in bronchioles and small bronchi. Scarring in these airways results in the obstruction of the airways, and the periphery of these airways undergoes a cystic dilation, accompanied by metaplasia of the epithelium; again, bronchiolization and metaplasia, even squamous metaplasia, can occur. Mucus produced by the epithelium cannot escape, and therefore, the cystic spaces increase in size, being finally visible at CT magnification. These cystic air spaces form the honeycomb lesions. In UIP, fibroblastic foci can be seen in the different stages of development: early stages with lots of myxoid matrix, immature collagen, and many proliferating myofibroblasts and old stages with lots of mature collagen and fibrocytes. Honeycomb lesions may present in a florid stage where some fibroblastic foci can be seen within the cysts, or older lesions with lots of mucus, or old lesions with secondary bacterial pneumonia overlying UIP. Also, the scars can be old, devoid of inflammatory infiltrates, or younger with scattered fibrocytes and lymphocytes. This together forms the temporal heterogeneity of UIP.

Fibroblastic foci are often used as the dominant and even sole diagnostic criterion for the diagnosis of UIP. This can result in an incorrect diagnosis since other interstitial diseases can also present with fibroblastic foci.

In the presentation, we will review several cases presenting with fibroblastic foci, which can closely mimic and might give rise to an incorrect diagnosis of UIP.

006

Pitfalls in drug-induced lung pathology

*B. Murer**
*Italy

Wednesday, 1 September 2010, 08.00–11.00,
Sala Wystawowa B

WGS-06 Molecular pathology on formalin-fixed paraffin-embedded tissues—European Network of Archive Tissue biobanking for translational research

Chairpersons: G. Stanta, Italy
F. Bosman, The Netherlands

001

Biobanking and biomolecular resources research infrastructure

*K. Zatloukal**
*Medical University of Graz, Institute of Pathology, Austria

002

The role of pathologists in translational research for patients and industry

*M. Dietel**
*Universitätsmedizin Charité, Berlin, Germany

Background: Due to continuous technical developments and new insights into the complexity of many diseases, e.g. cancer, molecular pathology is rapidly growing gaining center stage in the clinical management of tumors and pharmaceutical development of new anti-cancer drugs. Activated signaling components are the targets for the newly developed inhibitors, e.g. small molecules (gefitinib, erlotinib) and therapeutic antibodies (panitumumab, cetuximab). However, the application of the compounds in clinical trials has revealed promising results only when predictive procedures have been available for determining which patients will benefit from targeting therapy, so-called eligibility or predictive tests, e.g. Her2 in breast cancer, KRAS and EGFR mutations in colorectal cancer and non-small cell lung cancer. For the pharmaceutic industry, predictive tissue-based assays are of increasing importance in the development of new targeted drugs. FDA and EMEA stressed this issue several times during the process of approval, and pathology based analyses will become an essential factor in drug development. **Conclusion:** For pathology, the situation to become a partner in the clinical decision, which drug shall be given to the patient and which assay can be made available for a new, is a chance and a challenge in one. The issues to be solved are: (1) Morphology, in particular immunohistochemistry and in situ hybridization, must become measurable, e.g. by virtual microscopy. (2) The molecular assays must be done absolutely reliably and be reproducible. (3) Quality control, i.e. ring trials/round robin tests, must be passed by all active labs. If these challenges (and some others) are met, molecular pathology is facing an excellent development.

003

The role of biobanking and translational research for the pathologist

*F. Carneiro**
*IPATIMUP, Porto, Portugal

Among biobanking initiatives, tumour banks play a pivotal role in biomedical research. The general aim of a tumour bank is to acquire neoplastic and control non-neoplastic samples in standardized conditions for research (basic, clinical or translational). A tumour bank is a vital new resource for cancer research providing high-quality, well-characterized tissue. It is possible for pathologists to collect fresh tissue prospectively during their routine dissection procedures. In this way, the specimens can be optimally sampled and stored for both diagnosis and research purposes. Ideally, specimens are sampled immediately after surgery, prior to fixation, to ensure optimal preservation of proteins and nucleic acids. Retrospective collection of tumour tissue for study and banking purposes is feasible also because in most countries, pathology laboratories have been legally obliged to file, for at least some years, the formalin- fixed and paraffin-embedded samples that were analyzed. Over the last decade, tumour banks acquired a pivotal role in translational research in the field of oncology, providing tools for the evaluation of new predictive factors; evaluation of the value of a known target in a new entity; search for new therapeutic targets; validation of new diagnostic markers; and implementation of new diagnostic procedures, namely development of tissue-based diagnostic tests for guidance of therapy with new drugs introduced in clinical practice. In this scenario, it is a priority to emphasize the central role that pathologists play in translational research, specifically in tumor banking, by the establishment of a bridge between clinicians and basic researchers.

004

Limits and perspectives for standardization of tissue processing

*G. Bussolati**
*Italy

The paper by Pupo and colleagues is a well-conducted study on the role of the gasotransmitter hydrogen sulphide in angiogenesis. The increased effect of hydrogen sulphide in tumor-derived endothelial cells and its possible effect as a VEGF second messenger are of interest in the field of tumor angiogenesis. Criticisms: (a) It would be important to show the specificity and non-toxicity of hydrogen sulphide synthesis inhibitor on endothelial cells. (1) Experiments using the cystathionine lyase inhibitor alone on TEC proliferation and cytotoxicity are required. (2) Experiments testing the effect of cystathionine lyase on TEC motility induced by pro-angiogenic factors (i.e. FGF, HGF, nitric oxide donors, etc.) are suggested. (b) The authors should speculate on the possible reason(s) explaining the different responses of different endothelial cell types. (c) In the references, the same author (Fiorio Pla) is quoted in two different ways. (d) In "Material and Methods", the origin of a furnisher (Lonza) should be specified.

005

Standardization and controls of methods for molecular analysis in archive tissues

*G. Hoefler**
*Medical University of Graz, Institute of Pathology, Austria

Background: Formalin-fixed paraffin-embedded (FFPE) samples represent the vast majority of tissue specimens available from tissue archives, available routine diagnostics, as well as research purposes. These tissues vary among institutions with respect to pre-fixation time, formalin fixation time, concentration and buffer systems used. Therefore, it is of utmost importance to standardize the methods for molecular analyses to obtain consistent and comparable results.
Method: The first and maybe the most important step is the isolation of nucleic acids (DNA, RNA) from FFPE samples. In a recent multi-centric study performed by 13 European laboratories of the IMPACTS group, the results obtained by different laboratories varied significantly, even when the same commercial kit was used. The DNA extraction protocols used by the laboratories ranged from homemade protocols with and without purification steps to commercially available kits. The extractions were performed using the same FFPE specimens.
Results: For array applications or tests that require accurately determined DNA input, silica-based adsorption columns for DNA recovery are recommended. For RNA extractions, the best results were obtained for chromatography column-based commercial kits, with respect to quantity and quality. Quality testing resulted in the successful amplification of 200- to 250-bp PCR products from most tissues. Modifications of the protocol, especially with regard to proteinase-K digestion, led to significant improvements, also for the performance of commercial kits.
Conclusion: The results emphasize the need for standardization and control of methods for molecular analysis in archive tissues to allow the generation of valid and comparable results in both diagnostic and in research settings.

006

Approaches to the development of a European Network of Archive Tissues

*G. Stanta**
*University of Trieste, A.C.A.D.E.M., Italy

FFPE tissues taken from patients are stored, sometimes for a very long time, in the pathology archives. With this material, it is possible to do very important translational research. Any strategy is possible, such as that of large number analysis or rare entity collections. There is the necessity to transform our pathology biorepositories into a biobanking network. This can be done through a European organization that could be developed within the ESP and starting from the "IMPACTS" groups, which gained a large experience in molecular analysis validation and standardization of this kind of tissues. A European archive tissue biobank network depends on the willingness of the pathologists to participate, considering that this can significantly improve the diffusion of molecular methods, translational research and consequently acceleration of clinical application of molecular methods among pathologists. This can be done by a voluntary and collaborative participation of pathologists in specific projects, as scientific collaborators. The pathology archives will always be clinical biorepositories, and their function is going to change into a biobank function after accepting participation in a specific project, with the anonymization of the personal data of the patients. The networking system is based on low-cost activities with an easy governance and management of the biobanking system and with guarantees of protection of human material and data. Qualification, education and training will be implemented by the network organization and with the collaboration of the ESP.

Wednesday, 1 September 2010, 08.00–11.00, Aula Mała

WGS-07 Hereditary cancer

Chairpersons: J. Lubinski, Poland
R. Scott, Australia
S. Narod, Canada

001

Clinical genetics of breast/ovarian cancers

*S. Narod**
*Women's College Research, Familial Breast Cancer, Toronto, Canada

002

Chemoprevention of hereditary cancers

*J. Lubinski**
*Pomerian Medical University, Szczecin, Poland

003

Response to therapy with cisplatin in BRCA1-positive breast cancer patients

T. Byrski, J. Gronwald, T. Huzarski, E. Marczyk, P. Blecharz, J. Mitus, K. Urbanski, J. Lubinski, S. A. Narod*
*Pomeranian Medical University, Szczecin, Poland

Background: The aim was to assess the frequency of complete pathologic response after neoadjuvant treatment with cisplatin chemotherapy in women with breast cancer and a BRCA1 mutation.
Method: Forty patients with breast cancer and a BRCA1 mutation, who presented with stage I to III breast cancer between December 2006 and March 2010, were treated

with cisplatin 75 mg/m^2 every 3 weeks for four cycles, followed by mastectomy and then conventional chemotherapy. Surgery occurred 3 weeks after the final dose of cisplatin. The excised breast tissue and lymph nodes were examined for the presence of residual disease. Pathologic complete response was determined by a review of surgical specimens.

Results: Forty patients were enrolled in the study. Twenty-four patients had tumors of >2 cm and 12 patients had positive lymph nodes at diagnosis. Thirty-seven patients completed four cycles of cisplatin and three patients completed two cycles. Clinical complete response was observed in 31 patients (77%). Pathologic complete response was observed in 29 patients (72%).

Conclusion: Platinum-based chemotherapy is effective in a high proportion of patients with BRCA1-associated breast cancers. Clinical trials are warranted to determine the optimum treatment for this subgroup of breast cancer patients.

004
Clinical outcome of ovarian cancer in respect to polymorphism of low-penetrance genes

E. Grzybowska, K. Tcza, J. Pamua-Piat, S. Jdru, E. Telka, Z. Kosza, B. Zema*
*Centre of Oncology-MSC Memoria, Dept. of Molecular Biology, Gliwice, Poland

Objective: We wanted to analyze the influence of modifying genetic factors on the risk of BRCA(+) and BRCA(−) breast and ovary cancers and on clinical parameters as the age of onset and survival.

Method: The study includes three hundred forty-eight anonymous healthy women (control group), 229 persons with ovary cancer, 45 persons with breast and ovary cancer (case group). The patients under study developed breast cancer prior to ovary cancer. RFLP and ASA PCR were used to analyze mutations in BRCA genes and polymorphisms in PGR, MDR1 and TP53 genes.

Results: The presence of allele T of 660 PGR significantly decreased the risk of second malignancy (breast cancer; OR= 0.44, p=0.039). Heterozygote CT of MDR1 gene C3435T and genotypes AG and AT of G2677T/A were at lower risk of developing ovary cancer. Genotype CT of C3435T polymorphism had protective effect against developing the second malignancy, while allele TT increased the risk of breast cancer. Carriers of allele C for TP53Arg72Pro polymorphism had a significantly lower risk of developing ovarian cancer (OR=0.72, p=0.04) The best survival was found for patients carrying germline mutation in BRCA1 gene; the worst survival was observed for the group of patients with breast and ovary cancer and without germline mutation in BRCA1 gene. Alleles of TP53Arg72Pro significantly modified the survival of the group of patients who were diagnosed with breast cancer prior to ovarian carcinoma. The worst survival was connected with rare allele C.

Conclusion: Polymorphic variants of PGR, MDR1 and TP53 genes under study modified more the clinical course and the risk of second malignancy than the risk of developing ovary cancer alone.

005
DNA testing for high risk of cancers

*B. Górski**
*Poland

006
Genetics of colorectal cancers

*R. Scott**
*Discipline of Medical Genetics, School of Biomedical Sciences, Newcastle, Australia

The study of rare inherited conditions with high penetrance that predispose to colorectal cancer has significantly advanced our knowledge of the genetic basis of disease and represents a paradigm that has served to better understand the molecular mechanisms that underlie the development of disease in persons who have no apparent genetic predisposition. Inherited predispositions to colorectal cancer can be divided into essentially two groups: those that present with a premalignant phenotype (such as familial adenomatous polyposis (FAP) and the hamartomatous polyposis syndromes) and those like hereditary non-polyposis colorectal cancer, Lynch syndrome and Muir–Torre syndrome. For predispositions where the underlying genetic basis of the disease is known, much has been learned about disease penetrance and the expected spectrum of disease as increasing numbers of mutation carriers are characterized. This has lead to the reclassification of some defined syndromes as a result of the inherited predisposition being identical. Improved knowledge about disease penetrance has also resulted in a number of questions being raised with respect to what factors influence disease expression in patients known to harbour a mutation in a colorectal cancer susceptibility gene. Of particular interest are additional genetic factors (termed modifier genes) that have been correlated with disease manifestation. More recently, the focus of attention has shifted from familial aggregations of disease where there is a well-defined inherited component to population studies aimed at identifying common low-penetrance disease alleles that are associated with small affect sizes. These studies are significant not only because they are identifying genetic factors associated with colorectal cancer risk but also potential

modifier genes that are important in modulating disease expression in persons who harbour a genetic predisposition.

Wednesday, 1 September 2010, 14.30–16.30, Aula Duża B

WGS-08 Gastrointestinal tract eosinophilia
Chairpersons: K. Geboes, Belgium
G. Klöppel, Germany

001
Eosinophils in the gastrointestinal tract: function and dysfunction
G. Pineton de Chambrun, P. Desreumaux, M. Capron*
*Association de Gastroentérol., HP Huriez, Lille, France

Eosinophil functions have been associated for a long time with effector activity in adaptive immune responses during parasitic infections and inflammatory processes in allergic manifestations as well as in mucosal responses. The production by eosinophils of a vast array of cytokines as well as their expression of innate receptors and mediators confer to eosinophils a unique contribution both in inflammatory and adaptive responses but also in immunoregulation and innate immunity. Eosinophils are customary inhabitants of the gastrointestinal tract, except for the oesophagus. Beneficial function of intestinal eosinophils was their ability to defend host against helminths. However, eosinophil accumulation in the gastrointestinal tract is a common feature of numerous gastrointestinal disorders, including inflammatory bowel diseases, intestinal vasculitis, gastroesophageal reflux disease and food allergy. Primary eosinophilic gastrointestinal disorders, defined as disorders that selectively affect the gastrointestinal tract with eosinophil-rich inflammation of unknown etiology, include eosinophilic esophagitis, eosinophilic gastroenteritis and eosinophilic colitis and are occurring with increasing frequency. Decrease in the rate of parasitic infections in the developed world is associated with a rise in atopic/allergic disorders and increased hypersensitivity responses to allergens and may represent a driving factor towards the recruitment and activation of gut eosinophils in those disorders. Although the pathogenesis of those disorders is still poorly understood, new findings on gastrointestinal eosinophil proliferation and migration mechanisms involving IL-5 and eotaxins provide a rationale for specific disease therapy. The present review will summarize knowledge on the physiology of gut eosinophils and will illustrate some aspects of eosinophilic disorders of the gastrointestinal tract.

002
Eosinophilic oesophagitis
M. Genevay-Infante, L. Rubbia-Brandt, A.-L. Rougemont*
*Switzerland

Gastro-oesophageal reflux disease (GERD) and eosinophilic oesophagitis (EE) are the two main non-tumoral diseases involving the oesophagus and can develop in both children and adults. The former, the more frequent, is present in up to 20% of the Western population. It is the consequence of a massive flow of gastric contents back into the oesophagus, leading to symptoms or organ damage. Eosinophilic oesophagitis, first reported in 1978 and defined as a specific clinicopathological entity in 1993, is characterized by eosinophilic infiltration of the oesophagus. Its cause or causes are unknown despite its frequent association with an allergic setting. In 2007, a consensus meeting of the American Gastroenterological Association Institute and the North American Society for Paediatric Gastroenterology, Hepatology and Nutrition recommended diagnostic criteria for EE. Despite this increased knowledge of both pathologies, GERD and EE present considerable clinical and pathological overlap. Their distinction remains, however, crucial as their clinical outcome and treatment modalities are quite different. The pathologist has to be aware that the diagnosis cannot be based solely on pathological features. The aim of this presentation was to provide pathologist both clinical and histological diagnostic clues in order to propose the most accurate diagnosis and treatment.

003
Eosinophilic enterocolitis
*A. Driessen**
*The Netherlands

Eosinophilic gastro-enterocolitis, consisting of eosinophilic gastroenteritis (EG) and eosinophilic colitis (EC), belongs to eosinophilic gastrointestinal disorders whose diagnosis is based on gastrointestinal symptoms, an eosinophilic infiltration in the gut and the exclusion of secondary causes of eosinophilia (Collins MH 2009, Furuta GT 2008, Shifflet A 2009). EG and EC are both rare entities. EG is a predominantly benign disease occurring at any age (peak incidence, third–fifth decade). It may affect any part of the gastrointestinal tract, most commonly the stomach (25–80%) and small intestine (28–100%). EC is more uncommon, involving the colon and less frequently the rectum. Symptoms vary in function of the affected layer of the wall: mucosa (25–100%): abdominal pain, diarrhea; muscular layer (13–70%): intestinal obstruction; serosa (12–40%):

ascites. The ethiopathogenesis is not fully clear, but is sometimes related to a food allergy. Diagnosis requires a panendoscopy with biopsy. Histologic examination may, however, be hampered by the patchy distribution of the mucosal inflammation and the presence of a normal mucosa in association with muscular/serosal involvement. In this case, a full-thickness biopsy may be necessary for diagnosis. Microscopic features are the presence of numerous eosinophils in the lamina propria, extending into the epithelium (crypt abscesses) and the submucosa, associated with architectural abnormalities. In mural or serosal EG/EC, eosinophils are predominantly situated in the muscular or peritoneal layer. Differential diagnosis includes other disorders with eosinophilia, e.g. inflammatory bowel disease, celiac disease, parasitic infection, vasculitis. Several treatment modalities have been described in literature, such as restriction diets, corticosteroids or antihistamines. Surgery is restricted to the resection of stenosed segments in mural enterocolitis.

004

Systemic eosinophilic disorders and the GI tract

*A. Hoorens**
*UZ Brussel, Dept. of Pathology, Belgium

Secondary eosinophilic disorders, including infectious, inflammatory, hypersensitivity and neoplastic illnesses, always require exclusion before making the diagnosis of eosinophilic oesophagitis/gastroenteritis/colitis, particularly in case of peripheral blood eosinophilia. Parasite infections are well known to present with eosinophilia of the gastrointestinal mucosa. A drug-induced aetiology should also always be considered. Gastrointestinal eosinophils may be a feature of connective tissue disease, especially scleroderma, and can accompany vasculitis in polyarteritis nodosa and Churg–Strauss syndrome. With very pronounced peripheral eosinophilia, hypereosinophilic syndrome (HES) with gastrointestinal involvement, clonal eosinophilia and lymphocytic variant hypereosinophilia should be considered. HES is defined as eosinophilia ($\geq 1.5 \times 10^9$/L) for at least 6 months, no known cause of eosinophilia, and evidence of organ involvement. The gastrointestinal tract is affected in 25%. When only the digestive tract is involved, it may prove difficult to distinguish HES and eosinophilic gastroenteritis. Involvement of the intestinal tract in HES has been associated with limited prognosis and, in some, a fatal outcome. Eosinophilic infiltration of the GI tract in HES should be distinguished from eosinophilic infiltration of the GI tract in lymphocytic variant hypereosinophilia where eosinophilia is associated with phenotypically abnormal and/or clonal T lymphocytes. Clonal eosinophilia is characterized by neoplastic proliferation of eosinophils as part of an underlying myeloid malignancy and can accompany any one of the myeloid malignancies. Two distinct subcategories of clonal eosinophilia are recognized: chronic eosinophilic leukaemia, NOS and myeloid/lymphoid neoplasms with abnormalities involving PDGFRA/PDGFRB or FGFR1. Accurate diagnosis of all these conditions requires the correlation of endoscopic and biopsy findings together with a careful clinical examination.

005

GI eosinophilia in paediatrics

*M. Walker**
*United Kingdom

Eosinophils are powerful innate immune cells home to the gastrointestinal tract and play a major role in both host immunity to luminal pathogens and maintenance of homeostasis of intestinal epithelium in the normal gastrointestinal tract (GIT). Normal numbers at different GIT sites are defined in children. However, if in excess, eosinophils may play a key role in the pathogenesis of disease of the GIT, including primary eosinophilic gastrointestinal disease (EGIDs). Data from the World Wide Web-based registry of EGIDs show that these have a strong genetic and allergic component, 80% having coexistent atopic disease, 62% food sensitisation and 16% with a family member with similar disorders. The most studied EGID in children is eosinophilic oesophagitis; symptoms include feeding intolerance and GERD symptoms. Endoscopy shows a characteristic linear furrowing, and histological features include ≥15 eosinophils/1 HPF (peak count). There is a male preponderance and an allergic and genetic component. Around 7–8% of children are affected by food allergy, most commonly cow's milk allergy and egg and peanut allergies which may manifest as eosinophil-induced GI disorders. Eosinophilic gastroenteritis is manifest as allergic eosinophilic gastroenteritis, allergic proctocolitis and food protein-induced enterocolitis syndrome (FPIES). Eosinophilia is also seen in helminth infection, inflammatory bowel disease, coeliac disease and graft vs. host disease where eosinophil density can correlate with disease severity. Recent work has implicated duodenal eosinophilia in functional conditions, particularly paediatric dyspepsia, with success in treatment aimed at the eosinophil–mast cell axis.

006

Parasites and eosinophils in the GI tract

*G. De Hertogh**
*UZ Leuven, Dept. of Pathology, Belgium

Background: Human endoparasites belong to four groups: the protozoa and the nematodes, cestodes and trematodes. About 12 protozoan and 15 helminthic species can cause gastrointestinal (GI) pathology. Eosinophils are frequently associated with the lesions present in these conditions.
Method: PUBMED-based review of the associations between GI parasites and eosinophils.
Results: Protozoan parasites may be located on the mucosal surface or in the bowel wall in an intra- or extracellular position. Eosinophils can be increased in blood and tissues, notably with *Isospora belli*. *Dientamoeba fragilis* infection may even masquerade as allergic colitis. Invasive amoebiasis (*Entamoeba histolytica*) on the contrary has been associated with a decreased number of tissue eosinophils. Worms may be observed in the egg, larval or adult stage buried in or attached to the bowel wall. Helminthic infections are classically associated with blood and tissue eosinophilia, which can be limited to the place of attachment of the organisms. The number of eosinophils in biopsies can be very high and even suggestive of primary eosinophilic enteritis, e.g. in trichuriasis, anisakiasis and enterobiasis, and with *Ancylostoma caninum* and *Angiostrongylus* infections. Alternatively, chronic inflammation with eosinophilia may be confused with ulcerative colitis or Crohn's disease, the latter especially if granulomas are present (as with *Schistosoma mansoni* infection and more rarely in strongyloidiasis, anisakiasis and enterobiasis).
Conclusion: Eosinophils are involved in the defense against many protozoan and helminthic GI parasites. The resulting histological picture may at times be confused with other disorders such as primary eosinophilic enteritis and the idiopathic chronic inflammatory bowel diseases.

Wednesday, 1 September 2010, 14.30–16.30, Aula Średnia A

WGS-09 Emerging concepts on borderline melanocytic lesions and melanoma

Chairpersons: D. Massi, Italy
A. Zembowicz, USA

001
Emerging concepts regarding borderline melanocytic tumors

*A. Zembowicz**
*USA

002
Critical differential diagnoses in the evaluation of melanocytic lesions

*T. Brenn**
*USA

The diagnosis of invasive melanoma is straightforward in most cases. It is based on the recognition of a malignant junctional, in situ component in addition to an invasive dermal component. Classical diagnostic criteria are established mainly for superficial spreading melanoma, being the most common melanoma subtype. The morphological spectrum of melanoma is, however, wide, and distinction from both melanocytic naevi as well as non-melanocytic tumours may be challenging in individual cases. In particular, some benign melanocytic naevi may show concerning histological features overlapping with those of melanoma, resulting in significant potential for overdiagnosis. Melanocytic lesions notoriously difficult to separate from melanoma include dysplastic naevi, naevi of special sites, halo naevi, recurrent naevi, mitotically active naevi and deep penetrating as well as blue naevi. Non-melanocytic tumours may mimic melanoma by recapitulating a junctional in situ as well as a dermal invasive component showing similar cytological and architectural features, including epithelioid, spindle cell, small cell or desmoplastic differentiation, in addition to immunohistochemical expression of the so-called melanoma markers such as S100, HMB-45 and Melan A. These tumours may be of epithelial, haematolymphoid as well as mesenchymal lineage ranging from benign to outright malignant. Awareness of the critical entities in the differential diagnosis of melanoma and their differentiating features and diagnostic clues is important to avoid misdiagnosis, especially as the treatment modalities and clinical behaviours may differ significantly.

003
Molecular basis of melanocytic lesions

*A. Batistatou**
*Greece

Molecular studies of melanocytic lesions are necessary in order to identify additional prognostic and predictive biomarkers and establish the pathways of relevance for targeted therapy. The advent of biotechnology has also enabled the utilization of molecular testing as a diagnostic adjunct in the microscopic evaluation of difficult melanocytic lesions. The molecular multistep process of melano-

magenesis has been correlated to the Clark model for melanoma development. The first step of melanocytic hyperplasia and nevus formation has been linked to constitutive activation of the ERK-MAPK pathway as a result of somatic mutations of BRAF or N-RAS (or H-RAS or GNAQ). The development of cytologic atypia (dysplastic nevi) is related to alterations in cell growth, apoptosis and DNA repair; inactivation of CDKN2A; and PTEN pathways and TP53. Radial growth phase has been associated with decreased differentiation and deregulated expression of MITF, as well as deficiency in the p16INK4a-Rb pathway. Alterations in cell adhesion, such as reduction/loss of E-cadherin, increase of N-cadherin, $\alpha v\beta 3$ integrin, MMP-2 and increased expression of osteopontin are associated with the development of radial growth phase and metastatic melanoma. Alternatively, some melanomas (including acral and mucosal) arise de novo and harbor mutations/amplifications of KIT and amplifications of cyclin D1 or CDK4 genes. Other less frequent, pathogenetic pathways have also been proposed. In the near future, distinct molecular signatures of gene expression may be useful in identifying melanocytic lesions as melanomas or nevi. For melanomas, refined classification systems based on molecular analysis could provide more accurate prognostic markers and enable targeted therapy.

004

Novel targeted therapies in melanoma and the pathologists' role in therapeutic selection

*A. Lazar**
*USA

Background: When melanoma metastasizes, it is a deadly disease with unsatisfactory treatment options. It has become clear that melanoma is composed of multiple genetic subsets of disease that have different oncogene additions and thus distinct therapeutic vulnerabilities.
Method: Many clinical trials and application of targeted therapies outside of the trial setting depend on melanoma subtype and the genetic features of an individual case. Pathologists are absolutely critical in this area of testing and in therapeutic selection to enable precision medicine.
Results: Targeted therapies based on the molecular genetic features of individual melanoma cases is beginning to drive targeted therapy selection and can greatly enrich the rate of response to particular treatments.
Conclusion: This lecture will focus on the genetic features of melanoma currently most relevant to treatment and how testing drives therapeutic selection. In the very near future, such testing will be employed for treatment in virtually all cases of metastatic melanoma.

Wednesday, 1 September 2010, 14.30–16.30, Aula Średnia B

WGS-10 Native kidney disease: news and views
Chairpersons: W. Schürch, Canada
A. Halon, Poland

001

Nephron number and essential hypertension

*K. Amann**
*University Erlangen-Nürnberg, Dept. of Pathology, Germany

Nephron number as determined during nephrogenesis may be one determinant of renal disease and hypertension in later life. Various genetic and epigenetic factors, but also maternal or environmental causes, are known to influence nephron number. An involvement of the kidney in the development of hypertension has been postulated for a long time and supported by experimental studies in rats and sheep. Brenner and colleagues supposed a direct association between nephron number and blood pressure in humans. Their so-called Brenner hypothesis postulated that any reduction in nephron endowment leads to hyperfiltration of the remaining glomeruli, followed by glomerular enlargement with glomerular and then systemic hypertension, resulting in glomerulosclerosis, thereby establishing a vitious circle. In line with this theory, an association of low nephron number and development of hypertension was shown in different animal models as well as in two autopsy studies in humans. Keller et al. found in Caucasian patients with essential hypertension a significantly lower number of glomeruli per kidney than in matched controls. In parallel, mean glomerular volume was more than twice as high in hypertensive patients as in normotensive control patients, indicating compensatory glomerular enlargement. These results were confirmed in a Caucasian American population, whereas in African Americans, there was no association between glomerular number and blood pressure. The pathomechanisms linking low nephron number and hypertension are only partly understood. Among others, inappropriate activation of the renin–angiotensin system, impaired tubular salt handling leading to salt and volume retention, or post-glomerular structural changes have been discussed.

002

The new classification of lupus nephritis: a critical reappraisal

*A. Halon**
*Wroclaw Medical University, Poland

Background: Systemic lupus erythematosus (SLE) is the prototype of human autoimmune multisystemic disease and has a wide spectrum of clinical manifestations, of which renal failure is the most common and severe. About 50–80% of patient with SLE suffer from lupus nephritis (LN), and it is one of the major causes of morbidity and mortality. LN manifests as diverse patterns of immune complex-mediated renal disease involving all tissue compartments: glomerular, vascular and tubulointerstitial.
Method: The histopathological manifestations of LN are classified into several categories, originally designated by the WHO in 1982. In 2002, an international group of pathologist, nephrologists and rheumatologists formulated a new classification of LN in order to standardize definitions and improve diagnostic procedures. This review will focus on the most recent, widely practiced classification, the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 Classification of Lupus Nephritis, which includes various changes from predecessors.
Results: Histological prognostic factors proposed in LN including ISN/RPS 2003 Classification, Activity (AI) and Chronicity (CI) indices may not be sufficient to predict renal failure progression and response to therapy. New markers of glomerular and interstitial response to injury and renal scaring progression, such as α-SMA, PGM1 (CD68), E-cadherin, collagen IV, caspase-3, Bcl-2 and p53, can become new prognostic factors for LN.
Conclusion: The lecture will be devoted to the revision and reappraisal of a new classification of LN and the potential role and predictive ability of new markers.

003

The Oxford classification of IgA nephropathy: a review based on the Polish renal biopsy registry

A. Perkowska-Ptasinska, M. Wagrowska-Danilewicz, M. Danilewicz, A. Halon, E. Komuda, H. Karkoszka, A. Andrzejewska, K. Okon, I. Kurnatowska, M. Krasnicka, T. Hryszko, M. Kusztal*
*Poland

IgA nephropathy is a disease of a diverse course and outcome. There were several attempts to create a morphological classification that would serve as a tool efficiently defining the potential responsiveness to the immunosuppressive treatment as well as prognosis in individual cases. The last of these proposals, The Oxford classification of IgA nephropathy, was published in July 2009. The aim of our study was to compare the utility of few functioning classification systems, including Oxford and Hass classifications and Japanese histological grading, as well as our own morphological index of biopsies with IgA nephropathy. On the basis of data collected in Polish Renal Biopsy Registry, we selected 135 IgA nephropathy cases that were characterized by at least 2 years of post-biopsy follow-up, as well as satisfactory tissue material for light microscopy evaluation. Microscopical grading was performed by a group of experienced nephropathologists. The lecture will be devoted to the results and conclusions of this study.

004

The myofibroblast. From wound healing to neoplasia. With special emphasis on tissue fibrosis and fibrocontractive conditions. Twenty-five years of reflexions

*W. Schürch**
*Canada

The myofibroblast (MF) was discovered in 1971 in electron micrographs from experimental granulation tissue. This cell was shown to share features of fibroblasts and smooth muscle cells, hence its name. In due course, it was found to be the principal cell to effect wound contraction, i.e., wound healing. This unique cell was later defined at the histologic, ultrastructural, immunohistochemical, biochemical and pharmacological levels. For the surgical pathologist, the MF is best defined by its ultrastructure. Immunohistochemical studies demonstrate a heterogeneous pattern of cytokeletal phenotypes regarding actin isoforms and intermediate filaments. Five immunophenotypes were identified. Ultrastructural features that define the MF include: (a) stress fibers, i.e., bundles of microfilaments with interspersed dense bodies; (b) well-developed cell-to-stroma attachment sites, i.e. fibronexi, intercellular intermediate and gap junctions; and (d) abundant production of extracellular matrix. MFs have been observed in normal tissues, in granulation tissue and in several pathologic settings which will be discussed in detail. Numerous cells can modulate into a MF phenotype: foremost, the local resident fibroblasts, followed by pericytes, vascular smooth muscle cells, liver perisinusoidal stellate cells, kidney mesangial and tubular epithelial cells, bone marrow stromal cells and mesothelial cells. For the development of the MF phenotype, a two-stage model was proposed. Following tissue injury, complex changes in the microenvironment occur with the release of cytokines and the concerted action of a permanent feedback between intra- and extracellular tension, transforming

progenitor cells into proto-MFs and then into differentiated MFs.

Wednesday, 1 September 2010, 14.30–16.30,
Sala Wystawowa A

WGS-11 Lung tumours: mimics between benign and malignant

Chairpersons: R. Attanoos, United Kingdom
B. Papla, Poland

001
Distinguishing benign and malignant lymphoid lesions of the lung

*R. Attanoos**
*United Kingdom

002
Controversies in pulmonary epithelial proliferations: defining neoplasia from hyperplasia and metaplasia

*R. Langfort**
*National TB & Lung Disease, Research Institute, Warsaw, Poland

Three types of pulmonary preneoplastic changes (PPC) are accepted: squamous dysplasia and carcinoma in situ (SD/CIS), atypical adenomatous hyperplasia (AAH), and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH). SD/CIS are associated with the development of squamous cell carcinoma (SCC). AAH is a pre-invasive lesion for adenocarcinoma (ADC), and DIPNECH may progress to carcinoid. Recently, adenocarcinoma in situ (AIS, formerly bronchioloalveolar carcinoma (BAC)) was included among PPC. Pathogenesis of SCC and ADC is a multistep and multicentric process involving the transformation of the normal bronchial mucosa and alveolar lining cells through a continuous spectrum of lesions. The sequence of PPC for SCC includes: hyperplasia > metaplasia (SM) > SD > CIS > SCC; for ADC, it is hyperplasia > AAH/BAC > ADC; and for carcinoid, it is DIPNECH > tumorlet. Numerous genetic and molecular abnormalities occur in the very early stages of lung carcinogenesis, including hyperplasia and metaplasia and even in normal-appearing bronchial epithelium. It is not known which genetic changes are the most important or at what stage the process is irreversible. SM and hyperplasia can be either a repair or a reactive and reversible process of injured bronchial epithelium. It is found in purely inflammatory settings. These reactive processes usually do not progress to dysplasia and carcinoma. That is, morphology is a gold standard in the diagnosis of both reactive and PPC, and no ancillary studies can be used as a diagnostic aid. The strict histologic criteria are used to assist in the recognition and grading of mucosal lesions.

003
Handling the problematic pleural biopsy in suspected mesothelioma cases

*S. Anttila**
*HUSLAB Jorvi, Dept. of Pathology, Espoo, Finland

The most challenging diagnostic problems in pleural biopsies include distinguishing between benign mesothelial hyperplasia and epithelioid malignant mesothelioma and between reactive pleural fibrosis and sarcomatoid or desmoplastic mesothelioma. In addition, sometimes rare tumor types, such as lymphohistiocytoid malignant mesothelioma or epithelioid hemangioendothelioma, may mimic reactive processes. The first question to be considered when handling a problematic pleural biopsy is: Is the biopsy material adequate and representative as can be judged from clinical, imaging and thoracoscopic findings? If in doubt, a new biopsy should be recommended. The distinction between benign and malignant mesothelial proliferation can be made on morphological grounds only, although immunostains, e.g., for EMA and desmin, may favor either a benign or malignant lesion. The most reliable criterion of malignancy is invasion. Immunostains for broad-spectrum cytokeratins may aid in recognizing invasion both in epithelioid and spindle cell lesions. In spindle cell lesions, cytokeratins also help recognize the growth patterns of spindle cells. In reactive pleural fibrosis, a layer of cytokeratin-positive cells may be seen parallel to the pleural surface, whereas in sarcomatoid or desmoplastic mesothelioma, the pattern of spindle cells is haphazard or storiform. Negativity of immunostaining for cytokeratins in a spindle cell lesion should not automatically lead to a conclusion of a benign process as, e.g., sarcomas and about a quarter of sarcomatoid or desmoplastic mesotheliomas are cytokeratin-negative. Correlation of histological and immunochemical findings with clinical and imaging findings is of utmost importance for a correct diagnosis without unnecessary delay.

Wednesday, 1 September 2010, 14.30–16.30,
Sala Wystawowa B

WGS-12 Hematopathology (EAHP): newly defined entities in WHO classification of hematopoietic and lymphoid tissues

Chairpersons: R. Maryniak, Poland
C. Meijer, The Netherlands

001

Histiocytic and dendritic cell tumours

*S. Pileri**

*Bologna University, Dept. of Haematology and Oncology, Italy

These rare tumours stem from mononuclear phagocytes or antigen-presenting dendritic cells. The latter belong to different cell lineages (either haematopoietic or mesenchymal). Histiocytic sarcoma (HS) affects adults and is characterised by an aggressive clinical course in most instances. HS more often occurs at extranodal sides and consists of pleomorphic, atypical, large cells that are $CD68^+$, $CD163^+$, $lysozyme^+$, $CD45^+$, $CD45R0^+$, and $HLA\text{-}DR^+$. Partial expression of S-100 is recorded. Tumours derived from Langerhans cells (LC). They maintain the phenotypic profile ($S100^+$, $CD1a^+$, $Langerin^+$) and ultrastructural features ($Birbeck\ granules^+$) of LC. They include LC histiocytosis and LC sarcoma. The former represents a well-known entity, more commonly observed in children: Its behaviour varies according to the stage. The latter is a very aggressive disease of adulthood with overt cytological atypia and dismal prognosis. Interdigitating cell sarcoma (IDCS) is a very rare neoplasm of adulthood that cannot be differentiated from follicular dendritic cell sarcoma morphologically, both being composed of oval fusiform cells growing in fascicles, storiform pattern or 360° whorls. The diagnosis is based on S-100 and vimentin positivity in the absence of other lineage markers. Follicular dendritic cell sarcoma (FDCS) occurs in adults and more often presents in the lymph node, at times within the context of Castleman disease. In 90% of cases, it has an indolent behaviour. The diagnosis of FDCS relies on the expression of at least two FDC-associated markers, including CD21, CD23, CD35, CNA.42, clusterin, and CXCL13. A special variant is the so-called inflammatory pseudo-tumour-like FDCS characterised by regular EBV infection.

002

Diffuse large B cell lymphoma versus Burkitt lymphoma

*P. Kluin**

*UMCG, Dept. of Pathology, Groningen, The Netherlands

Burkitt lymphoma (BL) is a well distinct clinicopathologic entity. In children of western countries, it mainly presents at extranodal sites such as the ileocecal region. However, some diffuse large B cell lymphomas (DLBCL) share one or more pathologic, molecular and/or clinical features with BL. In the new WHO classification of 2008, it was felt that cases in which no solid diagnosis of BL or DLBCL can be made deserve more scientific attention and, therefore, should temporarily be put in a separate category. Many lymphomas in this category are the so-called double-hit (or even "triple-hit") lymphomas with a MYC/8q24 in combination with a BCL2/18q21, a BCL6/3q27 (or sometimes even a CCND1/11q13) breakpoint. By definition, a MYC/8q24 breakpoint should always be present. These double- or triple-hit lymphomas make up approximately 30–40% of all (previously diagnosed) BL in patients >60 years and represent highly aggressive tumours. Many patients fail to enter stable complete remission, even after high-intensity therapy as given for BL. Other cases that fall into this novel WHO category are DLBCL with a MYC breakpoint and other features of BL such as a very high Ki-67 proliferation index and expression of CD10 and BCL6, or cases of otherwise regular BL with an unexplained abnormal phenotype such as strong BCL2 expression. Several cases will be discussed during the session.

003

Lymphoproliferative disorders in immunodeficient patients

*H. van Krieken**

*UMC St Radboud, Dept. of Pathology, Nijmegen, The Netherlands

004

Grey zones between Hodgkin and non-Hodgkin lymphoma

*P. Kluin**

*UMCG, Dept. of Pathology, Groningen, The Netherlands

Hodgkin lymphomas (HL) consist of two main categories, classical HL (CHL) and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). The latter occasionally shows large diffuse areas. In particular in small biopsies, this generates a difficult differential diagnosis of T cell/histiocyte-rich large B cell lymphoma (THRLBCL). Immunohistochemistry with a large panel of antibodies may be of help, but cannot solve all problems, and clinical aspects should always be taken into account as well. In view of the important clinical (therapeutic and prognostic) consequences, such cases should also be evaluated by an expert hematopathologist. The second problem is the distinction between CHL and primary mediastinal large B cell lymphoma (PMBL) or diffuse large B cell lymphoma (DLBCL) NOS. There may be various situations: a mediastinal mass with a synchronous composite CHL and PMBL with divergent morphology and immunophenotype. This situation may be more frequent than thought since usually, only a small sample from the mediastinal mass is taken. Both lymphomas may also develop metachronously. The coincidence of both lymphoma types is explained by the fact that both lymphomas likely have a common (thymic) origin. Indeed, they share many genetic and molecular features as well. More difficult to

understand and diagnose are the cases that arise outside the mediastinum with mixed features of cHL and DLBCL-NOS and cases of otherwise CHL that strongly express CD20 and/or other B cell markers. In such cases, other diagnoses should be considered as well, such as EBV + DLBCL of the elderly. Several cases will be discussed during the session.

005
Peripheral T cell lymphomas: a new frontier

S. Pileri, C. Agostinelli, P. P. Piccaluga*
*Bologna University, Dept. of Haematology and Oncology, Italy

Peripheral T cell lymphomas (PTCLs) account for about 12% of lymphoid tumours worldwide. Almost half of them show such a morphologic and molecular variability as to hamper any further classification and to justify their inclusion in a wastebasket category termed "not otherwise specified (NOS)". The latter corresponds to neoplasms with aggressive presentation, poor response to therapy and dismal prognosis. Conversely to B cell lymphomas, PTCLs have so far been the object of a limited number of studies aiming to elucidate their pathobiology and identify novel pharmacologic approaches. Herewith, the authors revise the most recent contributions on the subject based on the experience they gained in the extensive application of microarray technologies. PTCLs/NOS are characterised by the erratic expression of T-cell-associated antigens, including CD4 and CD52, recently proposed as targets for ad hoc immunotherapies. They also show variable Ki-67 marking, rates >80% heralding a worse prognosis. Gene expression profiling (GEP) studies reveal that PTCLs/NOS derive from activated T lymphocytes, more often of the $CD4^+$ type, and bear a signature composed of 155 genes and related products that play a pivotal role for cell signalling transduction, proliferation, apoptosis and matrix remodelling. This observation seems to pave the way to the usage of innovative drugs, such as tyrosine kinase and histone deacetylase inhibitors whose efficacy has been proven in PTCL primary cell cultures. GEP does also allow better distinction of PTCL/NOS from angioimmunoblastic T cell lymphoma, the latter being characterised by follicular T-helper lymphocyte derivation and CXCL13, PD1 and VEGF expression.

Wednesday, 1 September 2010, 14.30–16.30, Aula Mała

WGS-13 Pathologists in favour of developing countries—presentation of ongoing projects and results of the European Meeting in Turin in April 2010

Chairpersons: D. Soldini, Switzerland
L. Viberti, Italy

001
Unity is strength: Telepathology as effective tool against the shortage of specialists

*A. M. Ferrari**
*Casa di cura "S. Pio X", Milan, Italy

The South Saharan African countries are low-resource settings where the histological diagnosis of diseases and consequently the correct managing of the patient are difficult to obtain. Telepathology allows doctors working in remote locations to obtain a definite diagnosis through the transmission of tissue specimen via remote telecommunication. In particular, the "virtual slide" system allows capturing a visual image of an entire slide of a specimen which is then forwarded to another location for diagnosis. Association Pathology Beyond Borders (APOF) in 2005, in a small rural hospital in Zambia, to supply to the lack of pathology skilled personnel, started a project to train local staff to become technicians, able to prepare histological and cytological slides and to screen conventional Pap smears. The two Zambian technicians signed out negative Pap smears, and in the presence of abnormal findings, they took pictures of significant diagnostic fields. Then the images were sent through Internet to experts in Italy who, on a rotating roster, were responsible of the final diagnosis. Since 2007, the technicians were also able to prepare histological slides from the surgical specimen: Digital images of the slides were taken through a scanner and saved in a database in a local server. Through a satellite connection and a made-to-measure archival software and web site, pathologists in Italy were able to examine the specimen, record their diagnosis and transmit it directly to Zambia. In low-income countries where no other possibilities are available, telepathology seems to be a reliable and secure diagnostic tool.

002
Leave a mark: organizational models in building and managing a pathology service in developing countries

*P. Giovenali**
*Ospedale S. Maria, della Misericordia, Perugia, Italy

The main aim of the NGO Association Pathologist Beyond Borders (Associazione Patologi Oltre Frontiera, APOF) is the improvement of activities related to anatomical pathology in developing countries to raise the health standard in those areas through the performing of histological and cytological diagnoses for therapeutic and preventive purposes, such as screening programs. An essential part of every project managed by APOF consisted in the construction and organization of new pathology services or in the implementation of already existing laboratories. While taking into

account every particular local environment, needs and available resources, APOF decided to export the same models that have proven effective in our context: The technical histological and cytological process, from the surgical sampling to the final report, was set in detail trough precise guidelines with the inclusion of procedures dedicated to process control. Similar guidelines were used to update and adjust the diagnostic parameters according to international consensuses. The allocated funds were mainly used for the purchase of equipment and consumables and for the organization of updating courses dedicated to the technical and medical staff. In places where no skilled personnel were available, longer and more in-depth classes were organized for local staff, both in the place or in Italy. The staff, once trained, passed also an examination to obtain an official and specific degree. The main purpose of this kind of management was to build a reliable system that could then be directly managed by local staff at the end of the project.

003

The great debate about screening models in developing countries: matching needs and opportunities

*S. Guzzetti**

*Ospedale Evangelico Valdese, ASL TO1, Turin, Italy

Background: Association Pathology Beyond Borders (Associazione Patologi Oltre Frontiera, APOF) started in 2005, a project with the Mtendere Mission Hospital (MMH) at Chirundu, Zambia, to build and organize a Pathology Department then to set up a screening program for cervical cancer involving women of the MMH catchment area. The aim of this study was to discuss the reliability of Pap smear in screening programs for cervical carcinoma in low-resource settings.

Method: Two local staff members were trained by some APOF members to prepare histological and cytological slides and to screen conventional Pap tests. Subsequently, resident physicians were trained by two Italian gynecologists for colposcopy. Negative smears were directly reported by the two Zambian technicians. Digital images of selected microscopic fields of suspect cases were sent over a special web site and reported by a group of experts. Finally, all slides were sent to Italy and blindly reviewed by a single expert. Positive cases were then directly managed at MMH by colposcopy, bioptic confirmation and treatment.

Results: Pap smears results are summarized in Table 1. During the first 2 years of activity, inter-observer reproducibility was analyzed using two statistic indexes: Cohen's *k* and Gwet's AC1 (the agreement between the Zambian technicians with the expert was 0.77 and 0.87 and that between the web site and the expert was 0.57 and 0.65).

Conclusion: Pap smears can be an effective screening method even in low-resource settings focusing on local human resources.

TABLE 1 – PAP SMEARS PERFORMED AT MMH IN YEARS 2006 – 2008 AND RESULTS

Diagnosis	2006	%	2007	%	2008	%
UNSATISFACTORY	50	9.2	80	8.1	133	5.7
NEGATIVE CASES	366	67.6	742	74.8	1895	81.3
OVERALL POSITIVE CASES (≥ ASC-US)	126	23.2	170	17.1	303	13.0
ASC-US	16	2.9	23	2.3	38	1.6
AGC	7	1.3	2	0.2	7	0.3
L-SIL	64	11.8	70	7.0	136	5.8
ASC-H	8	1.5	25	2.6	23	1.0
H-SIL	17	3.1	40	4.0	82	3.5
SCC	13	2.4	9	0.9	16	0.7
ADENOCARCINOMA	1	0.2	1	0.1	1	0.1
TOTAL	**542**	**100.0**	**992**	**100.0**	**2331**	**100.0**

004

Managing unusual diseases in unusual settings: Project Uganda and haemopathology

*L. Leoncini**
*University of Siena, Italy

Although in the developed world the importance of the correct diagnosis is appreciated as a critical issue, this is still an evolving concept in some of the developing countries, especially in Africa. In particular, there are striking differences in the turnaround time for histopathological diagnosis, in the accuracy of diagnosis that has a profound impact on patients' management and ultimate outcome. The current problems in practice of lymphoid/lymphoma diagnosis include—basing treatment decisions on fine needle aspiration cytology in a large proportion of cases, poor quality histology in a minority of cases where biopsies are performed, complete lack of immunohistochemistry and other supportive investigations, and lack of an update on the current criteria for the diagnosis of various lymphoid pathologies. In Africa, a majority of the laboratories still use the Working Formulation for Clinical Usage, a lymphoma classification from the early 1980s which is based on morphology alone and does not include many entities recognized in the last 20 years. Without accurate diagnosis, any research project and effective patient management cannot be instituted. Though there are no magic answers for an issue of this magnitude, on which other aspects are critically dependent, twinning between institutions in the developed countries and developing countries seems to be the most likely long-term approach. Examples of twinning approach to childhood cancer diagnosis and treatment have been Africa. These programmes have led to improvements in the diagnostic accuracy through capacity building and joint research projects with both direct and indirect technology transfer.

005

The role of APOF in low-resource settings: present and future projects for the development of surgical pathology in developing countries

*V. Stracca-Pansa**
*Italy

In the last years, the cancer issue in most African countries is more and more dramatic. The WHO AFRO and the resolution of the 58th World Health Assembly made cancer one of the health priorities in developing countries and pushed African countries to formulate National Cancer Control Programmes. Once considered a disease of the rich, the pendulum has swung dramatically, and some 70% of new cancer cases in the next decade will be in the developing world. Many poor countries are unable to cope with the accelerating burden of cancer. Furthermore, to establish any reliable oncologic treatment, it is mandatory to obtain a complete, documented histological diagnosis. Patologi Oltre Frontiera (POF, Pathologists Without Borders), a newly established Italian NGO, has the mission to strengthen or build laboratories for anatomical pathology in developing countries, train local personnel, doctors and technicians for future autonomy of the laboratory, and provide medical and technical resources for histological and cytological diagnosis in the countries where departments of pathology are completely absent or based on a very small number of pathologists. Since 2000, Patologi Oltre Frontiera, which counts on tens of volunteers (pathologists, biologists and technicians), have launched numerous projects in Tanzania, Cuba, Zambia, Kosovo, Palestine, Egypt, Uganda, Madagascar, Congo and Gibuti. Every project is tailored on the particular needs of the place, but each has to respond to some strict criteria of efficacy and sustainability, with the final goal to leave local, well-trained personnel and a modern well-equipped laboratory.

Wednesday, 1 September 2010, 17.00–18.30, Aula Duża A

WGS-14 Breast pathology business meeting

Chairpersons: T. Tot, Sweden
C. Wells, United Kingdom

001

The Breast Cancer Pathology Report in 2010

*C. Mies**
*USA

The essential purpose of the breast cancer pathology report is to communicate pathologic findings that aid in prognostication and guide the selection of appropriate local and systemic treatment for patients with breast cancer. To be clinically useful, the content of the report must change as new prognostic and predictive factors are validated and others become obsolete. In 2010, the list of useful features—those which are grounds for specific therapeutic actions—is relatively short: the anatomic extent of disease in the breast and axilla (AJCC/UICC stage), the specimen margin status, and measures of certain cancer cell proteins (hormone receptors, HER-2/neu) that predict the likelihood of response to specific adjuvant therapies. Histological subtype, carcinoma grade, the presence of lymphatic tumor emboli, the finding of Paget disease in a mastectomy specimen are also often reported, but do not inform specific treatments the way that stage, margin status and predictive markers do. In patients with node-negative, estrogen receptor-positive breast cancer, the pathologist selects a paraffin-embedded tumor sample for the Oncotype DX assay, which is used to predict the risk of

recurrence in patients treated with tamoxifen and may addend the recurrence score to the surgical pathology report. The breast cancer pathology report in 2010 is vital to appropriate breast cancer treatment, but can be expected to evolve.

002
Working on national guidelines: European guidelines
*C. Wells**
*United Kingdom

003
Working on national guidelines: British guidelines
*I. Ellis**
*United Kingdom

004
Working on national guidelines: Swedish guidelines
*D. Grabau**
*LabMedicin Skåne, Dept. of Pathology, Lund, Sweden

In order to assure the quality of pathology in all of Sweden, the Swedish Society of Pathology has instituted organ-specific quality assurance and standardization groups. These groups consist of dedicated pathologists from many subspecialty areas and are called KVAST groups. They meet regularly and their principal work is to formulate and maintain a document with guidelines which has a common framework for all the organ-specific groups. Our group prepares the guidelines for breast pathology. The mandatory sections in all the guidelines include clinical background information, instructions to clinicians as how to handle the specimens, what information is needed on the requisition form, gross description, analyses, what information must be reported by the pathologist (gross and microscopic), recommended classification system, administration and miscellaneous. Sweden has a national cancer strategy programme emphasizing a patient perspective by focusing the patient process instead of piecing the treatment process together through independent specialities, as has been done previously. In 2008, a group of breast cancer clinicians established a national quality register for breast cancer patients called INCA which also include a pathology section. In collaboration with the Swedish Breast Cancer Group and the breast KVAST group, another group called SweQA works with quality assurance programmes of the biomarkers used in routine pathology; estrogen and progesterone receptors, HER2 status, and histologic grade. The guidelines for breast pathology will require the breast pathologists to participate in the breast cancer patient process by focusing on the standardisation of requisition and pathology reports and quality assurance of biomarkers.

005
Working on national guidelines: Hungarian guidelines
*G. Cserni**
*Hungary

Background: All pathologists feel that the establishment of pathological diagnoses is somewhat subjective. Writing national guidelines is useful in order to achieve better consistency in reporting at the level of a geographic area, Hungary. As not all countries have their national guidelines on breast reporting, it was thought useful to provide some examples of how guidelines can be constructed and/or adopted.
Method: The text of the Hungarian guidelines on breast pathology reporting was written by a committee of pathologists with expertise in breast pathology. The basis of the new text was a consensus document from 10 years ago. This was rewritten, circulated, and modified several times, and the updated text, ready for wider discussion, was put on the web (Internet) along with four other texts (diagnostic radiology/imaging including nuclear medicine; surgery including reconstructive surgery; radiotherapy; and systemic therapy). The URL allowing access to the texts was widely circulated on discipline-specific web sites. A Consensus Conference was organized for live discussion, and written comments were also welcome both from the writing committee members of the other texts and from the wider medical community (the other texts were discussed similarly to allow better congruence). All relevant comments were incorporated and the pre-final text was discussed at the Consensus Conference. The writing committee finalized the text on the basis of the relevant comments discussed at the conference. The pathology Guidelines were scheduled for publication with the other texts in the national oncology journal, Magyar Onkologia (Hungarian Oncology).
Results: NA.
Conclusion: NA.

006
Working on national guidelines: Polish guidelines
*E. Chmielik**, W. P. Olszewski, J. Rys*
*Oncology Institute, Dept. of Pathology, Gliwice, Poland
Objective: The pathologists from the three major oncology centers have worked out the guidelines for breast cancer pathology reporting.
Method: The proposed report was mainly based on the European and American guidelines as well as on the seventh version of TNM classification and was prepared in cooperation with radiologists and surgeons.

Guidelines of the pathological reporting of breast cancer were accepted by the multidisciplinary board of the main national specialists representing different medical professions such as pathology, oncologic surgery, medical oncology, chemotherapy and radiotherapy, and afterwards, they were described in the supplement of the Polish Journal of Pathology (2009, vol 60, issue 3) The guidelines include the rules of interpretation of needle core biopsies and other diagnostic procedures, as well as the recommendations of gross description and processing of surgical specimens depending on the type of surgical treatment. Especially, pathology report of breast carcinoma after neoadjuvant chemotherapy was proposed. Special techniques used for diagnosis of breast lesions, their performance and interpretation are also included.
Results: Finally, the histopathological evaluation form of breast cancer has been proposed.
Conclusion: In a year after the supplement publication, it is planned to conduct a survey to find out the practical use of those guidelines. On the basis of both the answers to those questions and medicine-based evidence, Polish guidelines are going to be compiled.

Wednesday, 1 September 2010, 17.00–18.30, Sala Wystawowa B

WGS-15 Haematopathology (EAHP): indolent lymphomas

Chairpersons: P. Kluin, The Netherlands
E. Campo, Spain

001
Indolent B cell lymphomas

*E. Campo**
*Hospital Clinico, Dept. of Pathologic Anatomy, Barcelona, Spain

Indolent lymphomas are a heterogeneous group of neoplasms with different clinical and pathological features that share some common features. The tumor cells are small B and grow predominantly in the topographic sites where the corresponding normal counterpart is localized. The proliferation rate is relatively low, and the cells tend to expand towards the adjacent tissues with limited destructive capacity. Clinically, these tumours have a long indolent course and the patients may not need chemotherapy for long periods of time. However, the tumours may not be cured with the current therapies and slowly progress, increasing the tumor burden and relapsing successively during the evolution of the disease after an initial response to the therapy. In some cases, transformation to a more aggressive diffuse large cell lymphoma may occur, and then the evolution is rapid with poor response to savage therapies. Indolent lymphoid neoplasm includes chronic lymphocytic leukemia (CLL), lymphoplasmacytic lymphoma (LPL), follicular lymphoma (FL), extranodal marginal zone lymphoma of the mucosa associated lymphoid tissues (MALT), nodal marginal zone lymphomas (NMZL) and splenic marginal zone lymphomas (SMZL). CLL and SMZL present usually with a leukemic phase, whereas FL and NMZL are nodal diseases. FLs in extranodal sites such as skin and duodenum have distinctive clinical and biological characteristics that differ from the nodal counterparts. MALT lymphomas develop on a previous chronic inflammatory background that in most occasions is related to certain infections or autoimmune disorders. The morphology, phenotype, molecular alterations and genetic features of the tumours are distinctive, allowing a precise characterization.

002
Primary cutaneous lymphomas

*C. Meijer**
*The Netherlands

Wednesday, 1 September 2010, 17.00–18.30, Aula Mała

WGS-16 Constituting Meeting of the Thymus and Mediastinal Pathology Working Group

Chairpersons: W. D. Travis, USA
A. Marx, Germany

001
Problems of the 2004 WHO Thymoma Classification that need to be solved: a practicing pathologist's view

*T. Molina**
*France

002
Refinement of morphological WHO criteria for thymoma diagnosis

*W. D. Travis**
*USA

003
New diagnostic markers for improved delineation of thymoma subtypes

*P. Ströbel**
*Germany

004

Translational aspects of thymic epithelial cancers: could anti-angiogenesis be an option?

M. Marino, G. Palmieri*
*Italy

005

Has the time come for a new WHO Classification of mediastinal tumours?

*H. K. Müller-Hermelink**
*Germany

Thursday, 2 September 2010, 08.00–11.00, Aula Duża A

WGS-17 Progress in soft tissue and bone pathology

Chairpersons: P. C. W. Hogendoorn, The Netherlands
P. Dei Tos, Italy

001

Histopathological diagnostic discrepancies in soft tissue tumours

*C. Fisher**
*Royal Marsden Hospital, Dept. of Histopathology, London, United Kingdom

Background: For the management of soft tissue sarcomas, it is essential to make a correct pathological diagnosis and grade. However, these tumours are rare, with an incidence of about 25 per million population, and a general pathologist might encounter only one or two per year. Furthermore, diagnostic criteria evolve, particularly concerning ancillary investigations such as immunohistochemistry and molecular genetics.
Method: We reviewed the pathology of all patients with soft tissue tumours diagnosed elsewhere and referred for management to a specialist sarcoma unit in a 1-year period. This did not include cases sent for second opinion directly from other pathologists.
Results: In 349 specimens, we found discrepancies in referring diagnosis or grade in 27% of cases, including major discrepancies in 11% of cases and minor discrepancies in 16% of cases. Benign/malignant discordances accounted for 5% of all discrepancies. The most common discrepancies occurred in the diagnosis of gastrointestinal stromal tumours and leiomyosarcoma and in the subtyping of other spindle cell sarcomas as well in grading.
Conclusion: The findings support the guidelines by the National Institute for Health and Clinical Excellence in the United Kingdom that diagnostic review of soft tissue tumours should be performed by specialist soft tissue pathologists, i.e. those who regularly report a significant number of cases, take part in multidisciplinary team meetings and participate in an accredited external quality assessment scheme.

002

The role of fine needle aspiration cytology in the examination of soft tissue tumours

*H. Domanski**
*Labmedicin Skåne, Dept. of Pathology, Lund, Sweden

Background: Open biopsy usually provides sufficient tissue for the diagnostic workup of soft tissue tumours. Some disadvantages to open biopsy are risks of complications, high costs and occasionally delay in therapy initiation. An increasing use of minimally invasive diagnostic procedures has resulted in better acceptance of fine needle aspiration cytology (FNAC) in the diagnosis of soft tissue tumours.
Method: FNAC has been part of the diagnostic workup of patients admitted to the Sarcoma Center of Lund, Sweden since 1972. The Center has been responsible for the treatment of patients with musculoskeletal tumors in the South Sweden Health Care Region comprising 1.7 million inhabitants.
Results: The combined evaluation of clinical and radiological data and FNAC has been sufficient for making a treatment decision in most patients with sarcomas. For patients who were referred with a clinical suspicion of malignancy but in whom it was later proved to be a benign soft tissue lesion, only one visit to the Center was necessary for a therapeutic decision to be made.
Conclusion: Compared to open biopsy, FNAC is an outpatient procedure, well tolerated by patients, and with negligible risks for serious complications. Correct diagnosis of soft tissue tumours is facilitated when cytological diagnosis is based on strict cytological criteria and ancillary techniques and when FNAC is used in the context of the clinical findings. On-site, immediate evaluation of smears stained by DiffQuick is an optimal way to ensure that an adequate sample for both routine examination and for ancillary studies has been taken.

003

Lipomatous tumours that does not need MDM2 and/or CDK4 analyses

*F. Collin**
*France

004

Clinicopathological and molecular pathology of dermatofibrosarcoma

*S. Bague**
*Spain

005

Histological variants of Schwannomas

*B. Liegl-Atzwanger**
*Austria

Schwannomas are benign nerve sheath tumors (BNST) composed of cells with distinctly schwannian characteristics. The four major schwannoma subtypes are conventional, cellular, plexiform and melanotic schwannoma. However, other morphologic variants including ancient schwannoma, epithelioid schwannoma, arguably glandular schwannoma and hybrid schwannoma/perineurioma, hybrid schwannoma/neurofibroma and the microcystic/reticular schwannoma have been described. The anatomic distribution of these tumors is wide, with the majority arising in the subcutaneous tissue of the distal extremities or the head/neck region. Rarely, schwannomas occur in visceral locations. Classic schwannomas are encapsulated and are composed of two morphologically distinct components known as Antoni A and Antoni B tissue in various proportions. Verocay bodies as well as the common occurrence of thick-walled hyalinized vessels are helpful diagnostic features. Characteristically, the tumor cells express S-100 protein and the perineurial capsule can be highlighted with EMA. In contrast, schwannomas arising in visceral locations are unencapsulated, have pushing margins/focally an infiltrative growth and a characteristic morphology. It is worthy to note that there is a tendency to misdiagnose schwannomas and to overestimate their grade. Diagnostic challenges are cellular schwannomas, which may mimic a malignant peripheral nerve sheath tumor (MPNST), melanotic schwannomas, which are often mistaken as melanomas and the plexiform schwannoma, particularly in the cellular form and when occurring in the childhood simulates MPNST. The aim of the lecture will be to focus on the mentioned diagnostic challenges, the recently described microcystic/reticular schwannoma, a distinctive morphologic variant with predilection for visceral locations, as well as on BNST with hybrid features of schwannoma and soft tissue perineurioma or neurofibroma.

006

Molecular understanding of Ewing sarcomas and potential targets for treatment

*E. de Alava**
*University of Salamanca-CSIC, Spain

Bone and soft tissue sarcomas are an infrequent and heterogeneous group of mesenchymal tumors, including more than a hundred different entities. Sarcomas are quite resistant to conventional chemotherapy (anthracyclines and ifosfamide), with the exception of some subtypes such as Ewing sarcoma (ES). New drugs with proved efficacy against sarcomas include taxanes, gemcitabine, and ET-743. Preclinical studies have also identified key molecular events leading to the progression and development of ES which are good candidates to targeted therapy. Inhibitors of tyrosine kinase receptors, such as IGF-1R, c-kit, PDGFR, VEGFR, or the mTOR signaling pathway, proteasome, angiogenesis, and stress response proteins are under clinical evaluation against ES. This particular neoplasm, characterized by chromosomal translocations that originate gene fusions (EWS-FLI1, EWS-ERG), is an example of a good chemotherapy responder tumor whose survival rate shows a plateau in recent years, especially in metastatic disease. Preclinical studies have identified that new targets such as HSP90 are of relevance to ES. On the other hand, recent studies showed the role of cancer stem cells (CSCs) in sarcomas and the relevance of the identification of reliable molecular markers and possible therapeutic targets. New therapeutic approaches could be directed against CSCs. This talk describes more recent targeted therapy in sarcomas, with special emphasis on ES and the role of CSCs. We also emphasize the role of high-throughput genomics/proteomics techniques in identifying new therapeutic targets. Acknowledgements: Research at Enrique de Alava's lab is supported by the European Commission (Network of excellence on sarcomas EuroBoNet), the Ministry of Science of Spain, and the Maria Garcia-Estrada Foundation.

007

Molecular understanding of EWS translocations: implications for tumours other than Ewing sarcoma

*P. Dei Tos**
*Italy

Thursday, 2 September 2010, 08.00–11.00,
Sala Wystawowa A

WGS-18 Pathologists meet transplant physicians

Chairpersons: M. J. Mihatsch, Switzerland
A. Perkowska-Ptasinska, Poland

001

Indications for and clinical consequences of renal biopsies in kidney transplants

*H. Hopfer**
*University of Basel, Institute of Pathology, Switzerland

The transplant nephrologist decides to biopsy a given patient on the basis of clinical symptoms and laboratory data. The renal pathologist then makes a histological diagnosis which allows an evidence-based approach to specific therapy, which again will be decided on by the clinician. Renal transplant

diagnoses generally fall into one of five categories: preexisting donor-related diseases, rejection, drug toxicity, infection, recurrent or de novo renal diseases. Although many diagnoses are typically encountered during a certain phase after transplantation, clinical information alone will often not suffice to differentiate between diagnoses, which require a totally opposite treatment regimen. The timing of the biopsy is most important to achieve a successful treatment and to avoid irreversible damage to the transplant. The rule of thumb at our center is to biopsy early in delayed graft function if there is a change in serum creatinine of more than 0.5 mg/dl or there is a newly diagnosed or increased proteinuria. Sufficient biopsy material, adequate workup including C4d and SV40 immunohistochemistry, and an experienced pathologist are the other prerequisites. A close collaboration between nephrologist and pathologist with regular case discussions not only helps understand the viewpoint of the clinical/pathological partner but will also aid in patient management.

002

Pathology in zero-hour biopsies and clinical consequences: Hungarian and Polish experience

A. Perkowska-Ptasinska, E. Kemeny*
*Poland

At the University of Szeged, Hungary, we carried an original clinicopathological study to assess what type of vascular changes—if any—are associated with late graft dysfunction. We examined in 94 zero-hour biopsies the frequency and severity of nonspecific morphological lesions semiquantitatively. The wall thickness/lumen (W/L) ratio of each artery present in the biopsy was determined by morphometry. Among the arterial changes studied, only the intimal fibroelastosis (IFE) of moderate degree (frequency, 26.6%) revealed a significant correlation with serum creatinine at 48 months ($p<0.01$). In IFE, there was also a significant correlation with the frequency and severity of tubular atrophy and interstitial fibrosis ($p<0.001$). By morphometry, a significant association was found between the mean W/L ratios of arteries and the degree of IFE ($p<0.01$). According to Hungarian experience, donor kidneys with a moderate degree of IFE do indeed have a higher risk of late graft dysfunction. The analogically defined Polish retrospective study revealed that the presence of arteriolar hyalinisation in the implantation biopsy was associated with more profound reduction of GFR at 3rd, 6th and 12th months after transplantation in those patients who experienced episodes of an acute rejection ($p=0.018$) within first 12 post-transplant months. This suggests that donor-derived arteriolar hyalinisation predisposes to more severe acute rejection-associated graft damage.

003

New techniques for the identification of HLA antibodies

*G. Boehmig**
*Austria

Background: It has become evident that antibody-mediated immunity plays a critical role in acute and chronic allograft rejection. According to the Banff scheme, serological alloantibody detection represents one of the major diagnostic criteria for antibody-mediated rejection (AMR). The design of sophisticated diagnostic tests for prediction and monitoring of AMR has become a major goal in transplant medicine.
Method: An important innovation has been the establishment of solid phase HLA antibody detection using flow cytometry, ELISA or Luminex technology. In this context, Luminex-based bead array technology represents an attractive strategy for a detailed analysis of anti-HLA reactivity patterns.
Results: In recent studies, solid phase detection of preformed donor-specific antibodies (DSA) was shown to predict AMR and graft loss. Nevertheless, the actual diagnostic significance of such assays is still ill-defined and currently under intense discussion. For supersensitive bead array technology, there is a need for well-defined and standardized test thresholds. In addition, the predictive value of distinct qualitative parameters, such as DSA binding strength or antibody-triggered in vitro C4d deposition, is still to be established. In the context of allograft dysfunction post-transplant, solid phase detection of alloantibodies, with or without C4d-fixing ability, may represent a valuable diagnostic adjunct. However, the predictive value of circulating DSA in stable recipients is still under discussion.
Conclusion: The implementation of innovative serological tests can be expected to substantially improve the diagnostic repertoire in transplant medicine. Future studies are needed to clarify the actual value of such tests for allocation, targeted recipient desensitization or diagnosis of ongoing or pending rejection.

004

Diagnosis and management of polyomavirus nephropathy

*J. Kowalewska**
*Medical University Bialystok, Dept of Pathology, Poland

Objective: Polyomavirus-associated nephropathy (PVAN) is a significant clinical problem that has emerged in the last decade of renal transplantation. It affects between 1% and 10% of renal transplant recipients and is a significant cause of allograft dysfunction and failure. BK polyomavirus (BKV) is the most common cause of PVAN. The virus resides in latent form in the urinary tract in the majority of healthy individuals and is known to reactivate in immunocompromised patients.

While seen in recipients of other solid organ transplants, it affects almost exclusively renal transplant receivers. BKV primarily infects epithelial cells, causes cell lysis, gets released into urine and/or blood, and elicits virus-specific cellular and humoral immune response.

Results: Currently, the monitoring for BKV reactivation/infection includes cytologic examination of urine specimen for shedded epithelial cells infected by the virus (decoy cells) and quantitative PCR analysis of urine and serum samples for the number of vial copies. The gold standard for the diagnosis of PVAN remains allograft biopsy that can illustrate characteristic viral cytopathic effect in the nuclei of the tubular epithelial cells and the degree of associated inflammatory cell response. The infection with BKV should be confirmed by either immunohistochemical stain for SV40 or by in situ hybridization. Proposed management of BKN includes: modification of immunosuppressive regimen and/or additional regimen with cidofovir, leflunomide, IVIg, fluoroquinolones, and others.

Conclusion: In summary, PVAN is a disease of the renal allograft. Immunosuppression is the main modifiable risk factor. To ensure optimum protection, screening of all patients is essential. Monitoring, early diagnosis, and therapy need standardization.

Thursday, 2 September 2010, 08.00–11.00,
Sala Wystawowa B

WGS-19 Joint meeting of WG of breast pathology and head and neck pathology

Chairpersons: A. Skálová, Czech Republic
T. Tot, Sweden

001

Recently described breast-like tumours of the salivary glands

*A. Skálová**
*Charles University, Faculty of Medicine, Plzen, Czech Republic

Salivary and mammary glands share an identical ductuloacinar architecture, and thus, it is not surprising that lesions and tumours arising in both organs share considerable histologic similarities. Salivary-type tumours are well known in breast; in contrast, breast-like lesions are rarely described in salivary glands. One of the commonest breast conditions, benign fibrocystic disease, was not thought to have a salivary counterpart, but recently, sclerosing polycystic adenosis (SPA) was described as a distinctive neoplastic lesion of the major salivary glands. Although SPA has many histologic similarities to its mammary counterpart, it represents a true neoplastic condition characterized by clonality, focal dysplasia, and a tendency to recur. Up till now, secretory carcinoma (SC) has been considered to occur only in the breast. Recently, we published a series of 16 salivary gland tumors with histomorphological and immunohistochemical features reminiscent of SC of the breast. This is an unusual, hitherto undescribed, distinctive salivary gland tumor, with some morphological features of both salivary acinic cell carcinoma (AciCC) and mammary SC, characterized immunohistochemically by strong vimentin and S-100 protein positivity. Microscopically, the tumours exhibit a lobulated growth pattern, and they are composed of microcystic and glandular spaces with abundant eosinophilic homogenous or bubbly secretory material, which is positive for PAS, mucicarmine, MUC1, MUC4, and mammaglobin. All but one analyzable case in our study demonstrated a t(12;15)(p13;q25) ETV6-NTRK3 translocation, exactly as in breast SC. For this tumour, we propose, therefore, a designation mammary analogue secretory carcinoma (MASC) of salivary glands. No translocation was found in any conventional salivary AciCC or other salivary gland tumours with undetermined secretory features. Thus, our results support the concept that MASC and salivary AciCC are distinct entities and should be recorded separately in salivary gland tumour classifications. We believe that our study has added another example to the list of similar tumours in both organs, with MASC as the salivary gland counterpart of mammary SC. Despite the shared morphologic and immunohistochemical properties, SC of the breast and MASC of salivary glands differ in their clinical behavior. Breast SCs have a rather indolent clinical course, with a propensity to local recurrence and prolonged survival. In contrast, salivary MASCs constitute a spectrum of clinical behaviour from indolent tumours to those with a rapid clinical course characterized by metastases and cancer-related death. Supported by: Grant no. 9725 of IGA MH CR (Internal Grant Agency of Health Ministry, Czech Republic).

002

The spectrum of salivary gland-type tumours of the breast

*F. Tavassoli**
*USA

003

Update on myoepithelial lesions of salivary glands

*R. Simpson**
* Royal Devon and Exeter Hospital, Exeter, United Kingdom

Myoepithelial cells are a normal constituent of the salivary acini and smaller ducts and are found between the epithelial cells and

the basement membrane. They can be recognised with various immunohistochemical markers, although none is specific or reliable in every case. S-100, alpha smooth muscle actin (αSMA), calponin, smooth muscle myosin heavy chain (SMMHC), cytokeratins 14, and p63 are the most useful in practice. Neoplastic myoepithelial cells in both benign and malignant tumours can take several forms, including epithelioid, spindle, plasmacytoid, clear and oncocytic, and this variability largely accounts for difficulties in histopathological diagnosis. Benign salivary adenomas form a spectrum with differing proportions of luminal, basal and myoepithelial cells and stroma. Whilst clinically similar, benign myoepithelioma differs from basal cell adenoma and pleomorphic adenoma only by being composed almost exclusively of myoepithelial cells, but the very different morphology justifies the separation of the entity in diagnostic practice. Benign myoepithelioma exhibits a considerable range of microscopic features, both of architecture and cytology, but nevertheless, there are several typical appearances, all reflecting the different forms that non-neoplastic myoepithelial cells can take. Solid, myxoid and reticular growth patterns may be seen, and the component cells may be epithelioid, spindle-shaped, plasmacytoid (hyaline), clear, or oncocytic. Many tumours show more than one growth pattern or cell type, but myoepitheliomas of the minor glands tend to be composed of plasmacytoid cells and those of the parotid, epithelioid or spindle cells. The clear cell variant is relatively rare. A minor degree of nuclear pleomorphism is allowable, but mitotic figures are generally scanty. The stroma is variable in amount and is usually hyaline, fibrous or myxoid, but occasionally, it may occasionally contain chondroid material or mature fat cells. Circumscription is sometimes incomplete, particularly in tumours of the minor salivary glands, but unlike their malignant counterpart, benign myoepitheliomas do not show destructive invasion. Myoepithelial carcinoma (malignant myoepithelioma) is a rare but probably under-recognised malignancy that may arise de novo or in a preexisting benign myoepithelioma or pleomorphic adenoma. There is an equal sex incidence and a wide age distribution. Most cases arise in the parotid, but any gland may be affected. It typically has a multinodular architecture. The range of cell types reflects that seen in benign myoepitheliomas and includes pure or mixed populations of epithelioid cells (the most frequent) often arranged in trabeculae or pseudo-acinar structures with cleft-like spaces, cells with clear cytoplasm (sometimes appearing signet ring-like), vacuolated (sometimes lipoblast like), hyaline (plasmacytoid) and spindle to stellate. The nuclei of malignant myoepitheliomas may be relatively uniform small- to intermediate-sized and composed of finely distributed chromatin, lacking obvious nucleoli, or there can be marked cytological atypia, with large nucleoli. Mitotic figures may be scanty to plentiful and include atypical forms. The tumour-related matrix is generally a prominent component and is hyalinized or myxoid. Metaplastic changes are frequent and include squamous areas, often with keratinisation. Other histological features include areas of necrosis seen in both high- and low-grade tumours, as well as perineural and vascular invasion. A tumour containing more than an occasional true luminal cell should not be diagnosed as a myoepithelial carcinoma. In one series, 40% of tumours were categorized as high-grade and 60% as low-grade. Almost all myoepithelial carcinomas express S-100 protein, vimentin and broad-spectrum cytokeratin antisera, either generally or in areas. Most show at least focal staining for more specific markers such as αSMA, calponin, SMMHC, cytokeratins 5/6 or14, and p63. The MIB1 (Ki-67) index is high, and in one series, a figure >10% was suggested as diagnostic of malignancy in a myoepithelial neoplasm. Myoepithelial carcinomas are locally aggressive, and about a third of patients develop metastases and die of disease. Myoepithelial cells are also found in greater or lesser numbers in a few other carcinomas and are an integral part especially in epithelial–myoepithelial carcinoma.

004

Myoepithelial tumours of the breast

*J. Reis-Filho**
*United Kingdom

005

Update on mucoepidermoid carcinoma

*I. Fonseca**
*Portugal

Mucoepidermoid carcinoma (MEC) is one of the most common subtypes of salivary gland malignant neoplasms that can also arise as a primary neoplasm at other organ sites including the bronchopulmonary tree, the gastrointestinal tract, the skin, the breast and scattered mucous/serous glands throughout the body. MEC is characterized by the presence, in variable proportions, of three cell types: epidermoid, mucin-producing and intermediate cells that can undergo clear and oncocytic change. Tumour histology remains the major criterion to establish the prognosis of affected patients, namely grade of differentiation. MEC composed by oncocytic and/or clear cells, as well as other variant features of the neoplasm, can raise difficult diagnostic questions with both benign and malignant epithelial neoplasms. MEC has a karyotypic profile, with recurrent rearrangements of chromosomal bands 11q21 and 19p13, that usually presents as a balanced t(11;19) translocation, frequently as the sole cytogenetic alteration. This chromosomal rearrangement creates a chimerical gene product that fuses the protein coding regions of CRTC1 exon1 in-frame with exons 2-5 of the MAML2 gene. More recently, another variant fusion

gene CRTC3–MAML2 was reported. CRTC1–MAML2 fusion is present in around 70% of MEC, but not in other salivary gland benign and malignant neoplasms. It has also been found in MEC at the skin, the breast and at the uterine cervix. Evidence points to accept CRTC1–MAML2 fusion as a prognostic indicator in MEC and allowing the segregation of a group with worse prognosis, including some grade II and most grade III fusion-negative tumours.

Thursday, 2 September 2010, 08.00–11.00, Aula Mała

WGS-20 Molecular diagnosis in pathology (part I)

Chairpersons: G. Bevilacqua, Italy
P. Liberski, Poland

001

Our changing view of the genome: implications for pathology

*P. Hall**
*Pathological Society, Journal of Pathology, London, United Kingdom

The decision to undertake the sequencing of the entire human genome stemmed from many sources, in particular Rrenato Dulbecco's 1986 seminal article in Science (1986; 231:1055–1056). Extraordinary technical advances were required to facilitate this, but few could have predicted the attendant conceptual revolutions. Since the publication in 2001 of the first draft of the human genome (and the parallel reports of the sequences of many other genomes), our view of the complexity of the organisation and regulation of genomes has increased. The EnCode project (Genome Res 2007;17:669–681) further highlighted the extraordinary diversity of transcription and demonstrated that the protein coding regions are but a fraction of the transcriptome. The importance of non-coding regions in genome regulation has become manifest, and the diversity of splicing events was previously unsuspected. The previous drip of new information has become a torrent, and 'next-generation' sequencing technologies promise to increase this driving cost of sequencing entire genomes to sub-$1,000 levels. The timescale of generating data similarly collapses to a mere fraction of that required even 2 years ago. The information load will be immense and new approaches will be needed to deal with the impact of this on clinical decision making. The consequences for these developments are perhaps unfathomable at present, but we are challenged to find ways to take advantage of these developments lest pathology be bypassed by other disciplines. Central to this will be educational programmes at the undergraduate and postgraduate level to 'future proof' tomorrows clinicians, including pathologists.

002

New approaches to molecular classification of lymphomas

*L. Leoncini**, *S. Lazzi, G. De Falco, C. Bellan, A. Onnis, V. Mourmouras*
*University of Siena, Italy

Background: The era of molecular diagnostics of lymphoid malignancies started with the cloning of the immunoglobulin and T cell antigen receptor genes. Southern blot analysis was applied in clinical laboratories to establish clonality of lymphoid proliferation. This was followed by the cloning of a number of translocation breakpoints in the more common lymphomas.
Method: The advent of polymerase chain reaction provided an alternative to Southern blot analysis as it is simpler and faster. In addition, the amount of clinical material required is much smaller, and it can be performed on archival paraffin-embedded materials.
Results: Gene expression (GE) analyses by use of microarrays (MAs) have become an important part of biomedical and clinical research. The resulting data may provide important information regarding pathogenesis and may be extrapolated for the diagnosis and prognosis of non-Hodgkin lymphoma (NHL)(19). This genomic technology has revealed that existing diagnostic categories of NHL comprised multiple molecular and clinically distinct diseases. In addition, gene expression profiling studies may lead to the identification of novel targets for the development of new therapeutic agents for NHL.
Conclusion: More recently, the discovery of a novel class of small non-coding RNAs, the microRNAs, has opened a new scenario in understanding the regulation of gene expression. MiRNAs control gene expression at the posttranscriptional level, and deregulation of their physiological function has been revealed to be crucial in cancer. MiRNA expression profile can be obtained by microarray and is even more informative than GEP as a few miRNA alterations can specifically identify a tumor (histo) type.

003

EGFR mutation analyses in NSCLC—experiences of a nationwide ring trial in Germany

*M. Dietel**
*Universitätsmedizin Charité, Berlin, Germany

Background: Since non-small cell lung carcinoma (NSCLC) is being predominantly diagnosed at advanced stage, the option of a curative therapy no longer exists in most instances. Roughly speaking, the first therapeutic standard measure to treat NSCLC consists of a platinum-based chemotherapy, reaching a response rate of 30–40%. Recently, new substances have been introduced to treat

NSCLC, i.e. erlotinib (Tarceva®) and gefitinib (Iressa®). Both inhibit the epidermal growth factor receptor (EGFR1).
Results: For the EGFR-inhibitors, it is possible to predict tumor response by EGFR mutation analyses. This is the reason why the EMEA has approved Iressa treatment only after such analysis, which has to be performed pre-therapeutically. The test has to be done by pathologists who (1) should reconfirm the diagnosis on an H&E slide; (2) he then should identify and mark the tumor area, (3) followed by manual microdissection to assure that at least 40% of the material is indeed NSCLC. (4) The selected tumor tissue then should be analyzed regarding mutations in exons 19 and 21 at a minimum; preferably, exons 18 and 20 should be included.
Conclusion: These analyses should be performed only in laboratories certified by a quality control organization, e.g. QuiP (Quality in Pathology, organized by the German Soc. of Pathology and the German Ass. of Pathology). By April 2010, there are 39 Institutes of Pathology in Germany which passed the QuiP ring trials on EGFR and performed approx. 3,000 tests. If an activating mutation was detected, the anti-EGFR tyrosine kinase inhibitor Iressa was recommended as a possible treatment.

004
An update of the ESP *k-ras* quality assurance program
*H. van Krieken**
*UMC St Radboud, Dept. of Pathology, Nijmegen, The Netherlands

Thursday, 2 September 2010, 14.30–16.30, Aula Duża B

WGS-21 Promoting excellence in immunohistochemistry
Chairpersons: D. Dabbs, USA
M. Vyberg, Denmark

001
Immunohistochemistry—the new challenges
*D. Dabbs**
*Magee-Womens Hospital of UPMC, Dept. of Pathology, Pittsburgh, USA

Objective: Immunohistochemistry (IHC) became a prominent means of diagnostic investigation in the early 1980s. IHC became fairly routine in surgical pathology and rapidly supplanted electron microscopy as a way of classifying tumors.
Method: Since the 1980s, the major focus for the use of IHC has been as a diagnostic tool, chiefly for the classification of tumors as carcinoma, melanoma, lymphoma, germ cell tumor, mesothelioma, or sarcoma. The explosion of molecular diagnostics, along with clinician demands, has channeled our focus on the current state of IHC in pathology.
Results: Molecular tests and gene expression profiles that purport to predict patient outcomes and drive therapeutic decisions are currently favored by oncologists. However, IHC is the only laboratory venue that supplies molecular morphology that may be directly visualized and interpreted. In addition to diagnostic IHC, theranostic and genomic applications are also now in the menu of the pathologist.
Conclusion: Pathologists must be able to emphasize the molecular morphology of IHC and how it can supply theranostic and genomic information in addition to diagnostic applications. Our IHC challenges include standardization of the total test and the ability to quantitate results for patient care.

002
Most common laboratory pitfalls in IHC
*S. Nielsen**
*Aalborg Hospital, Dept. of Pathology, Denmark

Most common laboratory pitfalls in IHC immunohistochemistry (IHC) is a well-established technique and used daily in virtually all departments of surgical pathology as a diagnostic, predictive and prognostic tool. However, IHC is an assay influenced by multiple parameters and the final result is highly dependent on the choice and performance of these variables. In the protocol setup for IHC, both the pre-analytical, analytical and post-analytical parameters will affect IHC staining, and it is of utmost importance to be familiar with these technical aspects in order to use IHC as a diagnostic tool. In the pre-analytical phase, fixation still is the key element for a reliable result, and it is essential that fixation is standardized with respect to the choice of fixative, time to and time in fixative in order to get consistent results and to avoid false negative or false positive reactions. The implementation of new tissue processing techniques based on, e.g. modified reagents, can also affect the staining result. Regarding the analytical phase, the three key elements are: (1) appropriate epitope retrieval, (2) a sensitive and specific primary antibody, and (3) a robust detection system. In external quality programs for IHC, it has been shown that most errors in IHC are related to epitope retrieval and/or the primary antibody. To validate the performance and consistency of IHC, it is necessary to use internal and external controls. Especially the use of multi-tissue blocks containing tissues with different levels of the antigen is superior to single control blocks.

003
Most common interpretation pitfalls in IHC
*J. Klos**
*Norway

Background: The real frequency of interpretation pitfalls in immunohistochemistry is difficult to assess and may vary greatly between laboratories and pathologists. The risk of misinterpretation increases with the greater use of immunostaining as an integral part of theranostic workup, the increasing number and varying quality of available antibodies, as well as the rapidly expanding body of information regarding the complexity of IHC profiles of tumours. Growing clinical expectations for pathological evaluation are an additional challenge.
Method: The presentation will address the most important elements regarding the correct interpretation of immunostaining using AAASPIN as a simple algorithm for safer approach.
Results: A—Adequate antibody panel: Selection of antibodies is often a consequence of considered differentials, but results of immunostaining may also influence the choice of antibodies in subsequent analyses. A—Antibody clone: The spectrum of reactivity and cross-reactivity should be known. A—Aberrant antigen expression/absence may be a challenge. S—Structures: The immunoreactivity has to be present on relevant structures/cells. P—Pattern: The appropriate staining pattern has to be present. I—Intensity of staining may depend on the amount of antigen and not only technical issues. N—Number of stained cells/structures. The results of immunostaining round cutoff values in oligobiopsies should be carefully interpreted since the distribution of antigen in tumour tissue may vary. The interpretation of immunostaining must always include the proper context of both morphological and clinical data.

004
Immunoquantitation
*J. Baak**
*Norway

005
Quality assurance in immunohistochemistry
*M. Vyberg**
*Aalborg Hospital, Institute of Pathology, Denmark

Background: Optimization of immunohistochemical (IHC) staining reactions to a common standard is vital for reliable and comparable results in diagnostic pathology. Yet, staining quality varies greatly between laboratories compromising diagnostic reliability. In an external quality assessment (EQA) system, staining results from many laboratories can be compared, allowing the identification of less successful antibodies (Abs) and insufficient protocols.
Method: The Nordic Immunohistochemical Quality Control (NordiQC) scheme, established 2003, comprises a general module with three annual runs catering 15–18 IHC markers and a breast cancer module with two annual runs catering hormone receptors and HER-2. Tests for 80 markers have been carried out up to nine times. General results are published on www.nordiqc.org, recommended protocols made available, and the producers encouraged to prune poor products. Individual results are e-mailed to the laboratories, which in the case of insufficient marks are given tailored advice to improve their performance.
Results: About 270 laboratories have attended the EQA scheme and >25,000 IHC stains assessed. The overall scores have been evenly distributed between optimal (36%), good (33%) and insufficient (borderline or poor, 31%). The causes of insufficient stains could be identified in more than 90% of the cases and largely ascribed to: less successful primary Abs; improper calibration of primary Ab concentration; and insufficient, inappropriate or missing epitope retrieval. Laboratories complying with the NordiQC recommendations improve in a following test for the same marker in about 70%.
Conclusion: There is still room for improvement in laboratory proficiency. EQA may be a useful tool in promoting excellence in IHC.

Thursday, 2 September 2010, 14.30–16.30, Aula Średnia A

WGS-22 Cytology for targeted therapy
Chairpersons: F. Schmitt, Portugal
W. Olszewski, Poland

001
Lung
*W. Olszewski**
*Oncology Center Warsaw, Poland

002
Accuracy of cytology in the diagnosis of lung cancer: a cyto-histologic correlation
A. Repse Fokter, I. Kern*
*Celje General Hospital, Dept. of Pathology and Cytology, Slovenia

Objective: The examination of cytological and histological specimens plays an important role in the diagnosis, therapy and prognosis of lung tumours. The purpose of this study was to evaluate the correlation between cytology results

and corresponding histologic diagnoses in tissue biopsies obtained from bronchoscopy.
Method: We studied the cytologic diagnoses, comparing them with histology reports in 241 consecutive patients who underwent fibre-optic bronchoscopy in the period between September 1, 2008 and September 1, 2009.
Results: First we compared cytologic and histologic diagnoses according to the presence/absence of malignancy. Agreement was found in 230 out of 241 cases (95.4%), with a kappa coefficient of 0.91. There were 118 patients with negative cytology, 18 cases were suspicious, and 98 were positive for malignancy. In seven cases, the samples were unsatisfactory. Histological diagnoses were as follows: 102 negative, 7 suspicious, 112 positive and 20 unsatisfactory. We achieved excellent agreement for small cell carcinoma (22/23) and non-small cell carcinoma (69/84), while agreement in subtyping of non-small cell carcinoma was not so high.
Conclusion: Cytology is of considerable diagnostic value in pulmonary pathology, although not as specific as histology in the subtyping of carcinomas.

003
Incorporation of molecular diagnostics into clinical oncology Russian experience
*E. N. Imyanitov**
*Petrov Institute of Oncology, St. Petersburg, Russia

Application of molecular tests in routine medicine is particularly challenging due to the high costs as well as problems in communication between clinical and laboratory specialists. Our activities were aimed to incorporate the most meaningful tests into practical oncology, with all necessary adjustments to economical and health care realities in the Russian Federation: (1) A number of the most clinically relevant molecular diagnostic tests have been selected based on the literature data. (2) Cost-efficient modifications of these tests have been developed. (3) Validation of these tests has been performed in selected series of subjects. (4) Practical achievements of molecular medicine have been presented in a number of national oncological conferences and schools. (5) Translational research issues have been incorporated in the courses for medical students as well as in the advanced training of certified oncologists. (6) A series of lectures have been published in various national journals, including freely available Internet-based "practical oncology". The panel consisting of seven non-expensive PCR tests has been developed for the diagnostics of breast–ovarian hereditary cancer syndromes. Due to a surprisingly strong founder effect in Russia, this panel was shown to have a nearly perfect sensitivity for the detection of BRCA carriers. The use of this panel allows identifying a hereditary cancer mutation in approximately 5% of non-selected breast cancer cases, 15–20% of high-risk (familial and/or early-onset and/or bilateral) breast cancer patients, and 15% of non-selected ovarian cancer cases. By now, almost 300 mutation-positive index patients have been identified, and their family members have been invited for genetic counseling and subsequent medical interventions. Another noticeable achievement includes the routine application of a simple mutation test, which allows selecting the responding patients for the treatment by EGFR tyrosine kinase inhibitors. This test does not require sequencing and is based on the combination of electrophoretic measurement of the length of amplified fragment and allele-specific PCR. This approach has already been applied to over 300 lung adenocarcinoma patients; as expected, nearly all mutation-positive subjects demonstrated an outstanding response to gefitinib. Detection of EGFR mutations has allowed optimizing the administration of expensive targeted compounds that eventually improved the accessibility of the latter to potential responders. We conclude that some of molecular tests can be introduced into clinical oncology with only limited spending of resources. Good cost/benefit ratio and activities in the dissemination of translational research knowledge are ultimate prerequisites for the acceptance of molecular diagnostics by medical oncologists.

004
Breast
*P. Vielh**
*France

005
Lymph nodes
*P. Zeppa**
*Italy

Thursday, 2 September 2010, 14.30–16.30,
Sala Wystawowa A

WGS-23 Joint meeting of Nephropathology WG and Polish Society of Transplantology
Chairpersons: M. Mengel, Canada
J. Kowalewska, Poland

001

Donor specific antibodies, C4d in renal biopsies and rejection

*H. Regele**
*Austria

According to the Banff Classification, antibody-mediated renal allograft rejection (ABMR) is defined by circulating donor-specific antibodies (DSA), C4d deposition in peritubular capillaries (PTC), and histologically detectable graft injury. These diagnostic criteria had primarily been developed and validated in biopsies from patients with early post-transplant dysfunction. Results from studies in patients without clinically detectable graft dysfunction, however, cast doubt on the universal applicability of these criteria. In my talk, I will address the following issues: (1) C4d deposits but no ABMR? Findings in protocol biopsies from patients with stable renal function indicate that C4d deposition is not necessarily always associated with renal dysfunction. The low predictive value of C4d in these patients might be due to the accommodation of grafts and highlights the fact that the diagnostic impact of C4d strongly depends on the clinical context. (2) ABMR but no C4d? Complement activation likely is not only of diagnostic importance but also part of the pathogenesis of ABMR. Results from gene expression profiling studies in human graft biopsies and from animal models, however, suggest that there also might be complement-independent mechanism of ABMR. Especially the lesions of chronic rejection might at least in part result from antibody-mediated complement-independent endothelial injury. (3) Are "atypical" staining patterns of diagnostic relevance? C4d can, in addition to the diagnostically relevant linear deposition in PTC, also be found in other locations and different staining patterns. It, however, appears that these other/additional staining patterns are of limited diagnostic value, at least for the detection of AMBR.

002

CIN toxicity: is it still a problem?

*M. J. Mihatsch**
*Universitätsspital Basel, Institute of Pathology, Switzerland

CNI toxicity (CNI-tox.) manifests itself in the tubuli as well as in the vessels, here practically exclusively in the arterioles. The epithelium of the proximal tubules mainly reveals isometric vacuolisation. Of much greater significance are the changes in the arterioles; these manifest either as TMA or much more frequently as so-called CNI arteriolopathy (CNI-Art.). CNI-Art. can be considered as a "form fruste" of TMA. The consequences of CNI-Art. can be: CNI glomerulopathy often in the form of FSGS, glomerular obsolescence, or interstitial fibrosis (striped form) with tubular atrophy. Tubular CNI-tox. is rapidly and completely reversible; CNI-Art. only early on. The severity of CNI-Art. increases with duration of therapy: mild > 270 days, moderately severe > 560 days and severe > 1,480 days. Twenty-five percent of cases of CNI-Art. are accompanied by glomerulopathy. With the increasing severity of CNI-Art. is a progressive increase in interstitial fibrosis, obsolescent glomeruli and FSGS. These changes are accompanied by a slow and irreversible rise in serum creatinine. CNI-Art./glomerulopathy was seen in 37 (5%) of biopsies between 1980 and 1989 and in 38 (7%) in 1990–1999. Between 2000 and 2004, a dramatic fall in CNI-Art./glomerulopathy cases to 19 (2%) was seen. A comparison between indication and protocol biopsies revealed CNI-Art. frequencies of 35% and 13%, respectively. In conclusion, in the last 25 years, the frequency of CNI arteriolopathy has fallen, but still occurs. A strict low-dose therapy helps avoid CNI toxicity and associated irreversible renal injury.

003

Patient selection, protocol biopsies and early steroid withdrawal

*J. Steiger**
*Universitätsspital Basel, Transplantationsimmunologie & Nephrologie, Switzerland

004

Is a protocol biopsy still helpful in patients with today's immunosuppression?

*D. Seron**
*Spain

005

Molecular transplant pathology

*M. Mengel**
*Canada

Background: Histopathology of allograft biopsies is standard for the surveillance of organ transplants. However, criteria for assessing histological lesions were empirically developed, and translating lesions into diagnosis represents arbitrary consensus. The advent of robust molecular techniques offers the opportunity for an objective measurement of the biological processes operating in the tissue.

Method: We did Affymetrix microarrays in 403 biopsies for cause, 107 six-week protocol biopsies from kidney allografts, and 105 endomyocardial allograft biopsies. The microarray results were summarized as pathogenesis-based transcript sets (PBTs). PBTs represent discrete biological events in organ transplants, e.g. infiltration by T cells. PBTs were developed in experimental models, thus representing a simple measurement of molecular changes in human tissue. The aims of our studies were to (1) define the molecular phenotype in allograft biopsies; (2) correlate the molecular phenotype with histological lesions and diagnoses; and (3) relate the molecular phenotype to clinical variables.
Results: We found a stereotyped molecular phenotype in all allograft biopsies: Increased expression of inflammation transcripts strongly correlated with the increased expression of injury transcripts and decreased expression of transcripts associated with normal parenchyma cell function. This robust molecular phenotype was associated with histological lesions of tissue inflammation, but not with current consensus for diagnosing rejection. An abnormal molecular phenotype was associated with allograft dysfunction and predictive of allograft loss.
Conclusion: The current diagnostic systems for diagnosing rejection frequently do not reflect the molecular phenotype. The assessment of the molecular phenotype in allograft biopsy can guide improvements of current diagnostics in transplant pathology.

Thursday, 2 September 2010, 14.30–16.30,
Sala Wystawowa B

WGS-24 Update in perinatal pathology
Chairpersons: P. Borralho Nunes, Portugal
E. Izycka-Swieszewska, Poland

001
Placental inflammation
*I. Scheimberg**
*Royal London Hospital, Dept. of Cellular Pathology, United Kingdom

002
Placenta and hypoxia
*J. Stanek**
*Cincinnati Children's Hospital, Dept. of Pathology, USA

There is no universally accepted system of classification of placental hypoxic lesions. Of 5,445 consecutive placentas from ≥20 weeks' pregnancies signed by the author, 2,564 placentas did not show hypoxic features (control group, GG), while 2,881 placentas showed hypoxic features (study group, SG). Five hundred seventy-four placentas showed fetal hypoxia (deep meconium penetration, erythroblasts in fetal blood), and 2,704 placentas showed placental hypoxic lesions, while 43 placentas showed both fetal and placental hypoxic lesions. Acute placental hypoxic lesions included villous infarctions (579), intravillous hemorrhage (64), and laminar membrane necrosis (736). Chronic placental hypoxic lesions included: global placental hypoxia (pre-uterine 295, uterine 238, and post-uterine 163), massive perivillous fibrin deposition (386), chorangiosis (738), microscopic chorionic pseudocysts of membranes and/or chorionic disc (516), decidual clusters of multinucleate trophoblastic giant cells (398), and excessive amount of extravillous trophoblasts (198). Three hundred ninety-seven placentas showed both acute and chronic hypoxic lesions. The SG featured more cases than the CG of preeclampsia (432 vs. 98), maternal diabetes mellitus (184 vs. 98), abnormal cardiotocography (533 vs. 343), intrauterine growth restriction (358 vs. 185), and cesarean sections (1,159 vs. 811), and less premature deliveries (1,616 vs. 1,659), congenital anomalies (178 vs. 200), and chorioamnionitis (875 vs. 1,109), respectively. In conclusion, hypoxic patterns of placental injury correlate with major types of high-risk pregnancy, fetal distress, intrauterine growth restriction, and cesarean deliveries. Non-hypoxic placentas are associated with congenital fetal anomalies and in utero infection.

003
Neuropathology of congenital infections
*C. Fallet-Bianco**
*Lagny-sur-Marne, France

During gestation, several microorganisms can infect the foetus and damage the developing nervous system, leading to severe neurodevelopmental sequelae. The acronym TORCH was introduced to refer to these organisms including toxoplasmosis, other microorganisms, rubella, cytomegalovirus and herpes simplex virus. TORCH infections share similar clinical features: The maternal infection is regularly asymptomatic, the clinical presentation of infected fetuses and neonates is quite identical, and, sometimes, a clinically silent infection in the neonatal period will be responsible for permanent neurological deficits occurring later in infancy. Extensive immunization programs, in developed countries, have led to an impressive decrease of congenital rubella syndrome, and the development of strategies of prenatal diagnosis and antimicrobial therapies has improved the management of congenital infections. Despite these significant advances, TORCH infections remain a worrying cause of vision loss, hearing loss, and

neurological disabilities in both developed and developing countries, and new syndromes have emerged such as congenital infections due to human immunodeficiency virus (HIV) and the human parvovirus B19. In addition, in the two last decades, growing evidence has supported the hypothesis of a significant association between choriamnionitis and cerebral palsy: brain lesions would result from a production of cytokines through a mechanism of toxicity. A wealth of literature has summarized current knowledge concerning epidemiology, microbiology, diagnosis and the management of congenital infections. In this presentation, we will focus on the neuropathological features of the most frequent intrauterine infections and underscore the invaluable contribution of neuropathological studies in understanding the pathogenesis of developing brain lesions.

004

Development and functions of the dura mater

*W. Squier**
*United Kingdom

The dura is more than just a fibrous covering for the brain; it has other functions, including control of venous outflow and uptake of CSF. Dural development is not complete at birth; it undergoes considerable remodelling in early life. The meninges develop from primitive mesenchyme derived from the neural crest. The dura has two leaflets, the outer periosteal and the inner meningeal which forms the falx and tentorium. Between them are the venous sinuses, which drain the entire venous outflow of the brain. Bridging veins are few and carry high blood flow. A muscular sphincter at their junction with the dura regulates blood flow when intracranial pressure increases. A venous plexus, most extensive at birth, is found in the posterior falx, tentorium and the parasagittal regions. Arachnoid granulations develop at 7 months of postnatal life. Originally thought to be responsible for drainage of CSF, they more likely monitor CSF homeostasis and pressure. Fluid channels in the dura may represent a system for the uptake of CSF. In early life, blood may reflux into these channels from the venous sinuses, causing intradural bleeding which is common in immature infants. Dural bleeding causes symptoms such as seizures and vomiting difficulties and is associated with age-related imaging changes in the underlying brain parenchyma. We do not yet understand the pathophysiology of these observations.

Thursday, 2 September 2010, 14.30–16.30, Aula Mała

WGS-25 Neuropathology

Chairpersons: B. Lach, Canada
B. Sikorska, Poland

001

Neuropathology of mitochondrial disorders

*B. Lach**
*Canada

Mitochondrial encephalomyopathies (ME) represent a heterogeneous group of diseases, secondary to respiratory chain dysfunction, impaired ATP production and energy crisis in the affected cells. ME could be associated with defective nuclear (nDNA) or mitochondrial (mDNA) genome, resulting in autosomal or maternal inheritance, respectively. Although multiple organ involvement is very common, the most affected are tissues with high oxidative metabolism such as CNS, myocardium and striated muscle. Myoclonus epilepsy with ragged red fibres (MERRF), mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), Leber's hereditary optic atrophy (LHOA), Kearns–Sayre syndrome and Leigh's disease represent the classical examples of ME. Precise phenotype/genotype correlations are very difficult due to the presence of overlap syndromes and atypical cases. The topography of lesions in the CNS determines symptomatology and varies in these conditions. Morphologically, ME are characterized by variably expressed mitochondrial abnormalities such as intra-mitochondrial crystalloid inclusions and ragged red fibres (RRF) in muscle biopsies, as well as selective neuronal loss in different CNS structures, laminar cortical necroses, microinfarctions and spongy degeneration in the grey or white matter. However, the common and diagnostically useful feature is the presence of COX-negative fibres, abnormal ultrastructure of muscle mitochondria and variably expressed vascular abnormalities. Although clinicopathological correlations are often problematic, the involvement of small caliber vessels may explain many focal CNS manifestations, while multisystem disease is very likely secondary to mitochondrial dysfunction in the neuronal component.

002

Kuru—50 years later

P. Liberski, P. P. Liberski*
*Medical University of Lodz, Dept. of Mol. Pathol. Neuropathol., Poland

Objective: Kuru, the first neurodegenerative human disease caused by infectious amyloids, was first reported to the Western medicine in 1957 by Gajdusek and Zigas.
Method: None.
Results: Kuru in the fore language means to shiver or to shake from fever and cold. Ritualistic endocannibalism

(eating of the relatives as a part of a mourning ritual, but not as an alimentary habit) was practiced not only in the kuru area but in many surrounding Eastern Highland groups which never developed kuru. The most striking neuropathologic feature of kuru was the presence of numerous amyloid plaques.

Conclusion: We may also speculate what would happen if kuru had not been discovered or did not exist. The infectious nature of Creutzfeldt–Jakob disease would probably not have been suspected until the beginning of the variant Creutzfeldt–Jakob disease (vCJD) outbreak in the UK. Creutzfeldt–Jakob disease and Gerstmann–Sträussler–Scheinker disease would have remained for decades as obscure neurodegenerations of merely academic interest. The familial forms of Creutzfeldt–Jakob disease would not have benefited from PRNP gene analysis, but only later would have been studied by linkage analysis and reverse genetics probably. The whole field would have probably remained of only arcane interest to veterinarians until the BSE epidemic began to exert its devastating effect. The discovery of vCJD would have been delayed as no surveillance would have been initiated for Creutzfeldt–Jakob disease. And, perhaps most importantly, the sea change in mentality that has led to the conception of 'protein misfolding diseases', including not only the neurodegenerative but also an increasing number of non-neurological disorders, would have been delayed by decades.

003

Cell autocannibalism in central nervous system. The role of autophagy in neurodegeneration and nervous system tumours

*B. Sikorska**
*Poland

Background: Autophagy is a process by which subcellular constituents and organelles are targeted for degradation in lysosomes. In macroautophagy, proteins and organelles are sequestrated into a double membrane-bound vacuole called autophagosome, formed by the ER membranes, under the direction of various proteins including MAP-LC3 (a microtubule-associated protein, light chain 3). In addition to maintaining cellular homeostasis, autophagy may also contribute to cell damage. Recently, autophagy was reported to be involved in many pathological processes including neurodegeneration, inflammatory processes and cancer. The role of autophagy in neurodegeneration is not only in removing protein aggregates but also in inducing the neuronal death.

Method: Autophagy in human neurodegenerative diseases and tumours of the CNS was studied using electron microscopy, immunohistochemistry and confocal laser microscopy.

Results: The presence of autophagy in prion diseases and other neurodegenerative disorders was shown. The extent of autophagy in tumours varied depending on histological type and malignancy.

Conclusion: Although the role of autophagy in neurodegenerative diseases remains unknown, at least three hypotheses must be taken into consideration: (1) removing protein aggregates, (2) one of the ways of neuron death, and (3) formation of spongiform change in prion diseases. The role of autophagy in cancer seems to be dual: On one hand, there is a growing body of evidence supporting the idea that autophagy may represent a tumor suppressor mechanism by reducing intratumoral necrosis, restricting oxidative stress and limiting chromosomal instability; on the other hand, autophagy may be an important process used by tumor cells to escape various types of stress and even therapeutic agents.

004

Advances in biology of gliomas

*W. Biernat**
*Medical University of Gdansk, Dept. of Pathology, Poland

Objective: Gliomas are the most common neoplasms of the central nervous system. The treatment of gliomas has been slowly changing for a few decades, and understanding of the glioma biology makes an important basic for the introduction of new treatment modalities.

Method: The most unfavorable prognosis concerns the group of diffuse gliomas which, due to the infiltrative growth, cannot be cured by surgery. The most common type of diffuse glioma is glioblastoma; this tumor may develop de novo (without preceding lower grade precursor lesion) or as a consequence of progression of malignancy.

Results: The molecular pathways most commonly seen in primary and secondary glioblastomas have been described, but recently, a new marker, isocitrate dehydrogenase 1 (IDH1), was defined as mutated early in the development of low-grade gliomas. The gene encoding this protein is located on chromosome 2q33. IDH1 catalyzes oxidative carboxylation of isocitrate to alpha-ketoglutarate. Nicotinamide adenine dinucleotide phosphate (NADPH) is the result of this reaction. IDH1 is somatically mutated in low-grade gliomas and glioblastomas. On the other hand, alterations of the ERK/MAPK intracellular pathway occur in circumscribed gliomas, e.g. pilocytic astrocytoma.

Conclusion: All these informations suggest that differential utility of these markersand will be presented.

005

Toward "fingerprinting" of brain tumours based on the synchrotron radiation X-ray fluorescence, Fourier transform infrared microspectroscopy (FTIRM) and discriminant analysis

D. Adamek, M. Szczerbowska-Boruchowska, M. Lankosz*
*Medical College Krakow, Dept. of Neuropathology, Poland

Objective: The neuropathologic diagnosis of brain tumours remains burdened by their well-known heterogeneity and difficulty to eliminate subjectivity in diagnosing.
Method: Synchrotron radiation-based techniques were applied to biochemical micro-imaging of brain tumours of different types and various grades of malignancy. The specimens were cryosectioned, mounted on appropriate sample supports and freeze-dried. Synchrotron radiation X-ray fluorescence was used for elemental analysis of samples. The level and distribution of P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb was determined. The biomolecular composition of neoplastic tissues was determined by Fourier transform infrared microspectroscopy (FTIRM). The composition of the tissues was used to construct a diagnostic classifier for brain tumours using discriminant analysis (DA).
Results: It was found that Cu, S, Cl, K, and Zn are the elements of the highest importance for the discrimination of tumor grade, as well as the tumor type. Elemental analysis allowed 99% accordance with histological type and grade of tumor. The DA applied to infrared absorption spectra indicated that lipids, amide I and amide II, as well as phosphate group are of the highest importance for the discrimination of tumor type and tumor grade. The model obtained allowed differentiation between all investigated tumours and control samples as well as correct group classification in 88%.
Conclusion: It is difficult to speculate on the meaning of our findings with the biochemical perspective; however, the DA based on elemental and biomolecular composition of tissue may be a potentially valuable method assisting the recognition and maybe grading of brain tumours.

Thursday, 2 September 2010, 18.00–19.30, Aula Duża B

WGS-26 Advances in pathology of the endometrium

Chairpersons: F. Nogales, Spain
J. Kupryjanczyk, Poland

001

The endometrium in the perimenopause

*F. Nogales**
*Universidad de Granada, Dept. of Pathology, Spain

002

Endometrial iatrogenic changes

*H. Hollema**
*UMC Groningen, Dept. of Pathology, The Netherlands

003

Current controversies in endometrial carcinoma

*M. Wells**
*Univ. of Sheffield Medical School, West Riding Yorkshire, United Kingdom

Thursday, 2 September 2010, 18.00–19.30, Aula Średnia A

WGS-27 Ongoing European Project on serrated polyps. Digestive diseases WG business meeting

Chairpersons: D. Tiniakos, Greece
A. Ensari, Turkey

001

European serrated polyp project: an initiative of the ESP working group of digestive diseases

A. Ensari, B. Bilezikci, F. Carneiro, G. B. Dogusoy, A. Dursun, J. F. Flejou, K. Geboes, G. De Hertogh, C. Langner, A. Jouret-Mourin, I. Nagtegaal, J. Offerhaus, J. Orlowska, A. Ristimaki, J. Sanz, B. Savas, M. Sotiropoulou, M. Tuncyürek, V. Villanaci, N. Kursun*
*Ankara University Medical School, Dept. of Pathology, Turkey

Objective: The family of serrated polyps comprises sessile serrated adenomas/polyps (SSA/Ps), traditional serrated adenomas (TSAs), hyperplastic polyps (HPs), and mixed hyperplastic/adenomatous polyps (MPs). The lack of consensus about the definition, nomenclature, and biology of these lesions creates considerable confusion among pathologists. The aim of this study was to reach a consensus on the definition and histopathological criteria of serrated polyps on a European scale.
Method: H&E slides of 15 cases of serrated polyps were circulated among the participating pathologists across Europe in the first round. A round table discussion was made on the preliminary results in order to reach a consensus on the diagnostic criteria. In the second round, H&E slides of 55 additional cases were examined by the same group of pathologists. The participants were asked to fill in a worksheet for each case for the presence of the listed criteria together with the final diagnosis after each round. Data were evaluated for interobserver agreement using kappa statistics.

Results: Paired kappa values for the first round ranged 0.09–1.00, 0.00–0.73, 0.07–1.00 and 0.63–1.00 for HP, SSA/P, TSA, and MP, respectively. In the second round, the range of paired kappa values was 0.17–1.00, 0.04–0.96, 0.05–1.00, and 0.06–1.00 for HP, SSA/P, TSA and MP, respectively.
Conclusion: This initiative offers a better interpretation of the diagnostic criteria of serrated polyps and helps improve interobserver agreement across Europe.

Thursday, 2 September 2010, 18.00–19.30, Aula Średnia B

WGS-28 Diagnostic pathology of thyroid cancer: old and new entities

Chairpersons: M. Sobrinho-Simoes, Portugal
D. Lange, Poland

001

Cytology (FNAB) and frozen sections: when do you use them? What is their usefulness?

*G. Tallini**
*University School of Medicine, Ospedale Bellaria, Bologna, Italy

Cytology and frozen section evaluation are traditional parts of the management of thyroid lesions. Their role and usefulness are dictated by some basic facts about thyroid nodules: (1) Nodules are very common; (2) they are benign in the majority of cases; and (3) the diagnosis of malignancy is based on cytologic features (papillary carcinoma) or on the presence of invasion of the tumor capsule or of blood vessels (follicular carcinoma). The common occurrence of benign thyroid nodules mandates an effective method for preoperative screening. The diagnosis of papillary thyroid carcinoma, by far the most common thyroid malignancy, is based on the identification of specific cytologic features. Therefore, fine needle aspiration biopsy (FNAB) has easily emerged in the past 30 years as the most accurate and cost-effective tool, indeed a true cornerstone, for the preoperative management of thyroid nodules. Standardized terminology to report diagnoses is highly recommended and is being implemented worldwide. The type of genetic alterations in thyroid cancer and the very nature of FNAB samples make them ideally suited for molecular analysis. On the other end, the importance of intraoperative frozen section diagnosis has been constantly decreasing over the years as a direct consequence of the widespread application of FNAB. It is now usually performed with cases that are suspicious after FNAB and may be useful in some cases with indeterminate cytologic diagnosis.

002

How to separate follicular adenoma from follicular carcinoma and follicular variant PTC?

*M. Sobrinho-Simoes**
*Portugal

Background: The differential diagnosis between FA, FC and FVPTC is, at present, the most frequent reason for any consultancy practice on thyroid tumours. We will review this differential diagnostic problem taking into consideration that it only concerns the encapsulated type of FVPTC. The poorly circumscribed/infiltrative types of FVPTC, as well as the multinodular/diffuse type, do not create any major diagnostic doubts and are easily diagnosed, putting together the pattern of growth and the nuclear features.
Method: If one sticks to the differential diagnosis of FA, FC and encapsulated type of FVPTC, the histopathological diagnostic hints are two: invasiveness (vascular invasion rather than pure capsular invasion) and presence of PTC—nuclear features.
Results: We will discuss what to do whenever such histopathologic findings are not clear-cut enough to support a definitive diagnosis. We will review, in this context, the diagnostic value of immunohistochemical markers of differentiation and/or proliferation and that of molecular markers in a retrospective study of 240 cases of well-differentiated carcinomas of the thyroid that have given rise to metastases and/or to local, clinically aggressive recurrences.
Conclusion: The results obtained in this study will be used to determine the best diagnostic options and to support the conclusion that most, if not all, follicular tumours and well-differentiated tumours of uncertain malignant potential, as well as minimally FC without vascular invasion and noninvasive encapsulated type of FVPTC, carry a good prognosis even when treated by lobectomy alone.

003

Angioinvasive well-differentiated carcinoma, widely invasive follicular carcinoma, solid variant of papillary carcinoma and poorly differentiated carcinoma: from diagnosis to prognosis

*M. Volante**
*University of Turin, Clinic and Biol. Sciences, Orbassano, Italy

Malignant thyroid tumors are generally divided into well-differentiated and undifferentiated (anaplastic) carcinomas, the former usually having a low malignant clinical behavior and good prognosis, and the latter being almost all very

aggressive and rapidly fatal. Diagnostic criteria rely on the recognition of cytological characteristics (i.e. nuclear alterations specific for papillary carcinoma), architectural patterns (i.e. papillary, follicular, solid, trabecular, insular or diffuse), high-grade features (i.e. increased mitotic index, necrosis) and invasive properties (i.e. vascular and/or capsular invasion). All of the aforementioned features are alternatively used as diagnostic hallmarks of specific histotypes, as peculiarities of specific variants, or as markers of aggressiveness, and their recognition is therefore essential to classify and stratify prognostically each individual tumor. The presence of vascular invasion is essential to recognize the malignant nature of a follicular tumor, but its extent is the major prognostic parameter in follicular carcinoma and draws a line between minimally invasive and widely invasive forms that are characterized by distinctive clinical outcomes. Necrosis and high mitotic index usually occur in aggressive cases of papillary thyroid carcinoma, but together with the presence of solid/insular/trabecular growth patterns represent the diagnostic features of poorly differentiated carcinoma, a specific tumor entity that shows a clinical behavior intermediate between well-differentiated and undifferentiated carcinomas. At variance, the presence of a solid growth pattern in an otherwise typical papillary carcinoma depicts its solid variant, which is more often encountered in children and radiation-exposed individuals but has a clinical behavior usually similar to its conventional counterpart.

004

Medullary carcinoma and familial non-medullary thyroid carcinoma: when do you suspect the thyroid tumor is familiar?

*P. Komminoth**
*Zürich, Switzerland

Most thyroid carcinomas occur sporadically, and only a minority of cases is associated with inheritable cancer syndromes. The majority of familial thyroid cancers (FTC) occur in younger patients, are identified in both lobes and exhibit multifocality. Some of the FTC exhibit a characteristic growth pattern which we should look for, describe and make an appropriate comment in our report. Histological hints for FTC, the most important associated tumor syndromes and the involved genes (if known) are outlined below: Familial adenomatous polyposis (FAP; APC gene on 5q21): bilateral multifocal tumors with fibrosis, cribriform, solid and spindle-cell areas lacking typical nuclear features of papillary thyroid carcinomas (PTC) called cribriform-morular variant of PTC. PTEN hamartoma syndrome (PTHS/Cowden syndrome; PTEN gene on 10q23.3): multiple adenomatous nodules with a rim of fibrous tissue occasionally associated with follicular carcinomas (FTC) or PTC and C-cell hyperplasia. Carney complex (PRKAR1A gene on 17q23-24 and unknown gene on 2p16): multiple adenomatous nodules, follicular adenomas occasionally combined with FTC and PTC. Familial PTC (FPTC, unknown gene on 19p13): multicentric PTC and adenomatous nodules with or without oxyphilia. Familial PTC with renal papillary neoplasm (unknown gene on 1q21): classical PTC and renal papillary neoplasms. Familial non-medullary thyroid carcinoma type 1 (fNMTC1; unknown gene on 2q21): classical PTC without any distinguishing pathologic features. Familial multinodular goiter syndrome (FMNG; unknown gene on 14q): goiter and occasional PTC. Familial medullary thyroid carcinoma (MTC; multiple endocrine neoplasia type 2A and 2B, familial MTC; RET gene on 10q11.2): bilateral, multifocal MTC associated with diffuse and nodular C-cell hyperplasia.

Thursday, 2 September 2010, 18.00–19.30,
Sala Wystawowa A

WGS-29 Joint meeting of nephropathology WG and Polish society of transplantology—discussion of unsolved cases presented by Polish pathologists and physicians

Chairpersons: M. J. Mihatsch, Switzerland
A. Perkowska-Ptasinska, Poland

Thursday, 2 September 2010, 18.00–19.30,
Sala Wystawowa B

WGS-30 Cytopathology WG business meeting

Chairperson: M. J. Mihatsch, Switzerland

Thursday, 2 September 2010, 18.00–19.30, Aula Mała

WGS-31 Molecular diagnosis in pathology (part II)

Chairpersons: G. Bevilacqua, Italy
P. C. W. Hogendoorn, The Netherlands

001

Molecular pathology of bone and soft tissue tumours

*P. C. W. Hogendoorn**
*The Netherlands

002

Molecular cytology in thyroid cancer

*I. Marchetti**

*Pisa University Hospital, Section of Cytopathology, Italy

Background: Thirty percent of thyroid nodule fine needle aspiration (FNA) diagnoses are indeterminate or suspicious for cancer. The prevalence of malignancy in FNA samples with these diagnoses varies from 10% to 52%. The analysis on cytological samples of the status of genes involved in thyroid carcinogenesis can represent an additional tool to improve the diagnostic accuracy. Our attention was focused on BRAF gene.

Method: DNA was extracted directly from cell scraped from stained smears of 140 patients with histological diagnosis of papillary thyroid carcinoma (PTC). The cytological diagnosis was of cystic nodule in one case, microfollicular proliferations without atypia in three cases, suspicious for papillary carcinoma (SPTC) in 54 (38%) cases and PTC in 83 (60%) cases. The BRAF V600E mutational status was determined by sequencing analysis in all patients.

Results: BRAF V600E mutation was detected in 61.1% (33/54) of cases with a cytological diagnosis of SPTC and in 75.9% (63/83) of cases with a diagnosis of PTC. Combining morphological with molecular analysis, the diagnosis of PTC rose to about 80% (111/140), with an increase of 20% in the sensitivity compared to cytology alone.

Conclusion: The morpho-molecular approach to preoperative analysis of PTC surely improves the diagnostic accuracy and possibly may have important implications in the management of these patients.

003

Clinical implications of ETS–family gene fusions in prostate cancer

*S. Perner**

*Germany

004

Brain tumours

*J. Haybaeck**

*Medical University of Graz, Institute of Pathology, Austria

As indicated by the World Health Organisation (WHO), histological grading is a way of predicting the biological behaviour of neoplasms. Especially in neuropathological settings, tumour grade is often the key factor determining therapeutic decisions. Clinical, radiological, macroscopic, histological and ultrastructural factors may already be summarized in a diagnosis that very well reflects the biology of the respective tumour entity, at least to a certain degree, but usually not in a personalized manner. This is exactly the stage where molecular biology comes into play. In recent years, much effort has been made on molecular characterization of brain tumours as there is an urgent need for specific and sensitive, highly reproducible tumour markers with a prognostic as well as predictive value. State of the art in modern neuropathology therefore ranges from basic immunohistochemical profiling, evaluation of genetic susceptibility, comparative genomic hybridization (CGH) approaches, fluorescence in situ hybridization (FISH), screening for genetic hallmark mutations, evaluation of mRNA expression of specific growth factor receptors, assessment of promoter methylation status and gene expression profiling to loss of heterozygosity (LOH) analyses. The challenge now for pathologists confronted with brain tumours is to integrate knowledge derived from the latest molecular biological methods into the established panel of pure morphology-based investigations.

Friday, 3 September 2010, 08.00–11.00, Aula Średnia A

WGS-32 Electron microscopy case studies in tumour pathology

Chairpersons: J. Lloreta-Trull, Spain
M. Santucci, Italy
W. Olszewski, Poland

001

Small renal tumour with widespread metastases: the birth of a new entity

*G. Herrera**

*USA

002

Unusual hepatic neoplasm. FNA in the diagnosis of hepatic masses and the role of electron microscopy

*E. Turbat-Herrera**

*USA

003

Plasmacytoid urothelial carcinoma of the bladder

*A. Franchi**

*Italy

Background: The plasmacytoid variant of urothelial carcinoma is characterised by the presence of single malignant cells having clear or eosinophilic cytoplasm and eccentrically placed, enlarged nuclei with small

nucleoli, which are often associated with areas of conventional high-grade transitional cell carcinoma. These tumours may be mistaken for myeloma or lymphoma both at primary and metastatic sites.
Method: A case of plasmacytoid urothelial carcinoma of the bladder occurring in a 53-year-old man was studied at the histological, histochemical, immunohistochemical and ultrastructural level.
Results: Histologically, the tumour presented a superficial component of conventional invasive high-grade urothelial carcinoma, while in the deeper component, the tumour was composed of dyscohesive oval to round cells with eccentrically located nucleus and abundant eosinophilic cytoplasm. Occasionally, neoplastic cells presented one or more cytoplasmic vacuoles, which contained PAS diastase and Alcian blue-positive material. Numerous atypical mitoses were identified and there was evidence of peritumour vascular invasion. Immunohistochemically, neoplastic cells were positive for cytokeratins (7, 20, AE1/AE3, 34betaE12) and focally positive for CD138. In addition, nuclear staining for GATA-3 was present. At electron microscopic examination, neoplastic cells were embedded in collagenous matrix and showed an oval shape, with regular contour. The cytoplasm contained a moderate amount of organelles, including rough endoplasmic reticulum cysternae and mitochondria. Bundle of tonofilaments were frequently observed, while a few neoplastic elements presented intracellular lumina, lined by microvillous projections. Intercellular junctions were not observed.
Conclusion: In conclusion, electron microscopy indicates that neoplastic cells in this peculiar variant of urothelial carcinoma show aspects of squamous and glandular differentiation.

004
More than a decade of experience in pathomorphological evaluation of pituitary tumours

M. Maksymowicz, W. Olszewski*
*Poland

Background: Phenotype of pituitary tumors as determined by means of functional, radiological and surgical data, histology, immunohistochemistry and electron microscopy (EM) may be relevant for prognosis and appropriate postoperative management. In the WHO 2004 classification of pituitary adenomas, immunohistochemical evaluation represents the "gold diagnostic standard". However, immunohistochemical profiles may overlap, but ultrastructural features of these tumors are essential for subtyping adenomas with different prognosis and response to treating.
Method: Among 1,919 pituitary tumors diagnosed between 1998 and 2010 at the Department of Pathology of Cancer Center in Warsaw, there were 1,643 (85.6%) pituitary adenomas. All cases were studied by review of clinical features, histology, immunostaining (for GH, PRL, ACTH, TSH, FSH, LH, alpha subunit) and EM. The proliferative index was determined by MIB-1 labeling.
Results: Ultrastructural evaluation was useful in: GH secreting adenomas, sparsely vs. densely granulated; GH and PRL secreting adenomas; clinically nonfunctioning immunopositive (silent) adenomas; immunonegative (null cell) adenomas: nononcocytic vs. oncocytic; unusual plurihormonal: monomorphous vs. mixed cell adenomas and invasive macroadenomas with no endocrine symptoms. The proliferative index had variable, low values (0–3%).
Conclusion: Evaluation of subtype remains the most important determinant of adenoma behavior. Currently, there are no accepted means for predicting an adenoma's invasiveness and long-term aggressiveness, but morphologic separation of some subtypes of pituitary adenomas by EM provided useful knowledge for classification and management. Proliferative markers, as MIB-1, may be helpful in the diagnosis of invasive, low differentiated tumors, but we did not find a correlation between this factor and adenoma behavior.

005
EM in description of epithelioid trophoblastic tumor and beta-HCG producing

A. Marszalek, J. Bręborowicz*
*Dept. of Clinical Pathomorpholy, Bydgoszcz, Poland

006
Hyalin globules in paucicellular leiomyomas of the gastrointestinal tract are distinct from skeinoid fibers—ultrastructural evidence

J. Schroeder, P. H. Wuensch, F. Hofstaedter, A. Agaimy*
*Universitätsklinik Regensburg, Institute of Pathology, Germany

Objective: Skeinoid fibres are globular brightly eosinophilic PAS-positive extracellular collagen deposits seen commonly in gastrointestinal stromal tumours (GIST) of the small bowel. Ultrastructurally, they display similarity to "skeins of yearn", hence the name. However, hyaline globules are occasionally encountered in leiomyomatous GI neoplasms and may be mistaken for true skeinoid fibres leading, to an erroneous diagnosis of GIST.
Method: We analyzed 93 histologically and immunohistochemically well-characterized true smooth muscle neoplasms of the GI tract for the presence of hyaline globules and examined representative examples of them

from formalin-fixed and deparaffinized tissue by electron microscopy.

Results: PAS—positive intracellular and interstitial hyaline globules were detected in all benign paucicellular leiomyomas of the muscularis mucosae (n=72) and the muscularis propria (n=14) irrespective of tumour size and site, and rarely also in the adjacent muscularis propria, but in none of the leiomyosarcomas (n=7) and cellular leiomyoma (n=1). Similar to surrounding tumour cells, hyaline globules expressed desmin, alpha-SMA and h-caldesmon, but were negative for CD117 and CD34. The mostly ovoid-shaped structures displayed at ultrastructural examination variably oriented bundles of filaments with a diameter of approximately 6–8 nm. At the periphery of these inclusions, altered filamentous material was recognized in different stages of degeneration with variable condensed matrix and occasional peripheral condensation suggestive of calcification. True skeinoid fibres were not detected.

Conclusion: The above findings are consistent with a multistep degenerative phenomenon affecting individual smooth muscle cells in paucicellular GI leiomyomas. Awareness of this finding should prevent misinterpretation as GIST, particularly in small biopsies.

007
Pseudotumours of the kidney

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Several clinicopathologic entities are grouped under the concept of pseudotumour: All of them usually result in a mass effect, therefore mimicking a neoplastic process grossly and sometimes microscopically. In the kidney and the urinary tract, the diseases most often presenting as a pseudotumour are malakoplakia and xantogranulomatous pyelonephritis. In fact, both of them can be considered inflammatory pseudotumours, i.e. inflammatory processes producing a mass effect. A more restrictive use of the term inflammatory pseudotumour applies to a subset of lesions that was initially known as myofibroblastic inflammatory pseudotumour and is currently referred to as inflammatory myofibroblastic tumour. Ultrastructural examination reveals the pathogenesis of malakoplakia as a defect in the mechanisms of lysosomal extrusion from histiocytes. Thus, the profusion of secondary lysosomes constitutes an optimal milieu for the deposition of calcium salts in typical Michaelis–Gutmann bodies. On the other hand, the foamy histiocytes of xantogranulomatous pyelonephritis are characterised by a profusion of myelin bodies due to the predominant phospholipid composition of the lysosomes, resulting from chronic destruction of renal tissue and bacterial degradation. Inflammatory myofibroblastic tumour is a true neoplastic lesion with myofibroblastic differentiation and a prominent inflammatory background. This is an important differential diagnosis for both malakoplakia and xantogranulomatous pyelonephritis that may have an extensive myofibroblastic component. Similar to its crucial role in kidney tumours, electron microscopy is a useful adjunct in the diagnosis of renal pseudotumours and has been essential in the elucidation of their pathogenesis.

Friday, 3 September 2010, 08.00–11.00, Sala Wystawowa B

WGS-33 Diagnosis of infectious diseases in cytopathology: extending the diagnostic scope—Joint Meeting of the WGs of Infectious Diseases and Cytopathology

Chairpersons: G. Cathomas, Switzerland
P. Hofman, France
P. Firat, Turkey

001
Cytologic and immunocytochemical methods in diagnostic investigation of infections

*V. E. Karev**
*Russia

Objective: Infectious complications have essential value at the conditions caused by primary and secondary immunodeficiencies, including connected with a HIV or immunosuppression therapy. Laboratory diagnostics of opportunistic infections in conditions inadequate formation of antibodies is directed on revealing of antigenes of the activator.

Method: One hundred fifty samples of washing waters of bronchial tubes are investigated at diagnostic or medical bronchoscopy of patients (children and adults) with acute lymphoid (47.8%) and myeloid (16.9%) leukemia, chronic lymphoid and myeloid leukemia, Hodgkin's and non-Hodgkin's lymphomas. Patients have been surveyed in different terms after transplantation of a bone brain. Cytologic preparations were painted H&E, Ziehl–Neelsen and PAS, ICH. The cellular structure, condition epithelial cells, a degree of expressiveness and character macrophages activity epithelial and non-epithelial cells, a degree of expressiveness and character inflammations and also the presence of specific activators were estimated.

Results: Data obtained by us testify to the prevalence bacteria (26.7%) and viruses (16.9%), inflammatory processes in respiratory ways. The changes connected with specific (tumoral), mycoses (aspergillosis, zygomycosis, a candidiasis) and pneumocystosis were less often observed by defeat. In one case, it was documented as mycobacteriosis. In 32.3% of cases of pathological changes, it is not revealed; in 8.4% of

cases, the changes connected with natural return development of pathological process were observed.
Conclusion: Carrying out complex research of a biological material for the definition of character and a degree of expressiveness of pathological process and also the proof of a role of the infectious agent in the development of pathological process is expedient.

002
Diagnosis of infections in fine needle aspiration (FNA) in immunocompetent and immunocompromised patients

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*Universitätsspital Zürich, Institute of Zytology, Switzerland

Fine needle aspiration (FNA) is a well-established method for the evaluation of the aetiology of superficial and deep masses. An infectious process has to be considered as a differential diagnosis in all cases, especially in the setting of immunosuppression (HIV infection, transplantation, chemotherapy, inborn defects). FNA allows a rapid distinction between neoplastic and inflammatory/infectious lesions. A wide range of pathogens including various viruses (CMV, EBV), bacteria (Gram-positive cocci, actinomyces, spirochetes, mycobacteria), fungi (*Aspergillus*, *Cryptococcus*) and parasites (toxoplasma, echinococcus) may be identified cytologically. Infectious agents may be visualised either direct in standard stainings (CMV, fungi), in special stainings (mycobacteria) or immunohistochemically (spirochetes), either on direct smears or in cell blocks. Molecular methods (in situ hybridization, PCR with sequencing) applied to FNA specimen help identify and often subtype the pathogen if required (EBV, mycobacterium tuberculosis vs. atypical mycobacteriosis). FNA is a particularly convenient method for fresh, sterile sample collection for microbiological examination with culture and drug resistance studies, playing an important role in treatment decision, e.g. in therapy of multiresistant tuberculosis. In some infectious diseases, the final diagnosis may be made by serology following indirect cytological hints in a FNA specimen (e.g. toxoplasmosis, HIV). Interestingly, infectious agents have been identified as aetiologically relevant factors in several tumor types (EBV in Burkitt lymphoma or lymphoepithelial carcinoma, HHV8 in Kaposi sarcoma, HPV in oropharyngeal carcinoma), so that the identification of the pathogens in FNAs may play an important role in the precise diagnosis of some neoplasias.

003
Usefulness of oral cytopathology in the diagnosis of infectious diseases in immunocompromised patients

*P. Hofman**
*France

Background: Cytology is an accepted and widely employed diagnostic methodology used in the early diagnosis of oral cancer. However, the diagnosis of many other specific clinical conditions of the mouth can be made from examination of smears, in particular infectious diseases.
Method: In recent years, the spectrum of infectious diseases of the mouth has changed. Firstly, oral infections observed in immunocompromised patients have dramatically increased owing to the widespread implementation of solid organ and bone marrow transplantation and in the increasing prevalence of HIV infections. Secondly, while the occurrence of many oral lesions has decreased significantly since the advent of highly active antiretroviral therapy, the incidence of oral warts has increased.
Results: In this regard, cytology can be used as a rapid, noninvasive, inexpensive and simple routine procedure in diagnosing infectious diseases of the mouth. The role of the cytopathology laboratory is diagnostic, i.e., to suggest or identify the presence of an infectious agent. However, exogenous structures that can mimic a variety of pathogens can pose a serious challenge.
Conclusion: The contaminants can be distinguished from microorganisms by their haphazard arrangement and lack of internal structure. Moreover, the absences of acute or chronic inflammation, of macrophages or multinucleated giant cells with or without granulomas, and the absence of necrosis are features that should alert the cytologist to the possibility of contamination. Finally, ancillary methods can be developed from cytological samples, which increases the specificity and the sensitivity for the diagnosis of infectious diseases in mouth.

004
The cytological peculiarities of urogenital viral infections

V. Zinserling, E. V. Lesjnak*
*Russia

005
Rapid detection of microorganisms in BAL by immunofluorescence

S. Savic, L. Bubendorf*
*Switzerland

006
Cytodiagnosis of pulmonary and extrapulmonary tuberculosis

*C. Ersoz**
*Turkey

Friday, 3 September 2010, 08.00–11.00, Aula Mała

WGS-34 Computational pathology

Chairpersons: M. Dietel, Germany
J. Szymas, Poland

001

The essentials in making a pathology diagnosis

*A. Batistatou**
*Greece

Pathology is the discipline concerned with understanding the nature of human disease. Pathologists' interpretations of tissue lesions become data, guiding decisions for patient management. Cognition, the sum of processes by which the visual input is transformed, reduced, elaborated, stored, recovered and used, is very important in pathology. Looking through the microscope at the stained tissue section (global impression and focal search) leads to perception of forms and colours. Various regions are examined under low and high magnifications. Then the pathologist puts the observed pieces of the colourful puzzle in place. An experienced pathologist proceeds promptly to pattern recognition and probably to diagnosis, while a novice takes more time, usually by not focusing on the significant areas but rather via an exhaustive search of the whole slide. Non-verbalized pattern recognition consists of short sequences which result from conversion of longer series of specific features. Alternative cognitive methods are diagnostic algorithms and the hypothetico-deductive strategy. Experienced pathologists perceive each case as a whole, constituted by parts varying in importance and relevance. And while novices prefer to use analytic reasoning strategies, which are conscious and controlled, experts often use implicit reasoning, which is unconscious and automatic and relies largely on tacit knowledge. Pathologists have faith in the analysis of morphology whose power has been appreciated even in the current molecular era. The use of virtual microscopy has a great advantage over the exchange of static images for diagnosis and training since it also allows for the initial steps to diagnosis, i.e. search, detection and perception.

002

Exploiting the O-axis of the Dutch KOPAC coding system for cervical smears

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Background: All Dutch cervical smears (800,000 per year) are centrally archived in the Dutch national pathology database, the PALGA, in the KOPAC coding system. The KOPAC O-axis contains inflammatory events, O1 for koilocytosis, O2 for *Trichomonas vaginalis*, O3 for dysbacteriosis, O4 for Candida, O5 for *Gardnerella vaginalis*, O6 for non-inflammatory flora, O7 for *Actinomyces* (mainly in IUD users), O8 for *Chlamydia*, and O9 or nonspecific inflammatory changes (leucocytosis).
Method: The participants of this study are screenees who had a smear taken in the Dutch national screening program, on the one hand screenees born in immigrant countries (immigrants) and on the other hand screenees born in the Netherlands. The database of the SBBW, one of the responsible screening organisations, encompasses over 500,000 KOPAC cytology reports of screenees, collected between 1996 and 2005.
Results: The variation of the cytoscores was maximal for *Trichomonas* (1.9–14.0‰), whilst for *Candida*, the values were between 10.0‰ and 29.0‰. Screenees from Surinam and the Dutch Antilles had high cytoscores for *Trichomonas* and *Gardnerella*, whilst Moroccan screenees had a maximally low cytoscore for *Gardnerella* of 0.8‰. In the analysis of our data, we focussed on the odds ratio (OR). The cytoscores for the pathogens *Trichomonas* and *Candida* and for the undesirable bacterium *Gardnerella* are by-products of cancer screening. The elevated risk for colonisation of *Gardnerella* in immigrants from Surinam, Turkey, and the Dutch Antilles is truly worrisome since it is related to the acquisition of HPV.
Conclusion: This study was possible by exploiting the O-axis of the Dutch coding system for cervical smears.

003

Exploiting the Dutch network and national data base for pathology (PALGA) of over 55 million pathology reports in patient care and quality control

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Background: The Dutch national pathology database, the PALGA, was started in 1971 by pathologists. As of 2010, all 59 Dutch pathology laboratories send their pathology reports each evening to the central database. The PALGA software can identify a patient by name, sex, and date of birth. At present, the PALGA database contains 55 million histology, cytology, and postmortem reports of 10.8 million patients, the Dutch population being 16 million.
Method: For 2009, the PALGA data traffic was analyzed.
Results: For the year of study, the data traffic comprised 2.1 million pathology reports and past pathology histories. For the Leiden Cytology and Pathology Laboratory (LCPL), exclusively serving general practitioners, 80,000 patients (out of 500,000) were added to the PALGA database, and 1,584

pathology diagnoses were asked for in the context of quality control of cervical cytology and dermatopathology.
Conclusion: In patient care, the 423 Dutch pathologists profit from the historical pathology diagnoses dating back to 1971. For quality control of the LCPL, the diagnoses of other pathology laboratories of the hospitals where the patients of the general practitioners were operated provided by the PALGA are of paramount importance. In 2010, the PALGA system will also be employed for the nationwide evaluation of the early detection of cervix, breast, and colon carcinoma.

004
Virtual microscopy in routine diagnostic procedure of pathology

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Background: Virtual microscopy (VM) is now widely applied in pathology. Many studies underline the diagnostic security, the technical robustness as well as the versatility of this method. The standardization organizations DICOM, HL7 and IHE call virtual slides as whole slide images (WSI) now and integrate the technology into their standardization strategy. In contrast to the increasing application in educations, the virtual microscopy is far from routine use in surgical pathology. This goes back to several reasons concerning technical and personal requirements: costs (scanning devices and storage), scanning time (between 1 and 5 min for a biopsy and between 5 and 20 min for a surgical specimen) and speed of virtual microscopes in comparison to conventional microscopy.
Results: Caching and prefetching may speed up image loading, the bottleneck in virtual microscopy. The positive effects of different prefetching and caching technologies which depend on the user's behaviour will be discussed. Further, the process of secondary diagnostic was evaluated using the "T. Konsult Pathologie" service of the Professional Association of German Pathologists within the German breast cancer screening program.
Conclusion: In summary, it could be shown that the safety of diagnostic on WSI is comparable to the conventional diagnostic based on glass slides and a classical microscope. Discrepancies go back to problems with the difficulty of the case itself and not to technical problems with virtual microscopy.

005
Virtual microscopy in teaching pathology in Pomeranian Medical University

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Objective: Microscopy is an important way of understanding the morphologic basis of diseases, and a slide seminar is part of the pathology program in medical schools worldwide. Virtual pathology which allows seeing the entire digitized microscopic slide on the computer screen has been introduced recently. Here, we share our experience in using digitized slides in teaching pathology and also discuss student's survey results. This has been the first implementation of a complete virtual pathology course for students of medicine in Poland.
Method: Two hundred twenty-four virtual slides for pathology course for medical students were produced by the use of Aperio Scanscope CS scanner and hosted in Spectrum database. Starting from the year 2006, a group of 223 Polish- and English-speaking students used glass slides and microscopes in the first year and then virtual slides in the second year. At the end of the second year, on completion of the pathology course, the students were given a questionnaire to evaluate both systems.
Results: Eighty-five percent of students preferred the screen over the microscope. Continuous and easy access to virtual slides via the Internet was indicated as one of the major advantages of virtual microscopy. Some technical problems having an adverse impact on learning efficacy were noted.
Conclusion: The move to virtual microscopy and computer-assisted pathology teaching appears to be well received by students and enhance their learning ability in the field of pathology.

006
Identity federations and image database authentication

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007
Latest development of telemedicine platforma

*S. Vinod**
*USA

Friday, 3 September 2010, 14.30–16.30, Aula Duża B

WGS-35 Cytology and pathology of the uterine cervix—Cytopathology and Gynaecologic Pathology WGs Joint Session

Chairpersons: P. Firat, Turkey
J. Ordi, Spain

001

The current status and future of cervical cancer screening in Europe

*C. Bergeron**
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002

Cytological characteristics of squamous lesions and the mimickers

*L. Di Bonito**
*Italy

High-grade squamous intraepithelial lesions are characterized by moderately sized parabasal (CIN2) or basal (CIN3) dysplastic cells with marked nuclear abnormalities. Basal dysplastic cells usually display scanty cytoplasm and large hyperchromatic, coarsely granular nuclei with irregular contours. Basal dysplastic cells can be mistaken for lymphocytes, macrophages or for small benign metaplastic or endometrial cells. Regressive changes in endocervical cells can be confused with HGSIL; repair cells can be a source of confusion, but they are characterized by active nuclei with nucleoli and even distributed chromatin. High-grade dysplastic lesions involving glandular structures may exfoliate in sheets and aggregates, arising diagnostic doubts with glandular lesions. High-grade squamous intraepithelial lesions may also have some special morphologic presentations in conditions such as postmenopause because atrophy and dryness have several effects on dysplastic cells. They appear enlarged, their nuclei appear pale, and the chromatine texture is not well discernible; diagnosis can be performed by a careful comparison of abnormal cells with adjacent dry, but normal cells. Low-grade squamous intraepithelial lesions (LGSIL) are characterized by the presence of superficial and intermediate dysplastic cells. Koilocytes occur in a great number of cases, confirming the close relationship of low-grade lesions with HPV infection. Koilocytes are mature squamous cells, usually intermediate type, with abnormal, enlarged and hyperchromatic single or double nuclei surrounded by large, sharply demarcated perinuclear clear zones or halos. Koilocytes must be differentiated from inflammatory cellular changes which may be responsible for slight nuclear abnormalities and narrow perinuclear clear zones.

003

Cytological characteristics of glandular lesions and their differential diagnosis

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Objective: Since 20 years, there is an increase of incidence and mortality of adenocarcinoma as compared to epidermoid carcinoma. This increase is particularly notable for women younger than 40 years. Adenocarcinoma equals one fourth of cancers of the cervix in some countries like the UK where there is an organized screening program.
Method: The Bethesda 2001 terminology has introduced for the first time the cytological diagnosis of adenocarcinoma in situ, the glandular counterpart of high-grade squamous intraepithelial lesion (HSIL).
Results: It is a rare lesion that corresponds to 5% of abnormal Pap smears and 0.1% of all Pap smear diagnoses. Adenocarcinoma in situ is characterized by periphery radial arrangement of feather-shaped nuclei, cigar stack-shaped pseudostratified strips, rosettes images, hyperchromatic nuclei with granular structure and clean background without necrosis. On the contrary, invasive endocervical adenocarcinoma is characterized by papillary groups, lost of cellular cohesion, polymorphism of the nuclei with one or two prominent nucleoli and tumoral diathesis with dirty background, cellular debris and fragmented red blood cells. The Bethesda 2001 terminology has maintained the grey zone for glandular cells abnormalities not well defined, included in the diagnosis of atypical glandular cells of endocervical, endometrial or undetermined.
Conclusion: The efficacy of cytological screening for detecting AIS will lead to the increase of the rate of detection of AIS. This will have an impact on the incidence and mortality of invasive adenocarcinoma in the coming years. The protective effect is beginning to be seen only recently for women younger than 30 years in Australia and in England.

004

Squamous lesions of the uterine cervix

*J. Bulten**
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Cervical cancer is a major cause of death in women and the second most frequent cancer throughout the world, accounting for almost 15% of all malignancies in women. In this course, an overview is presented of the precursors of squamous cell carcinoma, its mimics, microinvasive carcinoma and cervical squamous cell carcinoma. The CIN terminology (cervical intraepithelial neoplasia, grades 1–3) of Richart is most widely used for cervical cancer precursors. Nowadays, it is evident that CIN is not a continuum, but reflects merely a low-grade entity (koilocytosis, flat condyloma and CIN1) and conversely a true intraepithelial neoplastic process (CIN2-3 and carcinoma in situ). In diagnosing CIN, HPV testing is not recommended. On the contrary, post-treatment HPV testing can predict

treatment failure and thus residual CIN. In grading CIN and to differentiate CIN from its mimics as atrophy, immature metaplasia or reserve cell hyperplasia immunohistochemistry (Mib1 and P16) can be applied. Due to preceding biopsies, microinvasive carcinoma is frequently overdiagnosed. Colposcopy, cytology and immunohistochemistry are not very helpful in diagnosing microinvasive growth. As the diagnosis of (micro-)invasive growth has great clinical implications, cervical excision biopsies or cones should be totally embedded and serially sampled. There are three types of invasive growth, and the invasion depth should always be measured between the last cell of the deepest invasive focus perpendicular to the (expected) site of origin. Finally, the several types of squamous cell carcinoma and its mimics are presented.

005

Glandular lesions of the uterine cervix

*G. W. McCluggage**
*United Kingdom

Friday, 3 September 2010, 14.30–16.30, Aula Średnia A

WGS-36 Neuroendocrine tumours of digestive tract

Chairpersons: A. Nasierowska-Guttmejer, Poland
R. Y. Osamura, Japan

001

Pathology of neuroendocrine tumours: diagnosis, prognosis and therapy

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Background: The diagnosis of neuroendocrine tumors (NETs) is mainly established by morphology and immunohistochemistry (IHC). The NETs in digestive tract contain those of stomach, duodenum, ileum, appendix, colon and rectum. The NETs have been classified into: (1) well-differentiated neuroendocrine tumor (WDNET), (2) well-differentiated neuroendocrine carcinoma (WDNEC) and (3) poorly differentiated neuroendocrine carcinoma (PDNEC; WHO and ENTS). In addition to WHO classification, their biologic behavior is graded into G1, G2 and G3 (ENET) by Ki-67 index <2%, 3–20% and >20%, respectively, which correlates with prognosis.
Method: Our file of total 121 referred cases of GEPNETs for the IHC staining for somatostatin receptor (SSTR)2a, for somatostatin analogue therapy, indicates that the NETs of the digestive tract comprise 17.1% (21 cases) and that the majority of GEPNETs being pancreatic NETs. Among these digestive NETs, rectal NETs and duodenal NETs are prominent cases.
Results: The metastatic duodenal and rectal NEC showed a ki-67 index higher than 2%. SSTRa was positive on the cell membrane of the tumor cells of 20 cases, except for one case of rectal NET. It should be emphasized that all duodenal gastrinomas, which are well known as early metastases and sometimes as a member of MEN-1, revealed SSTR2a, which should serve as a good target molecule for the response of somatostatin analogue (SA) therapy.
Conclusion: In conclusion, it is the pathologist's role to make appropriate diagnosis (terminology), prognostication and therapeutic indication.

002

Gastric neuroendocrine tumours

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Objective: Neuroendocrine cells compose the diffuse endocrine system of the gastroenteropancreatic tract. Gastric endocrine tumours (GETs) are mainly composed of enterochromaffin-like cells. GETs are rare and constitute about 1% of all gastric neoplasms and about 9% of all gastrointestinal endocrine tumours. There are three types of GETs. Type 1 is the most common, well-differentiated neoplasm, polypoid, multiple usually benign, associated with chronic atrophic gastritis. Type 2 is a rare, multiple, polypoid tumor associated with the Zollinger–Ellison syndrome. Type 3 is the second most common GETs, sporadic, much larger than types 1 and 2 and have a high propensity to metastasize. Poorly differentiated tumours are rare and account for approximately 6% of GETs. Most patients have single, advanced and aggressive tumor. In 2000, the World Health Organization determined the classification for gastroenteropancreatic neuroendocrine tumours which categorizes tumours as: (1) well-differentiated neuroendocrine tumor—(a) benign and (b) uncertain malignant potential; (2) well-differentiated neuroendocrine carcinoma; and (3) poorly differentiated neuroendocrine carcinoma.
Method: In 2006, a new TNM classification system was proposed for the staging. T Primary tumor T is in situ tumor/dysplasia <0.5 mm confined to mucosa. T1 tumor invades lamina propria or submucosa or <1 cm. T2 tumor invades muscularis propria or subserosa or >1 cm. T3 tumor penetrates serosa. T4 tumor invades adjacent structures
Results: In the seventh edition TNM: T3—tumor invades subserosa, T4—tumor perforates serosa; invades adjacent structures.
Conclusion: Histopathologic grading was performed according to mitotic count and Ki67 index. Grade mitotic count (10HPF), Ki67 index (%) G1 < 2 <2; G2 2–20, 3–20; G3 > 20 > 20

003

Small intestine GEP NET

*A. Nasierowska-Guttmejer**

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Background: Neuroendocrine tumours of the small bowel are usually sessile nodules with a diameter between 0.5 and 3 cm. These are microscopically diagnosed as well-differentiated carcinoma. Clinically, the patients show primarily the liver metastasis, bowel obstruction or sub-ileus induced by the metastatic mesenteric tumor or systemic symptoms caused by the hormonal effects of serotinin called carcinoid syndrome. Secondarily, the primary tumours are discovered during an exploration of the gut.
Method: The prognostic relevance of the WHO, the new TNM classification system, clinical staging (CS) including other prognostic factors (gender, cellular proliferation rates according to Ki-67 labeling, G differentiation of the tumour cells, etc.) were analyzed retrospectively in 98 patients (mean age 58.3 years) with histologically proven small bowel neuroendocrine tumours (NET). Overall survival (OS) and progression-free survival (PFS) data were analysed using the Kaplan–Meier method. The differences between groups were evaluated using the Cox–Mantel test.
Results: There were five patients with WHO 1, 80 with WHO 2 and the rest with WHO 3. Including pTNM and stage, there were four cases of CS I, seven cases CS II, 31 cases CS III and the rest CS IV. Significant differences between median OS were noted in patients with CS I and II vs CS III and IV and WHO 1 vs WHO 3 and WHO 2 vs WHO 3 ($P<0.05$). Additionally, there was a significant difference between OS on patients with G1 vs G3 and those with G2 vs G3. Median OS and PFS, including all patients, were 44 and 18 months.
Conclusion: Histological type (WHO) and staging are the most important prognostic factors of small bowel NETs.

004

Large intestine GEP NET

*M. Lipidski**

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Objective: Large intestine neuroendocrine tumours should be classified (consensus 2007) into two anatomical groups: colon and rectum.
Method: When reporting neuroendocrine tumours, pathologic report should contain: tumor site, tumor size, focality, histologic type according to WHO group, mitotic count and Ki-67 index (grading), margins (proximal, distal, radial), lymphatic vessels invasion, pTNM staging, staining results. The pathologist should do at least three immunostaining procedures suspecting neuroendocrine tumor: synaptophysin, chromogranin A and MIB1 (anti-Ki-67).
Results: Colon tumours (mainly WHO groups 3 and 4) are rare, but due to the absence of early symptoms, they are discovered in stage of disseminated disease—30% of tumours have metastases at the time of diagnosis. These tumours metastasize to liver, lymph nodes, peritoneum and mesentery, with 5-year survival rate oscillating around 50%. Rectal neuroendocrine tumours (mainly WHO group 1, 2 or 3) have a better prognosis (overall distant metastases from rectal NETs occur in approximately 2.3% cases), in contradiction to their increase in number, probably due to the increase in reporting tumours removed endoscopically in polyps.
Conclusion: Thanks to prophylactic programs, there is larger opportunity to discover polypoid neuroendocrine lesions in the state of limited disease. However, it is essential to fully evaluate excision margins and to do this properly; endoscopists should carry out endoscopic submucosal dissection (ESD) as a procedure of first choice. Endoanal/rectal ultrasound is very useful in the preoperative assessment of tumor size, depth of invasion and presence of metastases in local lymph nodes. In the case of more advanced disease, other treatment options should be taken into consideration, although benefit from more aggressive surgery is unclear.

Friday, 3 September 2010, 14.30–16.30, Aula Średnia B

WGS-37 Pathologists in favour of developing countries—discussion of common projects

Chairpersons: D. Soldini, Switzerland
V. Canzonieri, Italy

Friday, 3 September 2010, 14.30–16.30, Sala Wystawowa A

WGS-38 Paediatric soft tissue and bone tumours

Chairpersons: M. Cohen, United Kingdom
J. Kobos, Poland

001

Rhabdomyosarcoma

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*Universität zu Kiel, Institute of Paidopathology, Germany

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood and adolescence. Currently, RMS is classified by the so-called International Classification of Rhabdomyosarcomas. This classification identifies three subgroups with different biological behaviors. Favorable prognosis include (botryoid subtype of embryonal rhabdomyosarcoma, spindle cell type of embryonal rhabdomyo-

sarcoma), intermediate prognosis ('classical' embryonal rhabdomyosarcoma), and unfavorable prognosis (alveolar rhabdomyosarcoma). Immunohistochemistry can be used to separate between embryonal and alveolar RMS. AP2 and p-cadherin are almost exclusively expressed in alveolar RMS, whereas EGF receptor and fibrillin 2 are found in embryonal RMS. In addition, the myf-4 expression of alveolar RMS is usually much stronger than in embryonal RMS. Genetic changes have been found for both embryonal and alveolar RMS. Most alveolar RMS have a balanced reciprocal translocation, t(2;13)(q35;q14). A smaller subgroup of alveolar RMS has a t(1;13) translocation. Two other translocations, a t(2;2)(q35;p23), which generates a fusion protein composed of PAX3 and NCOA1, and a t(2;X) resulting in a fusion protein PAX3/AFX, have been described in single cases. In embryonal RMS, a loss of heterozygosity (LOH) on chromosome 11p15.5 has been shown. The involved gene or genes have not yet been clearly identified, but several interesting genes are located in this area like the MyoD1 gene, the IGF-2 gene, the LDH (muscle subunit) gene, and the WT 1 gene. A possible imprinting of this gene has also been postulated.

002

Infantile fibrosarcoma and malignant fibrous tumours

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Background: Fibroblastic/myofibroblastic lesions account for 12% of pediatric soft tissue tumours and include benign neoplasms, reactive, pseudoneoplastic proliferations and, rarely, malignant tumours. The malignant lesions, registered in the Italian Protocol RMS-96, are reported to define their clinicopathologic and molecular features.

Method: Forty-eight patients with fibrosarcoma (congenital and adult) were reviewed (Table). Four cases, originally diagnosed as CIFS, with negative ETV6-NTRK3 transcript, were reclassified as composite fibromatosis (two), undifferentiated sarcoma (one) and rhabdomyofibrosarcoma (one) and excluded from this study.

Results: see Table 1 *No translocation legend: *CR* complete remission, *IICR* second complete remission, *LFU* lost at follow-up, *MTS* metastases, *DOD* died of disease

Conclusion: The identification of some recurrent chromosomal abnormalities has contributed to the redefinition of CIFS and identification of a subgroup of more aggressive fibrosarcomas lacking the typical transcript. Adult type fibrosarcomas may also occur in children. They involve more frequently the head/neck region and have a tendency to local relapse and distant metastasis.

003

Update on benign fibrous lesions

*J. Kobos**

*Medical University of Lodz, Dept.of Pathology, Poland

Some of these lesions are genuine benign neoplasms; others have a reactive nature. Nodular fasciitis is a fibroblastic/myofibroblastic proliferation mostly seen in the subcutaneous tissue, morphologically presenting a loose and culture-like growth pattern. Proliferative fasciitis (and proliferative myositis) is, likewise, nodular fasciitis, a fibroblastic/myofibroblastic proliferation with the presence of large ganglion-like cells. Myositis ossificans is a benign and reactive lesion. Fibrous hamartoma of infancy is a relatively common benign tumour of early childhood which presents three components: fibrocollagenous tissue, immature-primitive mesenchymal cells and mature fat tissue. Myofibroma/myofibromatosis is a benign tumour observed in newborns and children within the first 2 years of life, as well as in elderly patients. Fibromatosis colli is a rare benign lesion typically seen in the sternocleidomastoid muscle in infants. Microscopically, it is a proliferation of fibroblasts in between the muscle fibres forming a "scar-like" pattern. Juvenile hyaline fibromatosis is a very uncommon, non-neoplastic lesion. Fibroma of tendon sheath is a benign (possibly reactive) lesion related to tendons. Calcifying aponeurotic firbroma is the tumour more frequently seen in children. Gardner fibroma is an uncommon benign tumour mostly seen in infants, children and adolescents. Calcifying fibrous tumour is a rare benign tumor more frequently observed in children and young adults.

HISTOTYPE	SEX (m/f)	AGE RANGE (m)	SITE	Transcript ETV6-NTRK3	FOLLOW-UP (range 0-10yr)
9 Adult FS	5/4	4-16(10)	Head 5, Extremities 4	Negative	CR 5, IICR 1, LFU 2
23 CIF	14/10	0–2(0,54)	Head 1, Extremities 17, Trunk 4, Abdominal 1	12/16	CR 17, IICR 1*, LFU 5, MTS 1*
5 LGFMS	4/1	3-15(9,40)	Extremities 4, Trunk 1	Negative	CR 2, IIRC 1, MTS+LFU 1, LFU 1
4 SEF	2/2	3-9(6,5)	Head 2, Extremities 1, Trunk 1	Negative	CR 2, LFU 2
3 PMMTI	2/1	0,3-0,4(0,35)	Head 1, Extremities 2	0/3	IICR 1, MTS 1, DOD 1

004
ASPS, CCS, ES
*A. Kelsey**
*United Kingdom

005
Osteosarcoma
*P. C. W. Hogendoorn**
*The Netherlands

Friday, 3 September 2010, 14.30–16.30, Aula Mała

WGS-39 Computational pathology
Chairpersons: M. G. Rojo, Spain
Y. Yagi, USA

001
SNOMED CT in Europe
*M. G. Rojo**
*Spain

Background: A standard clinical terminology like SNOMED CT is important not only for pathology information systems but also to integrate these systems with many other hospital information systems (HIS, PACS, departmental systems, encoding, database patients) and primary care information systems.
Method: SNOMED CT includes two hierarchies (morphologic abnormality and disorder) with similar terms. A SNOMED CT implementation was designed taking into account both the difference between these hierarchies and the relationship between terms located in different hierarchies. This is especially necessary in the post-coordination of clinical expressions and the creation of subsets.
Results: The January 2010 edition of the subset of Pathology, which distributes the College of American Pathologists (CAP), contains 7,582 terms, but this subset needs to be improved to give it a clinical use. In SESCAM, 3,000 morphological concepts and 200 specimen concepts were selected to create a minimum basic set for use in pathology information systems. SNOMED CT is also used in the SESCAM telepathology portal (Project Serendipia), developed according to the IHE-Anatomic Pathology initiative and DICOM supplement-122 for medical imaging.
Conclusion: A SNOMED CT implementation strategy should not only include a list or subset of terms, but it must also consider the efficient use of relationships between terms and concept hierarchies, a mechanism for extension and update, and a plan for the coexistence or migration of legacy SNOMED II codes.

002
Latest developments in data storage devices applicable for virtual slides
*J. Görtler**
*Belgium

003
Structured reports in tumor pathology—how and why
*G. Haroske**
*Germany

Objective: Among all medical documents, pathology reports are often highly critical for patient care. In terms of information technology and knowledge engineering, presently, these reports are widely lacking adequate structure to support their usage in knowledge retrieval technologies for medical decision making, research, epidemiology, quality management and medical education.
Method: By means of literature surveys and by a questionnaire sent to vendors of pathology management systems (PMS), the degree of structure in pathology reports in PMS sold in Germany was analyzed and compared with user needs expressed by pathologists.
Results: Pathology reports are widely displayed, stored and exported as free text, identifiable by human beings, but without "observation identifiers" of a coding system not readable by a computer. A few systems allow textual information to be structured in sections partly identified by coding systems as SNOMED CT, etc. Within the sections, the information is still unstructured. At the very beginning are attempts to structure the report as a list of coded items based on templates, which are controlled for identifiers, version, and the underlying concepts and representations. This is by far the most granular structure for computer readability and knowledge engineering.
Conclusion: There are very different opinions about the aim and degree of structured reporting. Although standardization efforts have led to a series of reporting minimum requirements in tumor pathology, their implementation in structured data entry systems is still lacking. Tools for data mining in routine pathology will be decisive for all important progress in pathology informatics at all.

004
Experiences of virtual microscopy in TMA application
*G. Kayser**
*Germany

005

How to scale image information in virtual slides?

K. Kayser, S. Borkenfeld, J. Görtler, G. Kayser*
*Heidelberg, Germany

Objective: The application of virtual slides requires unequivocal definition and scaling of image information. Scaling of image information offers the evaluation of diagnosis and content-based image retrieval (CBIR).
Method: Theory in principle—image information can be extracted with or without knowledge of its content. Knowing its content (for example organ, potential diseases), we can segment biological meaningful objects (nuclei, membranes, etc.) and measure their features (size, gray values, derived parameters). The obtained results can be scaled and associated with the underlying disease. In reverse, knowing the applied algorithms and the object (and structure) features, CBIR can be applied. Without predefined knowledge, basic image information is mapped on spatial image units (pixels), called texture. They can be quantified by the application of stochastic geometry: Each biological meaningful object is composed of so-called primitives (points, fibers, rings, plateaus). Their mapping on an object can be used to detect knowledge-independent content and scalable image information.
Results: Object- and structure-based (1) and the texture-based (2) algorithms have been implemented and applied on more than 1,000 images obtained from H&E-stained glass slides of different organs (colon/rectum, lung, pleura, thyroid) in search for disease-significant areas (fields of view) and disease classification. The accuracy of the methods in field of view selection was computed to >98%, that of prospective disease classification >95%.
Conclusion: The developed algorithms permit a reliable scaling of image information that can be applied for CBIR and related tasks. References: K. Kayser et al. Diagnostic Pathology 2010, 5(Suppl 1):S12K. Kayser et al. Diagnostic Pathology 2008, 3:17.

006

Automation of image analysis in breast cancer

*J. McGee**
*United Kingdom

007

New generation of slide scanners open the way to digital pathology

*B. Molnár**
*Hungary

Background: Slide scanners with digital microscopy software solutions became available since the early 2000s. Digitalisation of the pathology laboratories and workflow process is still not achieved.
Aims: Identification, development and evaluation of digital slide scanner, laboratory, workflow, user interface and application needs including technical requirements for whole department digitalisation of the pathology laboratories.
Method: A new generation digital slide scanner was developed. It included a new dark light slide preview system for FISH scanning, then 100% safe loading mechanism, and enhanced scanning speed with frame rates of 50/second. Respective software applications (HL7 interface towards LIMS, server scanning, digital cockpit software for H/E, immunohistochemistry and FISH analysis) was developed.
Results: A middle-sized academic routine pathology department produces daily approx. 300 H/E, 200 IHC, and 35 FISH slides. Only one scanner for the H/E, for the IHC, and one for the FISH slides had to be placed without hiring any additional assistant workforce. Scanning could be performed in parallel to the sample preparation procedures due to the 10× increased scanning speed (20 s/cm^2) for any types of the slides directly to the digital slide server. The digital pathology workstation SW supported the parallel opening, viewing and evaluation of the respective slides and areas. Quantification modules became widely used for nuclear, membrane and FISH analysis.
Conclusion: The new-generation scanners make the digitalisation of the pathology laboratories financially also affordable. The software applications can contribute to the more robust, documented and comprehensive analysis of the digital cases.

Friday, 3 September 2010, 17.00–18.30, Aula Duża B

WGS-40 Mucinous tumours of the appendix

Chairperson: J. F. Flejou, France

001

Serrated and hyperplastic lesions of the appendix

*J. F. Flejou**
* Hôpital Saint-Antoine, Service Anatomie Pathologiqu, Paris, France

Hyperplastic lesions of the appendiceal mucosa are classically divided into two groups: diffuse hyperplasia and localized polyps. Localized lesions were usually diagnosed as hyperplastic polyps, but some of these lesions are now better classified as sessile serrated adenomas. Both diffuse hyperplasia and polyps are in most cases incidental findings on appendectomy specimens (performed for appendicitis or systematically sampled in a colectomy specimen). However, these lesions are significantly associated with colon adeno-

carcinoma, and therefore, their discovery in an appendectomy specimen is an indication of further examination to exclude colorectal neoplasia. Diffuse mucosal hyperplasia extends to more than one half of the circumference of the appendiceal mucosa. It exhibits elongated crypts with serrated architecture. The epithelium is composed of columnar cells alternating with large goblet cells in various proportions. The crypt bases are not serrated and are lined by regular cells with no atypia. Hyperplastic polyps are supposed to be rare in the vermiform appendix. They are localized lesions, with a histological aspect similar to the corresponding colorectal lesions. Similarly to diffuse mucosal hyperplasia, they are characterized by a serrated architecture restricted to the upper part of the crypts and by the lack of any dysplastic features. When lesions associate hyperplastic serrated features in one area and adenomatous (tubular and/or villous) features in another, they are designated as mixed adenomatous and hyperplastic polyps, as tumours of the colon and rectum. However, it is probable that at least part of these mixed lesions represent hyperplastic serrated polyps that have progressed to dysplasia. Sessile serrated adenoma (or sessile serrated polyp or serrated polyp with abnormal proliferation) is a more recently described type of colorectal polyp with a serrated architecture. They occur frequently in the right colon, and although they resemble to hyperplastic polyps, they are characterized by subtle architectural and cytological features, especially dilation and serration of the basal part of the crypts. They can be dysplastic or not. The relative frequency of sessile serrated adenomas and other serrated lesions of the appendix is not well established, but it is highly probable that many appendiceal lesions previously designated as diffuse hyperplasia or hyperplastic polyp represent cases of sessile serrated adenomas. Both types of lesions have been proven to show decreased expression of MLH1 and MGMT in a majority of cases and also BRAF mutation in a significant proportion of cases. These genetic and epigenetic alterations are similar to those observed in sessile serrated adenoma of the colon, which is now considered as a precursor lesion for a subgroup of colorectal cancer. The differential diagnosis of serrated lesions of the appendix also includes adenomas of colorectal type (more often showing villous architecture when compared to colorectal lesions) and low-grade mucinous neoplasms, often referred to as cystadenomas.

002

Adenocarcinoma, mucinous neoplasm of the appendix and pseudomyxoma peritonei

*A. Jouret-Mourin**

*Cliniques Universitaires ST LUC, Brussels, Belgium

Primary adenocarcinoma of the appendix is unusual. Some resemble typical colonic adenocarcinoma in their appearance and behaviour, but others are well-differentiated neoplasms or adenoma-like that may be associated with the pseudomyxoma peritonei syndrome without distant metastases. This pathological spectrum is confused because of terminological inconsistencies and the large variety of diagnostic criteria. Therefore, there remains a "grey area" between benign and malignant lesions. Some authors have recently proposed a new terminology based on the biological potential of the different lesions. Low-grade appendiceal mucinous neoplasm (LAMN) is defined as a subset of lesions that have low-grade cytological atypia and minimal architectural complexity. These lesions penetrate into or through the appendiceal wall often on a broad front, without overt invasion and may be associated with pseudomyxoma peritonei. Consequently, such LAMN category includes the old terms of "tumours of uncertain malignant potential, "pseudomyxoma with adenoma-like histology" and the "low-grade mucinous adenocarcinoma" which can also produce pseudomyxoma. Invasive mucinous adenocarcinoma (MACA) includes a destructive invasion of the appendiceal wall with high-grade atypia, complex proliferation (i.e. cribriform pattern), desmoplastic stroma, signet ring cells differentiation or undifferentiated tumours. Lymph node metastases occur lately, but peritoneal spreading is more frequent. Some recent publications have demonstrated that protein immunoexpression profiles offer predictive factors of adverse clinical outcomes, which should further facilitate the classification of appendiceal mucinous neoplasms. Appendiceal mucinous tumours can occur in patients who have suffered long-standing ulcerative colitis, familial polyposis and HNPCC syndrome. Most of them show microsatellite instability probably given the high frequency of serrated precursor. Pseudomyxoma peritonei syndrome is defined as the presence of abundant mucinous material on the peritoneal surface. This entity should be retained as a clinical term. Besides exceptional cases of pseudomyxoma reported in association with mucinous carcinomas at other sites, all cases of pseudomyxoma peritonei are related to appendiceal rupture with subsequent transcelomic spread of tumoral cells. Some authors have proposed that the same terminology should be used for the appendiceal primaries and the peritoneal lesions since LAMN lesions confined to the appendix have the same appearance as those that have spread to the peritoneum. In addition, any tumour capable of producing pseudomyxoma peritonei should be considered as adenocarcinoma. Therefore, the classification of pseudomyxoma perotonei includes low-grade and high-grade mucinous adenocarcinoma. The acellularity of the extra-appendicular mucin, based on extensive sampling, must be described in the pathological report because of its prognostic implications.

003

Mixed mucus-secreting and endocrine tumours of the appendix

*J.-Y. Scoazec**

*Hospices Civils de Lyon, Hôpital Edouard Herriot, France

Vermiform appendix is one of the most frequent sites of mixed endocrine–exocrine tumours in the gastrointestinal tract. One histologic type of mixed mucus-secreting and endocrine tumor is specific for the appendix: This distinctive tumour is known as goblet cell carcinoid. The others are mixed endocrine–exocrine carcinomas raising the same problems of diagnosis and management than in the other digestive locations. Goblet cell carcinoid is a highly distinctive clinical and histological entity. In contrast to the much more common pure neuroendocrine tumours of the appendix, which are usually discovered incidentally in young patients, goblet cell carcinoids present a peak of incidence in the fifth decade and are usually symptomatic. At macroscopic examination, goblet cell carcinoids present a very particular pattern of growth, resulting in the thickening of the whole appendicular wall and in a marked reduction in the calibre of the lumen, in the absence of visible tumour mass. This aspect is due to the predominant infiltration of the deepest part of the mucosa and the submucosa by tumour cells, which result in their fibrosis and thickening. At a higher magnification, tumor cells are grouped in small nests formed by large cells, with an abundant, pale, mucus-laden cytoplasm and an eccentric nucleus. These cells are strongly reactive with Alcian blue and PAS stainings. Small endocrine cells are closely intermingled with mucus-laden cells; they are usually difficult to identify by conventional stainings; they are much more easily demonstrated through the immunodetection of endocrine and neuroendocrine markers such as chromogranin A and synaptophysin. Finally, some amphicrine cells, with a combination of exocrine and endocrine markers, may be present. The number of endocrine cells admixed with mucus-laden cells is highly variable from one case to another and even from one region to another in the same tumour. There is no minimal amount of endocrine cells required for the diagnosis of goblet cell carcinoid, which is achieved by the combination of a typical macroscopic aspect, the presence of characteristic mucus-laden cells and the demonstration of even a few endocrine cells intermingled with the predominant mucus-producing population. From the molecular point of view, goblet cell carcinoids frequently harbour loss of heterozygosity in 11q, 16q and 18q. Goblet cell carcinoids carry a high risk of peritoneal dissemination, but a low risk of distant metastasis. Their prognosis is therefore much poorer than that of typical neuroendocrine tumours of the appendix: The 5-year survival is only 18% instead of 83%, all stages included; even for patients with localized disease, it is 55% instead of 94%. Importantly, the TNM classification of goblet cell carcinoid must follow the criteria used for the staging of appendicular carcinomas, not those recently proposed for appendicular "carcinoids". Mixed endocrine–exocrine carcinomas of the appendix combine an exocrine component, usually corresponding to a mucus-secreting adenocarcinoma of variable differentiation and an endocrine component, which may be well or poorly differentiated. The diagnosis of mixed tumour must be made with caution, only if the endocrine component is clearly neoplastic and represents at least 30% of the whole tumour. The prognosis and treatment are those of the most aggressive component.

Friday, 3 September 2010, 17.00–18.30, Aula Średnia A

WGS-41 Electron microscopy in neuropathology

Chairpersons: J. Lloreta-Trull, Spain
G. Cennacchi, Italy
A. Marszalek, Poland

001

Electron microscopy of muscle diseases

*C. Navarro**

*Spain

Electron microscopy has been relatively surpassed by immunohistochemistry in pathology, especially for the diagnosis of neoplastic processes. However, its use is mandatory in neurodegenerative and neuromuscular disorders of non-neoplasic origin. Ultrastructural examination has been proven to be useful in the following issues: (1) to understand the physiopathology of muscle disorders. Muscular dystrophies are produced by mutations in genes codifying proteins located at different subcellular levels. Ultrastructural alterations have been observed at the level where the defective protein is expressed, i.e. plasma membrane alterations in dysferlinopathies, caveolinopathies and sarcoglycanopathies, or nuclear envelope membrane defects in Emery Dreifuss muscular dystrophy and laminopathies. (2) To identify structures not visible with light microscopy. This is the case of intranuclear inclusions in oculo-pharyngeal muscular dystrophy, in inclusion body myositis, or tubulo-reticular inclusions seen in several autoimmune disorders and in AIDS. (3) To determine the origin of specific anomalies depicted with light microscopy. Ultrastructure of tubular aggregates, nemaline bodies, central cores or paracrystalline mitochondrial inclusions has been crucial to define the origin and organization of

such changes at the subcellular components of the muscle fiber. (4) To study in depth lysosomal storage disorders. The identification of different types of inclusions in neuronal ceroid lipofuscinosis according to the phenotype–genotype form of the disease has been especially useful, and so it was for the diagnosis of late-onset forms of glycogenosis type II, Fabry disease or others. In conclusion, electron microscopic examination is crucial to identify the subcellular structural involvement in some muscle disorders and to understand the underlying pathological mechanisms.

002

Electron microscopy of peripheral nerve

*A. Vital**
*Bordeaux University Hospital, Dept. of Pathology, France

Objective: Electron microscopic examination (EME) of a peripheral nerve biopsy (PNB) is particularly valuable to assess the type of nerve fiber lesions, endoneurial deposits and storage materials.
Method: A well-done PNB, as well as a reliable methodology for fixation and embedding of specimens, constitute a prerequisite for an informative EME.
Results: If most cases of hereditary peripheral neuropathies are now diagnosed by molecular biology, nerve lesion analysis can direct the search for mutations in specific genes. Prominent "onion bulb" formations are associated with PMP22 duplication. "Tomaculae" correspond to PMP22 deletion, but can also be observed in certain cases of P0 mutation. Other P0 mutations are mainly associated with irregularly uncompacted myelin lamellae. Discrete "onion bulb" formations in association with "pseudo-onion bulb" formations surrounding clusters of regeneration are much suggestive of connexin 32 mutation. Abnormal mitochondria point to a mitofusin 2 gene mutation. Ultrastructural evidence of macrophage-associated demyelination is very supportive in atypical cases of inflammatory demyelinating polyneuropathy. In some cases of paraproteinemic neuropathy, myelin lamellae may exhibit a regular spacing corresponding to the presence of immunoglobulin M. In others, granular immunoglobulin deposits are identified in the endoneurium. Occasionally, the endoneurial deposits have the tubular ultrastructure of a monoclonal cryoglobulin. The presence of osmiophilic lamellar inclusions in Schwann cells points to treatment intolerance. At last, EME of a PNB may help in the diagnosis of a hereditary storage disease.
Conclusion: EME can be decisive to establish the etiological diagnosis of a peripheral neuropathy and must be supported by a reliable methodology.

003

Electron microscopy of metabolic storage diseases

*I. Ferrer**
*Hospital Universitari de Bellv, Institut Neuropatologia, Spain

Metabolic diseases of the nervous system in infancy constitute a very complex group of variegated diseases with particular clinical, morphological, biochemical and genetic characteristics. Diagnosis of such disorders usually demands the collaboration of different specialists, methods, and skills. This is particularly important in order to avoid unnecessary probes and superfluous additional suffering related with inappropriate complementary examinations and visits. In recent years, molecular and genetic studies have facilitated a correct diagnosis during life, and improvements in therapy have permitted the application of new therapeutic tools in some cases. Morphological visualization of determinate lesions in skin, conjunctival, appendicular and, rarely, rectal mucosa, is extremely useful to support a clinical diagnosis. Lipidosis, mucopolysccharidosis, mucolipidosis, polyglucosan storage diseases and glycogenosis affect several cell types outside the central nervous system, including axons and Schwann cells, endothelial cells, pericytes, smooth muscle fibres, sebaceous and eccrine glands and ducts, and striate muscle. Deposits are usually too small to be visible with an optical microscope. Yet electron microscopy permits the observation of lysosomal inclusions filled with specific inclusions and deposition of abnormal material in different cells. In addition to metabolic diseases, other degenerative diseases affecting the nervous system in which the diagnosis is mainly based on key pathological markers are also subject to electron microscopy analysis during life. Nuclear neuronal inclusion body disease and infantile neuroaxonal dystrophy, among others, can be diagnosed by means of appendicular (or rectal) biopsies and by examining the nerve terminals and skin and conjunctiva, respectively.

004

Electron microscopy and neurodegenerative diseases/ prion CNS diseases

*P. Liberski**
*Medical University of Lodz, Dept. of Mol. Pathol. Neuropathology, Poland

Friday, 3 September 2010, 17.00–18.30, Aula Średnia B

WGS-42 Advances in head and neck pathology

Chairpersons: N. Gale, Slovenia
T. Helliwell, United Kingdom

001
The significance of HPV and HIV infections in sinonasal and laryngeal pathology

*L. Alos**
*Spain

002
Desmosomes in subtypes of squamous cell carcinoma of the head and neck

*N. Zidar**
*Slovenia

003
Recent advances in sinonasal inflammatory diseases

*T. Helliwell**
*United Kingdom

This presentation will cover a range of sinonasal inflammatory diseases, focussing on those where an improved understanding of pathogenesis illuminates diagnostic histopathology. The categorisation of sinonasal fungus-related diseases is important for prognosis and treatment. Fungi are common airborne allergens and can be cultured from the nasal secretions of most healthy individuals. The diagnosis of fungal sinusitis relies on the histological recognition of fungi in sinus tissue, although the morphology of fungal hyphae is of limited value in distinguishing between fungal species. Invasive fungal sinusitis is almost always encountered in patients whose immune system is compromised and is associated with necrosis and vascular invasion by fungi. Noninvasive fungal sinusitis includes sinus fungal balls and allergic fungal sinusitis, a disease characterised by so-called allergic mucin and scanty fungal hyphae. The pathogenesis of chronic rhinosinusitis involves a complex interplay between environmental factors and genetically influenced immune responses; eosinophils may be seen in both allergic rhinosinusitis and in patients with no evidence of allergy. The differential diagnosis of inflammatory polyps will be discussed. Pathologists should be aware of the many sinonasal diseases that may include a component of granulomatous inflammation including mycobacterial infection, cocaine-induced midline destructive disease and Wegener's granulomatosis, and of the value of ancillary investigations such as autoantibodies in their differential diagnosis. Autoimmunity to type II collagen is involved in relapsing polychondritis, a multisystem disorder in which destruction of the cartilages of the nose, ears and upper airway is prominent.

004
Classification and diagnosis of sinonasal adenocarcinomas

*I. Leivo**
*Haartman Institute, Dept. of Pathology, Helsinki, Finland

Salivary gland-type sinonasal adenocarcinomas can occur in the sinonasal tract, and they resemble the corresponding tumours in salivary glands. The most common types are adenoid cystic carcinoma and adenocarcinoma, not otherwise specified. Intestinal-type sinonasal adenocarcinomas mimic the histological appearances of intestinal adenomas, carcinomas, or rarely even the normal intestinal mucosa. Occurrence of the intestinal-type sinonasal adenocarcinoma has been associated with long-term exposure to hardwood (beech, oak) dusts. In the woodworking industry, these tumours can be 1,000 times more common than in the general population. The average exposure time is 40 years. Histologically, the most common appearances resemble colonic adenocarcinomas. If the resemblance is striking, the rare possibility of a metastasis from the gastrointestinal tract should be considered. Similar to colonic adenocarcinomas, intestinal-type sinonasal adenocarcinomas stain for CK20, CDX-2, and MUC2, but they also stain for CK7. Thus, the differential diagnosis between a primary intestinal-type sinonasal adenocarcinoma and a metastatic colonic adenocarcinoma cannot be based on histomorphology or immunophenotype alone. Then, the recommended approach is colonoscopy. The intestinal-type sinonasal adenocarcinoma is a high-grade malignancy. However, the prognosis of patients with hardwood dust exposure is better than in sporadic tumours. In addition, papillary growth patterns relate to a more favourable outcome. Low-grade sinonasal adenocarcinomas have not shown occupational or environmental associations. Histologically, they are papillary or glandular, with a single layer of uniform epithelial cells. Cytologically, they are bland, but malignancy is revealed by the complexity of architecture and invasive growth. Low-grade sinonasal adenocarcinomas do not express intestinal immunophenotypic markers.

Friday, 3 September 2010, 17.00–18.30, Sala Wystawowa A

WGS-43 Lymphoproliferative diseases Pediatric WG Business Meeting

Chairpersons: I. Scheimberg, United Kingdom
M. Pronicki, Poland

001

ID post transplant lymphomas: pediatric post-transplant lymphoproliferative disorders: pathology, etiology, tumor biology and epidemiology

*B. Ngan**
*Hospital for Sick Children, PediatricLabMed, Toronto, Canada

Background: Post-transplant lymphoproliferative disease (PTLD) is an important cause for morbidity and mortality in transplant recipients. Pathologically, PTLD is heterogeneous and is characterized by a spectrum of lymphoid proliferations that ranges from reactive lymphoid hyperplasia, non-clonal atypical lymphoid cells and or plasma cell proliferations to infiltrates of polymorphous, mono- or polyclonal lymphoid cells to monoclonal lymphoid cells with histopathogical features of non-Hodgkin or Hodgkin lymphoma of non-transplant patients.
Method: Pediatric PTLD biopsies in solid organ transplant recipients over a 30-year period were reviewed.
Results: There were 80 PTLD cases (12.1%) in 662 transplant recipients (217 liver, 203 heart, 197 kidney, 26 lung, 13 multivisceral and 6 small bowel). PTLD occurred in 16.5% liver, 13.8% heart, 5.1% kidney, 15.4% lung, and 23% multivisceral recipients. Thirteen PTLD had infectious mononucleosis-like and/or atypical EBV+ve lymphoid hyperplasia; five EBV-ve lymphoid hyperplasia; three EVB+ve plasmacytic hyperplasia; 14 EVB+ve polymorphous monoclonal B; 13 EVB+ve polymorphous monoclonal B, 16 EVB+ve monomorphous, monoclonal B, three were Burkitts with c-Myc translocation; five EVB-ve monomorphous, monoclonal B; 1 EVB+ve PTCL; three EVB-vePTCL; one EVB+ve Hodgkin; and one EBV-veHodgkin. Three PTLD cases had EBV+ve spindle cell tumor.
Conclusion: Histopathological diagnosis plays a central role in the identification of PTLD for the management and treatment of PTLD. It is also important in assessing the epidemiology and the understanding of the pathogenesis of PTLD. There is a high association with EBV. This study confirmed the higher prevalence of PTLD in children as compared with the published data on adults by others as well as UNOS 1995 on children.

002

Recent developments in neuroblastoma

*C. Cullinane**
*United Kingdom

Peripheral neuroblastic tumours are a group of paediatric tumours composed of cells of neural crest origin. The tumours arise in the sympathetic nervous system and range from the malignant neuroblastoma at one end of the spectrum to the benign ganglioneuroma at the other end, with an intermediate group of the tumours showing a mixed pattern. They are classified according to International Neuroblastoma Committee Classification (INPC). Their presentation and behaviour is variable, which may lead to challenges in diagnosis and management. In some patients, the disease is aggressive and lethal in spite of multimodality treatment, whereas others have an excellent outcome with minimal treatment even in the presence of disseminated disease. This talk will provide an overview of the current classification system and recent modification with regard to nodular ganglioneuroblastoma, discuss the recently introduced International Neuroblastoma Risk Group system, delineate the role of pathologist with regard to the diagnostic information required in current and future neuroblastoma trials, and present an update on favourable and unfavourable prognostic factors.

Friday, 3 September 2010, 17.00–18.30, Aula Mała

WGS-44 Computational pathology

Chairpersons: A. Batistatou, Greece
P. van Diest, The Netherlands

001

Experiences in digital pathology

P. dalla Palma, E. Bragantini*
*S. Chiara Hospital Trento, Italy

Background: Computer-assisted microscopy (CAM) offers proven solutions in immunohistochemical assessments, sharing digital slides with collaborators in different places, storing and organize pathological and clinical data and research.
Method: We initially developed an in-house system for the creation of digital slides and for remote diagnoses on frozen sections, suggesting that digital pathology would become the new standard in surgical pathology. In our institution, we adopt CAM using the Aperio Scanscope Xs for several applications:
Results: (1) Detection and semiquantitative measurement of equivocal Her2 immunoreactivity and of Ki67 in breast

cancer—our data on over 350 cases with comparison between CAM and human evaluation and with FISH analysis strongly support its feasibility and reliability in routine practice; (2) to store and analyze tissue microarrays with all associated experimental and follow-up data, permitting fast access to adjacent sections and comparing stains across multiple slides; (3) to store clinical–pathological data of patients for discussion in multidisciplinary meetings and courses.

Conclusion: In our opinion, CAM provides a unique opportunity for image visualization and analysis, archival and retrieval, which in the near future will probably replace the usual microscopy in routine practice. Bibliography 1. The virtual case. Virchows Arch. 2002, 441(2):159–164. 2. Robotic telepathology for intraoperative remote diagnosis. Am J Clin Pathol. 2001 116(5):744–752. 3. Digital Pathology: Science Fiction? Int J Surg Pathol. 2000 Oct;8 (4):261–263. 4. TMABoost: an integrated system for comprehensive management of tissue microarray data. Technol Biomed. 2006 Jan;10(1):19–27.

002

Quantification of immunohistochemistry in digital pathology

*J. Schmid**
*USA

003

Automated vision quantitative assessment of Ki-67 proliferation index in a nationwide series of patients with breast cancer

J. Konsti, M. Lundin, H. Joensuu, J. Lundin*
*University of Helsinki, Finland

Objective: An automated method for immunohistochemical assessment of Ki-67 proliferation index was developed and evaluated.

Method: Using an open source image manipulation tool, ImageJ, a macro called IhcJ was created for the assessment of immunohistochemistry stainings. The performance of the IhcJ macro was evaluated in a tissue microarray series of breast cancer specimens from 1,334 patients. The specimens were stained with an anti-Ki-67 antibody, counterstained with hematoxylin and digitized. The Ki-67 proliferation index was calculated both visually and automatically with the IhcJ macro (Fig. 1). The prognostic value of the Ki-67 proliferation index was evaluated in uni- and multivariate survival analyses.

Results: In a univariate survival analysis, the hazard ratio of distant recurrence during a median follow-up of 9.5 years for the automated Ki-67 medium area fraction was 1.77 (95% CI 1.31–2.37) and for the large area fraction 2.34 (1.76–3.10). Corresponding results for visual Ki-67 proliferation index were 1.41 (0.83–2.39) and 2.58 (1.52–4.37). In a multivariate survival analysis adjusted for known main prognostic factors, the hazard ratio for the automated Ki-67 area fraction was 1.62 (1.10–2.39) and 1.73 (1.19–2.51) for medium and large area fraction groups, respectively. The visual Ki-67 proliferation index was not a significant predictor in a multivariate survival analysis.

Conclusion: Multivariate survival analyses suggest that unlike the visual Ki-67 proliferation index, the automated Ki-67 area fraction is an independent predictor of outcome in breast cancer when other main prognostic factors are taken into account.

004

Five years of experiences with WebMicroscope for teaching basic and oral pathology in a practical course

J. Szymas, M. Lundin*
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During the past 5 years, we have completed a transition of the basic and oral pathology practical courses from light to virtual microscopy. After a pilot feasibility study, the entire training set of glass slides was digitized and located on WebMicroscope server. Giving access to the web, computers have become perfect companions of the students. The study material consists now of over 400 fully digitized slides covering 15 entities in basic pathology and 15 entities in oral pathology. Digitized slides are linked with still macro- and microscopic images, organized with clinical information into virtual cases. We undertake a comprehensive evaluation of this new approach at the end of every academic year. In these surveys, students rate the software, the quality of the images, handling of the images, and effective use of virtual slides during the time of the practical. Satisfaction surveys demonstrate a steady improvement over the past 5 years as various student suggestions were implemented. An overwhelming majority of our students considered using digitized slides at their convenience as highly desirable. Overall, the quality of the images was rated as very good. However, due to the limitation of resolution of few primary scans, we have rescanned these slides at ×40 objective and a 3,900 × 3,090-pixel camera to alleviate these problems. Our students and faculty consider the virtual microscope as a significant improvement. The administrators of our department consider virtual microscopy as innovative and cost-effective because there is no longer the expense of the

maintenance and replacement of microscope and glass slides.

005

A web-based examination system for students using WebMicroscopy for learning oral pathology

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We have developed and evaluated a user-friendly online interactive teaching and examination system for pathology courses. Already in 2006, at the end of the first course, students take a multiple-choice question exam (one correct answer per question). Every exam question is linked with a proper virtual slide and the time for the answer is unlimited. This examination system uses advanced HTML features, and the Web browser that can handle frames, JavaScript and cookies is required. Setting the screen to a resolution of 1,024 × 760 or higher and decreasing the size of the menu bars if necessary is helpful in viewing the digitized histological slide and the question at the same time. Specific instructions about answering the questions are also given at the beginning of the examination. A score is generated for questions which are correctly answered. The scores are accumulated until the student decides to quit the Web browser. In the last 5 years, an overall 92% concordance rate has been achieved on practical examination based on 50 virtual slides connected with 50 multiple-choice questions per student. The online practical examination system was evaluated by the students using a questionnaire. Students were asked to reply to a survey to evaluate the usefulness of digitized slides for practical examination. All students preferred the online examination to a traditional microscope and paper-and-pencil examination and all felt that the quality of digitized slides was superior to that of classical glass slide and allowed to make a more accurate diagnosis (rating 9.5 out of 10).

006

Use of gray level co-occurrence matrix in detecting corresponding fragments and evaluating quality of virtual slides

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Virtual slides may be acquired using various scanners, produced by different companies. Pathologists who examine digital images captured by different scanners may subjectively assess their quality. In this work, an algorithm which may objectively compare the quality of virtual slides is proposed and implemented. In the first step, the algorithm selects some fragments of the slide captured by one scanner. Then, it finds these fragments in virtual slides acquired by scanning the same glass slide using other devices. This is done by creating overviews of the whole slides (by zooming them out) and calculating gray level co-occurrence matrices (GLCM) for each selected fragment and for the overviews of other slides using windows of adequate size. Comparing some of the Haralick features of the matrices (especially measures related to orderliness) allows estimating the positions of the fragments in other virtual slides. Detected areas, in original resolution, are used to calculate GLCM and its Haralick features again. This time, measures for evaluating the quality (like contrast) are used. Aggregation of the calculated values allows comparing the relative quality of the virtual slides. This method is tested on two sets of ten virtual slides. They were created by capturing the same set of glass slides using two different devices: robotic microscope Axioscope2 (Zeiss) equipped with AxioCam Hrc CCD camera and DeskScan (Zeiss) with standard equipment. Acquired images were stitched and converted by specialized software based on advances in aerial and satellite imaging.

007

Color/Image quality standardization in whole slide imaging

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Free Papers Sessions

Wednesday, 1 September 2010, 14.30–16.30, Aula Duża A

FP-01 Free papers 1

001

Vacuum-assisted needle core biopsy in breast: correlation between the types of microcalcifications and the histopathology diagnosis and between the histopathology diagnosis of the biopsy and the definitive histology

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Objective: Vacuum-assisted needle core biopsy in breast (VANCB) is becoming the gold standard for non-palpable

breast lesions with microcalcifications (4). To analyze the correlations between: (1) types of microcalcifications (Le Gal classification) (1, 2) and the histological classification in B categories of the European Working Group on Breast Pathology (EWGBP) (3); (2) pre-operatory histological diagnosis of the VANCB and the definitive histological diagnosis after surgery.
Material and methods: Two hundred eight consecutive cases of VANCB of non-palpable breast lesions with microcalcifications were carried out from January 2006 to 0ctober 2009. VACORA Vacuum Biopsy System; 12–24 cores were obtained with 10-G needles. The cores and the respective paraffin blocks were X-rayed (5).
Results: (1) One hundred percent agreement of the type V Le Gal with B5 category of the EWGBP; 73.2%, 17.2%, 4.1% agreement of the type IV Le Gal with B4+B5, B2, B3 categories of the EWGBP, respectively; 42.0%, 37%, 6.9% agreement of the type III Le Gal 42.0%, 37%, 6.9% agreement with B5+B4, B2, B3 categories of the EWGBP, respectively. For type II Le Gal, 22.4%, 53.1%, and 8.2% agreement with B5+B4, B2 and B3 categories of the EWGBP, respectively. (2) One hundred percent agreement for B5 category of the EWGBP classification with the definitive histological diagnosis.
Conclusion: VANCB in breast lesions assisted the therapeutic decisions. A close interdisciplinary approach assured optimal results (6).

002

Careful initial clinical–radiologic–pathologic correlation should dictate clinical management of equivocal fibroepithelial lesions diagnosed on core biopsy

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Objective: Equivocal fibroepithelial lesions (FEL) diagnosed on core biopsy (CB) may prove to be either fibroadenoma (FA) or phyllodes tumor (PT) at excision, due to which their management is controversial.
Method: (1) To study the outcome of 136 patients with FEL on CB over a 7-year period at our institution. Consensus on initial patient's clinical management was reached during a multidisciplinary conference based on correlation of clinical data, radiology, and pathology. (2) To examine if any clinical, radiologic or histologic parameters could predict final classification, we retrospectively reevaluated imaging findings (mammography and sonography) as well as multiple histologic criteria (e.g. pattern, stromal cellularity, stromal atypia, mitosis, etc.) on CB and correlated these with diagnosis at excision.
Results: (1) Of 57 excised lesions, 18 were classified as PTs, 10 as benign cFELs and 29 as FAs. In 79 patients with no excision, no evidence of disease progression was observed (40±21 months, mean ± SD). FELs with indeterminate or suspect imaging findings, larger size, and an equivocal comment such as "cannot rule out PT" in the pathology report were excised more frequently (P=0.05, P=0.034 and P=0.01, respectively). (2) Final diagnoses did not correlate with retrospective evaluation of any clinical data, imaging findings or histologic parameters.
Conclusion: Careful initial clinicopathologic and radiologic correlation may select majority of clinically significant lesions for proper surgical management. Follow-up may be an appropriate alternative for a subset of patients, given a good correlation. No radiologic or histologic parameters or clinical data on their own are distinctive enough to predict final classification of FELs.

003

Interpretation of topoisomerase 2a immunohistochemical stains in breast carcinoma. How to set the proper cutoff for a positive status?

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Objective: Inconclusive results of the role of topoisomerase 2a (TOPA2a) in breast carcinoma biology and therapy are partially due to numerous thresholds of positivity measured by immunohistochemical (IHC) analysis. In the presented study, different criteria found in literature of the subject were compared with results of TOPA2a IHC-evaluated material (138 cases). In our study, we have the possibility to evaluate TOPA2a in breast cancer cells using two methods (immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH)) in all cases. As a part of a broader study, we also were able to correlate the results of TOPA2a in in situ carcinoma, benign lesions and breast tissue without pathological changes. We have also analyzed other prognostic and predictive factors as well as clinical outcome.
Method: Using IHC and FISH methods, we compared the percentage of stained cells, strength of nuclear stain, presence of co-expressed cytoplasmatic and membranous stain, amplification of TOPA2a gene and also response to anthracyclin-containing chemotherapy from clinical data.
Results: Analyzing the obtained data, we set our own cutoff points. We assumed that the best correlation both with genomic aberrations and other data listed above, which we considered important in this study, is when both the percentage and strength of stain are taken into account. In our study, a cutoff point for positive TOPA2a status evaluated by IHC method is at least 10% of carcinoma cells with strong (3+) nuclear stain or at least 50% of carcinoma with intermediate (2+) nuclear stain.
Conclusion: The presented criteria as defined above correlate with amplification (ratio ≥1.5) and response to

anthracyclin-based neoadjuvant therapy (measured by DFS and OS).

004

PARP1 expression in breast cancer including BRCA1-associated, triple-negative and basal-like tumours: implications for anti-PARP1 therapy

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Objective: Despite several ongoing trials of PARP inhibitors in the treatment of breast cancer (BC), PARP1 protein expression in BCs is not known. The purpose of this report is to assess the expression of PARP1 in BC including BRCA1-associated, triple-negative (TN) and basal-like (BL) tumors.
Method: Immunohistochemistry with a PARP1 antibody on tissue microarrays from 130 BRCA1-associated and 594 non-BRCA1-related BCs was used.
Results: Nuclear PARP1 expression was found in 93.1% (121/130) of BRCA1-associated and 97% (576/594) of non-BRCA1-related carcinomas. There was a significant difference between the mean nuclear PARP1 histoscore in BRCA1-associated versus non-BRCA1-associated carcinomas in all tumors ($p<0.0001$), in the BL group ($p=0.0086$), TN ($p=0.0015$) and non-BL groups ($p=0.016$), but not in the non-TN group. Among BRCA1-associated BCs, low PARP1 expression was found in 18.5% of all cases, 18.9% (17/90) of BL and 21% (22/105) of TN cancers. PARP1 negativity was determined in 6.9% of all tumors, 7.7% of basal-like and 7.6% of TN cancers. Among non-BRCA1-related tumors, low PARP1 expression was found in 8.8% of all cases, 3.1% of BL tumors, and 2.7% of TN cancers. Three percent was PARP1-negative.
Conclusion: PARP1 expression is significantly associated with BRCA1 status in BL and TN BCs. Because carcinomas with null or low PARP1 expression may show no or a partial response to PARP1 inhibitor therapy, the assessment of PARP1 expression in tumor samples by immunohistochemistry may improve the selection of BC patients for PARP inhibitor therapy.

005

A retrospective study of c-kit and PDGFRa mutations and EGFR amplification in endometrial stromal sarcomas: a report from the Spanish Sarcoma Group (GEIS-18)

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Objective: The expression of tyrosine kinases in endometrial stromal tumors is controversial. Several sporadic responses to imatinib have been observed in metastatic endometrial stromal sarcomas (ESS). We therefore aimed to perform a retrospective analysis of possible molecular targets in ESS: c-kit, PDGFRa and EGFR.
Method: Paraffin blocks from 67 patients with previous diagnosis of low-grade ESS and high-grade endometrial sarcomas from nine different institutions were examined and reviewed. Exons 9, 11, 13, and 17 of the c-kit gene and exons 12 and 18 of the PDGFRa gene were amplified by PCR and sequenced. The incidence and distribution of the KIT, PDGFRa, CD10 and Calponin expression were examined by immunohistochemistry, and EGFR amplification was assessed by fluorescence in situ hybridization (FISH).
Results: No mutations in c-kit and PDGFRa genes were detected. We observed several polymorphisms: I798I, exon17 (2/67); V824V, exon 18 (21/67); P567P, exon 12 (64/67); and one case with a double polymorphism V824V/G838G. Overexpressions of KIT, PDGFRa, CD10 and Calponin was detected in 2 (3%), 23 (34%), 30 (45%) and 26 (39%) cases, respectively, whereas amplification for EGFR gene by FISH was not detected.
Conclusion: Expression of KIT in cases of ESS is infrequent and always weak. PDGFRa expression was observed in 34% of the cases studied. We did not observe any mutations of c-kit and PDGFRa or amplification of the EGFR gene. So it is unlikely that patients with ESS can benefit from therapies with anti-EGFR and/or c-kit tyrosine kinase inhibitors.

006

Uterine tumors resembling ovarian sex cord tumors. A study of five cases

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Objective: Uterine tumours resembling ovarian sex cord tumours (UTROSCT) cause confusion with respect to nomenclature as well as diagnostic difficulties. These tumours belong to the group of low-grade malignant neoplasms, and their clinical course likely depends on the percentage of the sex cord-like component. Morphologically, they can be divided into two groups: those with <40% sex cord-like areas (type 1) and those with more than 40% (type 2).
Method: Five patients with primary UTROSCT underwent treatment in the Cancer Center, Warsaw, between 2000 and 2010. Biopsies or excisions from all tumours were examined microscopically and immunohistochemically

(IH). Treatment and follow-up were correlated to histopathological diagnosis.
Results: Patients ranged from 24 to 69 years of age and tumour size from 3 to 24 cm. Tumours contained 25% to 70% of sex cord component (two tumours of type 1 and three of type 2). By IH examination, the sex cord-like component was keratin-positive in all five cases, while the stromal component was positive for CD10 and negative for h-caldesmon. In addition, PGR positivity was found in all cases, while SMA, CKAE1/3 and inhibin was positive in three. Four patients were treated with gestagens in addition to surgery. No recurrences were noted in any of these four patients over a 2- to 10-year period of follow-up.
Conclusion: A correct subclassification of sarcomas of UTROSCT type is of crucial importance since most patients with these rare neoplasms respond well to gestagen therapy and have a good prognosis compared to other endometrial stromal sarcomas.

007
CD 56 expression in serous invasive ovarian cancer and serous ovarian borderline tumors

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Objective: CD56 is a cell surface glycoprotein that plays a critical role as adhesion molecule for the guidance of migration of neuronal cells during embryofetal development. It is also expressed on adult neuroendocrine cells, hematological neoplasms, as well as in various carcinomas.
Method: In this study, we retrospectively investigated the expression of CD56 in a series of 101 serous ovarian carcinomas and 37 serous borderline tumors and analyzed the significance of this marker with regard to patient outcome. Neuroendocrine cells were determined by immunohistochemical positivity of highly specific chromogranin A. Patients were treated homogenously by use of radical surgery and combined chemotherapy (carboplatin and taxol) in the case of carcinoma and of surgery alone for borderline tumors. Follow-up data were available for all patients.
Results: Sixty-seven cases (66%) of 101 invasive carcinomas, but only 1 of 37 (3%) borderline tumors, were positive for CD56 ($p>0.0001$), indicating that this phenomenon is significantly more present in invasive lesions. CD56 expression in the borderline tumor was accompanied by the presence of single scattered chromogranin A-positive neuroendocrine tumor cells, whereas in serous ovarian carcinomas, co-expression was only seen in less than a quarter of cases.
Conclusion: Our results demonstrate that CD56 positivity is much more frequently found in serous ovarian carcinomas than in serous borderline tumors; however, expression of CD56 had no prognostic effect. On the other hand, CD56 should not be used as a marker for neuroendocrine differentiation in serous neoplasm. Because of different chemotherapies in neuroendocrine ovarian carcinomas, specific neuroendocrine markers like chromogranin A or synaptophysin are recommended in these cases.

008
Are the cancer–testis (CT) antigens reliable markers of stem cells in squamous cell carcinoma of vulva?

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Objective: Vulvar cancer represents 3–5% of all gynecological malignancies. Squamous cell carcinoma is a predominating malignancy at this site as it accounts for approximately 85–90% of vulvar cancers. Analyses of humoral and cellular immune responses to autologous cancer determined cancer–testis (CT) antigens as potential targets for immunotherapy. As their expression has been identified in a variety of malignant neoplasms, they appeared as potential candidates for target therapy, i.e. T cell-mediated immunotherapy of cancer.
Method: Seventy-six patients with verified histopathological diagnosis and full clinical history were included into the study. The slides were incubated with the monoclonal antibody against MAGE-A1, MAGE-A4 and NY-ESO-1. All statistical analyses were performed with the statistical software Statistica 8.
Results: The expression of NY-ESO-1 was revealed in one case. The expression of MAGE-A4 and MAGEA-1 was identified in 66% and 12% primary tumors, 69% and 19% lymph node metastases and 54% and 33% local recurrences, respectively. Cytoplasmatic expression of these antigens was identified in suprabasal and squamoid cells of SCC (Fig. 1).
Conclusion: Vulvar SCC expresses CT antigens; however, their expression is restricted to the maturing squamoid cells of the intermediate level. This pattern of expression suggests that CT antigens are not reliable markers of tumor stem cells in vulvar SCC.

Wednesday, 1 September 2010, 17.00–18.30, Aula Duża B

FP-02 Free papers 2

002
Expression of c-kit protein does not correlate with prognosis in salivary gland carcinomas

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Objective: The objective was to investigate the expression of c-kit protein in salivary gland carcinomas and to correlate it to prognosis.
Method: Immunohistochemistry for c-kit protein was performed in 33 carcinomas using formalin-fixed paraffin-embedded sections. For the evaluation of reactivity of tumor cells, a combination of the cytoplasmatic and/or membranous staining and the percentage of positive cells were applied. Only cases without any staining pattern were considered negative. For prognostic correlation, univariate disease-specific survival curves were calculated by the Kaplan–Meier method and distributions were compared using the log-rank test.
Results: Of all 33 cases, only six were c-kit-negative, including three low-grade mucoepidermoid carcinomas, two carcinomas ex pleomorphic adenoma and one salivary analogon of secretory mammary carcinoma. The group of 27 (82%) positive tumors was dominated by nine adenoid cystic carcinomas (all but one revealing strong reaction), followed by five acinic cell, three each mucoepidermoid and salivary duct carcinoma, two carcinomas ex pleomorphic adenoma and five other tumors, in all of which positive staining ranged from 50% to 100%, with the immunoreaction varying from weak to strong. Disease-specific survival in c-kit-negative (n=6) carcinomas did not differ from that in positive (n=27) cases (χ^2 = 0.047).
Conclusion: Of all salivary gland carcinomas, only adenoid cystic carcinoma was regularly associated with c-kit expression. Immunoreactivity in other subtypes, considering our as well as published data, greatly varies. C-kit expression harbours no significant prognostic information.
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003
Oral lesions and candidiasis

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Objective: Chronic hyperplastic candidiasis (CHC) is an oral infectious lesion with a potential for progression to squamous cell carcinoma (SCC). If the histopathological features associated with candidiasis are overlooked and special techniques for fungal identification are not performed, diagnosis may be missed.
Method: During 2003–2010, all oral specimens were evaluated with periodic acid Schiff stain for structures suspicious for fungal microorganisms, serum exudation, bacterial colonization, parakeratosis, keratosis, superficial neutrophilic infiltration of mucosal or neoplastic epithelium was observed in H&E-stained sections. Forty-four histopathological materials from 30 patients with candidiasis and 28 materials from 24 patients suspicious but histochemically negative for candidiasis are included in this study. All biopsies were scored semiquantitatively for the aforementioned parameters. Candidiasis-negative and -positive cases were compared for demographical, clinical and additional histopathological features.
Results: The most frequent site for CHC or candidiasis-associated SCC was the tongue (n=25, 56.8%). Six cases (20%) were associated with SCC and one case was a SCC following long-standing CHC at the precise localization. Mild dysplasia was observed in 13 (29.5%) cases and moderate dysplasia was identified in one (2.3%) case of CHC. Among the histopathological features, the scores of parakeratosis and superficial neutrophilic infiltration was significantly higher in cases with candidiasis (Mann–Whitney U, p=0.036 and p=0.001). Only parakeratosis was more evident in cases with increased number of fungal microorganisms (Kruskal–Wallis test, p=0.033).
Conclusion: Parakeratosis and superficial neutrophilic infiltration are the most important clues for candidiasis of the oral mucosa. Association of candidiasis with pre-neoplastic and neoplastic lesions is also noteworthy.

004
Fine needle aspiration cytology of basal cell adenoma of the salivary gland. A cytohistologic correlation study of 36 cases

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Objective: Few cytological series describing BCA are available and diagnostic cytological criteria are not well established.
Method: This study was based on 42 cytology samples from 36 patients with BCA. Thirty-six of the aspirations procedures were performed preoperatively and six on tumor recurrence. All cases had a histopathological study.
Results: Aspirates were cellular showing groups with stroma and single cells. Groups had a variable configuration with two major forms. Some were large, three-dimensional and puzzle-like, with little ramifications. Cells were densely packed and intimately related to metachromatic stroma. Others were of medium size with prominent prolongations consisting of an inner core of hyaline stroma surrounded by tumoral cells. Ten cases showed cylindromatous stromal structures. Seven aspirations were cystic. Stroma was homogeneous with non-fibrillary morphology. Neoplastic cells showed a variable morphology from small, basaloid to larger ones with epithelioid morphology. Moderate pleomorphism was seen in five cases. There were two misdiagnosis of acinic cell carcinoma, and two cases were

considered as suspicious of malignancy. After a revision of these cases, two showed features of BCA, while in the remaining two, the suspicious of malignancy persisted because of minimal amount of stroma, predominant epithelioid cell morphology and cellular density.
Conclusion: BCA shows characteristic features that allow in many cases a precise diagnosis. It may be difficult to differentiate from epithelial-rich pleomorphic adenoma, and in these cases, a diagnosis including both possibilities seems preferable. In our series, the absence of stroma was responsible for the misdiagnosis with acinic cell carcinoma in two cases.

005

SDHB expression in paraganglioma–pheochromocytoma syndromes: advantages and limits

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Objective: Pheochromocytoma and paraganglioma are rare tumors arising from chromaffin cells. Almost 10% of them are part of typical familial syndromes: Von Hippel–Lindau disease (VHL), neurofibromatosis type 1 (NF1), multiple endocrine neoplasia type 2 (MEN2) and type 1 (MEN1), pheocromocytoma–paraganglioma syndromes (PGLs; SDHB, SDHC, and SDHD) and a newly described syndrome related to TMEM127 gene mutations. Recently, SDHB immunohistochemical analysis has been proposed as a promising molecular marker for succinate dehydrogenase mutation-related neoplasms (i.e. PGLs).
Method: All cases of reported pheochromocytomas (n=160) and paragangliomas (n=57) between 1988 and 2009 were retrieved from the archives of the Department of Pathology of Padova University. FFPE specimens were genetically characterized for familial syndromes. Syndromic cases and a control group were semiquantitatively (0, 1+, 2+) evaluated for SDHB immunohistochemical expression.
Results: Out of 217 cases, 21 cases showed SDHD (n=3), TMEM127 (n=3), RET (n=6), MEN1 (n=2), VHL (n=4), or NF1 (n=3) germline mutations. The other six sporadic cases were evaluated as control. The three SDHD-mutated cases showed either negative (n=1) or 1+ (n=2) SDHB immunostaining. Completely negative staining was also observed in a sporadic case. A strong SDHB immunoreaction was observed in 19 and a weak immunoreaction in four of the remaining cases.
Conclusion: SDHB immunohistochemical analysis, even not PGL-specific, can be used to triage genetic testing in pheochromocytoma/paraganglioma patients. Further multi-institutional studies should investigate the diagnostic power of this remarkable novel diagnostic tool.

006

Biochemical and histological effects of sitagliptin on Zucker diabetic fatty rat pancreas

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Objective: Inhibition of dipeptidyl peptidase-4 (DPP-4) activity by sitagliptin has been shown to improve glycemic control in patients with type 2 diabetes mellitus (T2DM) by prolonging the actions of incretin hormones, but the real impact of low-dose sitagliptin treatment on pancreatic lesions is almost unknown. This study aimed to evaluate the effects of sitagliptin on the biochemical and histological (pancreatic) parameters of Zucker diabetic fatty (ZDF, fa/fa) rats, an animal model of T2DM.
Method: Diabetic (fa/fa) ZDF male rats were treated with vehicle or sitagliptin (10 mg/kg body weight per day) during 6 weeks (n=8 each). The following parameters were assessed: serum glycaemia, HbA1c, insulin and lipid profile; serum and pancreas oxidative stress (MDA) and endocrine and exocrine pancreas histology, estimating and rating inflammatory infiltrate, fibrosis, vacuolization and congestion in a semiquantitative score ranging from 0 (minimal) to 3 (severe and extensive damage).
Results: Sitagliptin in diabetic ZDF rats promoted beneficial effects on dysglycaemia, dyslipidaemia, inflammatory profile and pancreatic oxidative stress. Endocrine and exocrine pancreas presented a reduction/amelioration of fibrosis severity, inflammatory infiltrate, intra-islet vacuolation, and congestion vs the vehicle-treated diabetic rats.
Conclusion: The favourable biochemical profile promoted by sitagliptin in the diabetic rats, together with protection against endocrine and exocrine pancreatic lesions, might represent a further advantage of low doses of sitagliptin in the management of T2DM.

Thursday, 2 September 2010, 14.30–16.30, Aula Średnia B

FP-03 Free papers 3

001

Defenders of the colon: comprehensive assessment of potential prognostic immunologic biomarkers in mismatch repair-proficient colorectal cancer

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Objective: Evidence suggests a confounding effect of mismatch repair (MMR) status on immune response in colorectal cancer. To date, the identification of cell types involved in innate and adaptive immunity which may complement the established prognostic effect of CD8 in patients with MMR-proficient colorectal cancers, representing 85% of all cases, has not been performed.
Method: MMR-proficient colorectal cancers from 1,406 patients treated at different European centres were assigned to a test group (n=839), validation group 1 (n=358) and validation group 2 (n=209) and mounted onto single- and multiple-punch tissue microarrays. Immunohistochemistry was carried out for CD3, CD4, CD45RO, CD56, CD68, CD8, CD163, FoxP3, GranzymeB, HLA-DR, iNOS, Mast cell tryptase, Mum1, PD1 and TIA-1. Independent prognostic effects were analyzed on the test group, and reevaluated using both validation cohorts.
Results: In the test group, CD3, CD8, FoxP3, Granzyme B, and TIA-1 had a significant (p<0.01) positive impact on survival while only $CD8^+$ (p=0.0021) and TIA-1+ (p= 0.0062) maintained this effect in multivariable analysis which included postoperative therapy. Among patients with $CD8^+$ tumours, TIA-1 contributed additional independent prognostic information. Patients with $CD8^+$/TIA-1 cancers experienced a 143 HR (95%CI, 109–187) increased relative risk of death compared to $CD8^+$/$TIA\text{-}1^+$ cases, a result affecting 35% of patients. Findings were confirmed in both validation cohorts.
Conclusion: The prognostic effect of combined CD8/TIA-1 phenotypes is independent of TNM stage and adjuvant therapy. The additional analysis of TIA-1 in patients with $CD8^+$ tumours translates into improved risk stratification for approximately 35% of all patients with MMR-proficient colorectal cancers.

002

Is there a proximal shift in the distribution of advanced adenomas of the colon?

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Objective: Several studies have suggested an increase in the incidence of right-sided colorectal carcinomas (CRC). If the CRC right shift is true, it is also likely that the incidence of right-sided advanced adenomas (RSAA) should increase in time.
Method: Two large endoscopic datasets (RETRO and PBP) have been compared. RETRO contains data from the Department of Gastroenterology between 1981 and 1994. PBP covers data from the Polish national colorectal cancer screening program (2000–2004). Only patients with advanced adenomas who underwent total colonoscopy were included into the analysis. Advanced adenoma was defined as: villous or tubulo-villous, adenoma with high-grade neoplasia or ≥1-cm lesion. Two definitions of proximality were applied. The first defined right colon is proximal to the splenic flexure and the second, additionally, the descending colon. A logistic regression analysis was used to compare the incidence of RSAA between groups, adjusted for patients' age and sex.
Results: Two hundred and 2,188 patients with advanced adenomas were found in two datasets, respectively. Both groups differed significantly according to age and sex. To adjust for possible confounders, the multivariate model was fitted.
Conclusion: There was no statistically significant increase in the proportion of patients with RSAA between both groups when adjusted for patients' age and sex.

	No of patients with RSAA	Percentage	No of patients with RSAA	Percentage
RETRO	41/200	20,5%	53/200	26,5%
PBP	550/2188	25,14%	705/2188	32,22%
	1st definition of proximality OR 1.32, 95% CI 0.82-1.89, p=0.131(adjusted for age and sex): no difference		2nd definition of proximality: OR 1.33, 95% CI 0.96-1.85, p=0.089 (adjusted for age and sex): no difference	

003

Loss of MSH2 expression in adenomas may predict a more aggressive tumor phenotype in patients with concomitant colorectal carcinoma

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Objective: Loss of the mismatch repair (MMR) protein MSH2 in normal colorectal mucosa has been linked to an increased risk of sporadic colorectal adenoma and with modifiable risk factors for colorectal cancer. The aim of this study was to determine whether loss of MMR proteins MLH1, MSH2, MSH6 and PMS2 in colorectal adenomas could predict a more aggressive tumor phenotype in patients with concomitant colorectal carcinoma.
Method: Two tissue microarrays (TMAs) were constructed from 49 cases of synchronous adenoma and matched colorectal cancer with full clinicopathological information and follow-up. The first included punches from carcinomas and the second from matched adenomas. TMA sections

were immunostained for MLH1, MSH2, MSH6 and PMS2 and, in negative cases, reconfirmed on corresponding whole tissue sections.

Results: Patients with MSH2-negative adenomas (n=10, 21.3%) experienced a significantly worse outcome compared to MSH2-positive (n=37, 78.7%) patients (p=0.043). Additionally, 50% of all deaths in the MSH2-negative subgroup occurred within the first 18 months after diagnosis. Evaluation of relative risks of death at 2 years of follow-up yielded a hazard ratio of 0.34 (95% CI, 0.1–0.99). Moreover, negative expression of either MLH1 or MSH2 further stratified patients by survival time with a relative risk of death of 0.3 (95%CI, 0.09–0.9; p=0.029).

Conclusion: Our preliminary results show that loss of MSH2 in adenomas from patients with synchronous colorectal cancers may predict a significantly worse outcome. If confirmed in larger prospective studies, MSH2 analysis may be useful as a screening tool to identify patients who may benefit from closer follow-up.

004

Immunological microenvironment of tumour buds in colorectal cancer stratified by mismatch repair status

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Objective: The aims of this study were to characterize the immunological microenvironment of tumour buds and evaluate the prognostic effect of immune markers and their ratios with tumour budding stratified by mismatch repair (MMR) status.

Method: Paraffin-embedded tumour blocks from 297 patients with colorectal cancer were cut in series and double-immunostained for CK22 with anti-CD138, -CD16, -CD20, -CD21, -CD56, -CD68, -CD8, -FOXp3, -Granzyme B, -Mast cell tryptase, -CD3, and -TIA-1 and stratified into MMR-proficient and -deficient cases. Tumour buds, immune cells and the ratio of immune cells/tumour buds was obtained.

Results: MMR-deficient tumours showed significantly less tumour budding (p<0.001) and greater amounts of $CD3^+$, $CD8^+$, and Granzyme B^+ cells (p<0.001). High amounts of $CD16^+$, $CD20^+$, $CD8^+$, $CD68^+$, $FOXp3^+$, Granzyme B^+ and $CD3^+$ were linked to improved survival (p<0.001). Prognostic effects differed between proficient and deficient tumours. Marker/budding ratios showed stronger relative risks of death when compared to most markers alone. In lymph node-negative patients, high marker/budding ratios of CD16 (HR, 0.29 (0.15–0.57)), CD68 (HR, 0.33 (0.17–0.63)), CD8 (HR, 0.31 (0.16–0.59)), FOXp3 (HR, 0.34 (0.18–0.64)) and Granzyme B (HR, 0.34 (0.17–0.65)) were independently linked to improved outcome.

Conclusion: Marker/budding ratios for $CD8^+$, $CD16^+$, $FOXp3^+$, Granzyme B^+ and $CD68^+$ are stronger prognostic features than the assessment of immune markers alone. These ratios are independent prognostic factors, particularly in lymph node-negative patients. Moreover, our findings underline the considerable confounding effect of MMR status in the evaluation of immune response in colorectal cancer.

005

Human crypt stem cell dynamics in juvenile polyposis (JPS) and Peutz–Jeghers (PJS) patients

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Objective: Pre-tumour progression is a process that precedes the adenoma–carcinoma sequence; colorectal mucosa appears normal, but cell kinetic and stem cell abnormalities suggest the existence of molecular alterations. Pre-tumour progression can be made visible by studying methylation patterns, or tags, in stem cells of the crypt. Shibata et al. (2004) showed higher crypt methylation heterogeneity in normal-appearing colonic crypts of familial adenomatous polyposis (FAP) patients compared to normal crypt mucosa of non-FAP colons. This indicates enhanced stem cell survival, which may be a predictive marker of increased neoplastic outgrowth since longer lived stem cells have the potential to gain more mutations. The objective of this study was to investigate stem cell survival in juvenile polyposis syndrome (JPS) and Peutz–Jeghers syndrome (PJS), a biomarker predictive of pre-tumour progression.

Method: DNA was isolated from laser-microdissected crypts of normal colonic JPS and PJS tissue and age-matched controls. DNA was bisulphite-converted and methylation tags were created by amplifying the CpG islands in the CSX gene. Sequence analysis of this pool of methylation tags allows characterisation of the heterogeneity among the different tags recovered from multiple stem cell pools.

Results: Preliminary results show that JPS patients with an identified germline defect harbour a greater number of unique methylation patterns when compared to normal controls. However, this appears to be germline mutation-dependent since patients with a BMPR1A germline mutation show a significantly greater number of unique methylation patterns, indicating enhanced stem cell survival, and JPS patients with a SMAD4 mutation do not. PJS patients do not show an increased number of methylation patterns.

006

LKB1 promoter amplification in sporadic Peutz–Jeghers polyps

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Objective: Peutz–Jeghers syndrome (PJS) is a dominantly inherited disorder characterized by gastrointestinal hamartomatous polyposis, mucocutaneous melanin pigmentation and an increased risk of cancer at a relatively young age. Inactivation of tumor suppressor gene LKB1 is the underlying germline defect in PJS patients. Cases of sporadic PJ polyps have been reported. It has been stated that these polyps are extremely rare and that the fact that no molecular data were found to support the PJS diagnosis is only due to failing techniques.
Method: We aimed to identify the molecular defect in six sporadic PJ patients. These patients are characterized by the presence of a distinct PJS polyp and most importantly the presence of arborizing smooth muscle. On these six, patients we performed LKB1 germline analysis and multiplex ligation-dependent probe amplification (MLPA) to detect exon deletions and amplifications.
Results: One-point mutation and two high-level amplifications of the LKB1 promoter were identified. Interestingly, this amplification was identified in an additional four PJS patients. To study the effect of this amplification on gene and protein expression, we performed a RT-PCR and WB in two patients. A significant reduction of LKB1 expression was found when PJS polyp, and normal tissue was compared to a panel of normal colon samples.
Conclusion: These results show that polyps that were based on histology sporadic PJ polyps are indeed PJS polyps, and these patients should be monitored accordingly. Interestingly, amplification of the LKB1 promoter resulted in decreased protein expression. The exact mechanism leading to this reduction remains to be determined.

007

Epidermal growth factor receptor and insulin-like growth factor 1 receptor expression predicts poor survival in pancreatic ductal adenocarcinoma

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Objective: Pancreatic ductal adenocarcinoma (PDA) is characterized by poor prognosis without effective treatment strategies. Epidermal growth factor receptor (EGFR) inhibitors in combination with gemcitabine are approved for the treatment of PDA, and there is an urgent need to identify patients who are likely to respond to targeted therapies. In this study, we evaluated the expression of EGFR and insulin-like growth factor 1 receptor (IGF-1R) in PDA and correlated it with survival and clinicopathologic variables.
Method: Ninety-eight cases of PDA were included in the study. Representative sections were stained with anti-EGFR and anti IGF-1R antibodies (Ventana, Tucson, USA). Cases were scored as negative (no staining), 1+ (incomplete membranous staining), 2+ and 3+ (complete membranous staining in <30% and >30% of tumor, respectively). For statistical analysis, EGFR and IGF-1R scores were dichotomized as low (0–2) versus high (3). IGF-1R gene copy number was evaluated by automated dual chromogen in situ hybridization (ISH, Ventana, USA). For ISH, chromosome 15 (red signal) and IGF-1R (black signal) were counted in 50 cells. Association between EGFR and IGF-1R expression and pathologic variables was analyzed using Fisher's, Wilcoxon and log-rank tests.
Results: Expression of IGF-1R protein correlated with gene amplification by ISH. Significant association was observed between the presence of lymph node metastasis and high EGFR expression ($p=0.038$). Univariately significant predictors of the overall survival included EGFR, IGF-1R, lymph node status and tumor size. In multivariate model, high EGFR expression was the strongest predictor of poor survival.
Conclusion: EGFR and IGF-1R expression are important for PDA aggressiveness. The relationship between two receptors may have therapeutic implications.

008

Expression of podoplanin, vascular endothelial growth factor C and D in esophageal cancer

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Objective: Lymph node metastasis is an important prognostic factor in esophageal cancer. Vascular endothelial growth factor C (VEGF-C) and vascular endothelial growth factor D (VEGF-D) are considered to induce both lymph node metastasis and lymphatic involvement in various cancers related to lymphangiogenesis. In many types of cancer, podoplanin (D2-40) immunostaining has recently been used to detect lymphatic vessel invasion (LVI), but invasion detected

using D2-40 immunostaining for a predictor of nodal metastasis was controversial. We examined the relationship between VEGF-C, VEGF-D and LVI and clinicopathological features in 53 patients with esophageal cancer who underwent esophagectomy.
Method: VEGF-C, VEGF-D and podoplanin expression was examined using immunohistochemical staining in surgically resected tissues. Differences in categorical data were assessed by a chi-square test or Fisher's exact test.
Results: Podoplanin expression was observed in the endothelial cells of lymphatic channels. None of podoplanin-positive vessels were blood vessels. Intratumoral lymph vessels were small and collapsed, while peritumoral lymphatic vessels with LVI were dilated and large. The expression of VEGF-C and VEGF-D protein was recognized in the cytoplasm of cancer cells, but not in the nucleus. We found that peritumoral LVI significantly correlated with lymph node metastasis (p=0.001), pathologic stage (p=0.003), tumor depth (p=0.038) and tumor size (p=0.014). The expression levels of VEGF-C and VEGF-D were associated with LVI.
Conclusion: VEGF-C, VEGF-D and peritumoral LVI may play an important role in lymphangiogenesis and the process of lymphatic metastasis of esophageal cancer.

Thursday, 2 September 2010, 18.00–19.30, Aula Duża A

FP-04 Free papers 4

001
16S rDNA heterogeneity analysis using RFLP and phylogenetics (SHARP-Screening) suggests a pathogenetic role of Alcaligenaceae species in pulmonal extranodal marginal zone lymphoma of MALT-type (BALT lymphomas)
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Objective: For several anatomical localisations of extranodal marginal zone lymphoma of MALT type (eMZCL), an association with chronic inflammation caused by microbiological agents (e.g. *Helicobacter pylori* in the stomach) has been described. In the lung, a link between lymphomagenesis and a defined causative organism is still missing.
Method: A comprehensive diversity survey using 16S rDNA library construction followed by restriction fragment length polymorphism (RFLP) analysis, sequencing and phylogenetic tree construction was employed on nine cases each of BALT lymphoma and control lung tissues (normal fetal lung, pneumonitis, cancer). This highly sensitive method, hereafter termed "SHARP-Screening", allows for the identification of the entire bacterial population in the tissue in a cultivation-independent manner. Following the results of SHARP screening, an Alcaligenaceae-specific PCR assay was employed on 20 additional BALT lymphoma cases and 21 control lung tissues.
Results: In eight of the nine cases of BALT lymphoma, bacteria of the Alcaligenaceae family (Alcaligenes, Achromobacter, AKIW733) were detected, whereas none of the control cases showed the presence of these clades. The subsequently performed Alcaligenaceae-specific PCR assay detected bacteria of the Alcaligenaceae family in 8 of the 20 independently investigated BALT lymphoma cases and in only one of 21 control lung tissues.
Conclusion: 16S rDNA library construction in combination with RFLP screening and phylogenetic analyses (SHARP-Screening) is a novel, molecular and cultivation-independent tool for the analysis of the microbial environment in chronic inflammation processes. Betaproteobacteria of the Alcaligenaceae family may be associated with and possibly involved in the lymphomagenesis of BALT lymphomas.

002
Biological signature of activated B cell receptor signaling in diffuse large B cell lymphoma
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Objective: Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma. Recently, major advances in the molecular characterization of DLBCL have been made through gene expression profiling studies that provided more insight into the biological heterogeneity within DLBCL. One of the identified molecular subgroups has been termed the "B-cell receptor (BCR)/proliferation cluster" due to the increased expression of BCR signaling cascade components including the spleen tyrosine kinase (SYK), which has emerged as an important therapeutic target within tumors of this subgroup. In fact, a recent clinical trial with a SYK inhibitor on uncharacterized DLBCLs has shown promising results, but only in a subset of patients.
Method: We employ a novel quantitative tissue-based immunohistochemical (IHC) methodology using markers

of activated BCR signaling to assess the activity of the BCR pathway in formalin-fixed paraffin embedded primary DLBCLs.
Results: Antibodies to phosphorylated forms of Lyn, SYK, BTK, BLNK and AKT as well as FOXO1 antibody represent attractive IHC markers to evaluate the activation of BCR signaling and comprise the basis of a biological signature of activated BCR signaling in DLBCL.
Conclusion: We report on the development of a quantitative tissue-based immunohistochemical (IHC) methodology employing activation-specific antibodies against multiple components of the BCR signaling pathway that will assess the activity of the BCR pathway in formalin-fixed paraffin-embedded primary DLBCLs. This approach will identify the subset of patient tumors that are actively signaling through the BCR pathway and, therefore, will predict therapeutic responsiveness to targeted inhibition of BCR signaling.

003

Enteropathy-associated T cell lymphoma: a clinicopathologic study focussing on prognostic factors and the frequency of defective T cell receptor chains

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Objective: Enteropathy-associated T cell lymphoma (EATL) is a rare highly aggressive T cell neoplasm divided into type I ($CD8^-$, $CD56^-$) and type II ($CD8^+$, $CD56^+$). We assessed clinical, histological and immunohistochemical features of EATL and their association with the outcome of patients.
Method: Twenty-one EATL patients were reevaluated for tumor type, stage, general performance, nutritional status, and survival. Immunophenotyping included CD3, CD4, CD7, CD8, CD30, CD43, CD56, CD11c, CD123, MUM1/IRF4, TCRalfa/beta, TCRgamma/delta, Ki-67, TIA-1, granzyme-B and perforin. The statistical analysis was performed using Student's *t* test and Cochrane-Cox model.
Results: A partial or complete loss of CD3 antigen was observed in 7 of 21 cases. Three lymphomas were of the alpha/beta and three of the gamma/delta TCR type, while 14 lacked TCR chains. TIA-1 was detected in 21 of 21, perforin in 16 of 21 and granzyme B in 13 of 21. MUM1/IRF4 (9/21) was significantly associated with an anaplastic or immunoblastic morphology ($p=0.023$). Dendritic cells were abundant in all specimens. A significantly prolonged overall survival (OS) was observed in patients with lower stage (I+II vs. IV, $p=0.02$) and type I EATL ($p=0.02$), with a trend for longer OS in patients in good general performance ($p=0.096$) and nutritional status ($p=0.12$).
Conclusion: Tumor stage and histological type were prognostically relevant for our EATL patients. An aberrant T cell phenotype with loss of CD 3 and TCR chains was frequent in the consistently $TIA\text{-}1^+$ tumors. MUM-1/IRF4 may be a promising therapeutical target for a subset of EATL.

004

Fatal case of Epstein–Barr virus primo-infection, haemophagocytic syndrome in a 26-year-old patient treated with azathioprine for Crohn's disease: an autopsy case

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Objective: A 26-year-old man with a history of Crohn's disease, treated with azathioprine since 2 years, presented an Epstein–Barr virus (EBV) primo-infection and exacerbation of digestive symptoms.
Method: An ileo-colectomy was performed, which showed a fatal EBV lymphoproliferation disorder along with a haemophagocytic syndrome. EBV DNA load in the peripheral blood persisted to be high loaded during hospitalisation (479,000 copies per milliliter) despite triple antiviral treatment.
Results: Autopsy revealed a systemic lymphoproliferation involving lymph nodes, gastrointestinal mucosa and solid viscera (heart, kidney, lungs, prostate, brain). This was compounded of a population of large polymorphic B cell, hypertrophic macrophages and T lymphocytes, associated to haemophagocytosis. These massive infiltrations mimicked macroscopically as ulcers in the intestinal mucosa and ranged from polymorphic with plasmocytic differentiation to monomorphic large cells. Autopsy results confirmed the absence of Crohn's disease reactivation. The EBV infection was observed in all organs within the large images of the B cell lymphoproliferations. Further postmortem investigations revealed a deficit of the azathioprine's metabolisation enzyme thiopurine methyltransferase (TPMT).
Conclusion: We report and discuss herein the observations of a complete autopsy case along with the postmortem identification of the EBV infection type and TPMT mutation in a patient treated by azathioprine for Crohn's disease. Autopsy findings and further investigations helped explain the complicate clinical evolution and the fatal issue of the patient.

005

Autophagy and its regulation in neuroblastoma

H. Sartelet, T. Inbriglio, C. Nyalendo, C. Barelli, S. Cournoyer, P. Teira, M. Duval, G. Vassal*
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Objective: Neuroblastoma (NB) is a frequent paediatric tumour. Despite high dose of chemotherapy, metastatic tumours have poor outcome. Autophagy is a cellular self-degradative process adapting to stress. By suppressing altered organelles by the intermediate of autophagosomes, autophagy maintains cell survival, but can play a role in programmed non-apoptotic cell death. In cancer, autophagy has an important function, but still controversial. The aim of this study was to determine if autophagy is present and could be regulated in NB.
Method: Five tissue microarrays containing 184 NBs were studied. An immunohistochemistry method was used to identify the expression of LC3, a specific autophagosome marker, and beclin 1, a positive regulator of autophagy. In addition, four NB cell lines were treated in vitro with six drugs (temozolomide, LY294002, rapamycin, vincristin, doxorubicin and cisplatin). Cell survival was measured by MTT cell proliferation assay. The autophagic vacuoles were labelled with monodansylcadaverine, and the result was measured by the intensity of fluorescence. Autophagosome formation was monitored by immunodetection of LC3 cleavage and beclin 1.
Results: Low levels of autophagy are present in a majority of NB, but the autophagy level (expression of LC3) does not represent a prognostic factor. In contrast, higher levels of beclin 1 were detected in NB with poor prognostic. In vitro, autophagy is inversely proportional to cell survival when neuroblasts are in contact with NB's classic chemotherapeutic agents and AKT path inhibitors. However, temozolomide induces autophagy without increasing cell death.
Conclusion: Autophagy is present and could be modulated by chemotherapy in NB.

Friday, 3 September 2010, 14.30–16.30, Sala Wystawowa B

FP-05 Free papers 5

001
EGFR mutation testing in non-small cell lung cancer (NSCLC)—recommendations of the IASLC-ETOP Workshop

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Objective: Gefitinib has received European approval for the treatment of patients with NSCLC with activating mutations of the epidermal growth factor receptor (EGFR) tyrosine kinase. In Europe, EGFR mutation testing is not well established, preventing access to new targeted therapies for NSCLC.
Method: An IASLC-ETOP European multidisciplinary workshop developed recommendations to facilitate the implementation of standardized EGFR mutation testing in routine practice.
Results: The treating physician requests EGFR mutation testing. The results should be available within seven working days. All patients with NSCLC may be tested, but exceptions can be made for carcinoids, squamous, large cell neuroendocrine, and mucinous adenocarcinomas—there is a low likelihood of mutations. No consensus agreement was achieved for prescreening algorithms, although non-mucinous adenocarcinomas most likely harbor an increased percentage of mutations. For diagnosis, the most accessible tissue is sampled; re-biopsy at recurrence or disease progression should be considered. More data on the potential heterogeneity between different biopsy sites are required. The use of cytology samples needs to be evaluated. Tumor cell enrichment of samples is often required, depending on the test used. The tumor-to-normal cell ratio is important, although the minimum tumor cell number is ill defined. No standard method exists to detect EGFR mutations. Some evaluate the known activating mutations; others detect all mutations. New mutations might be difficult to interpret since activation status of the receptor has been defined for the most common ones.
Conclusion: The pathologic report should contain tumor histology, sample size/quality, tumor cell percentage, methodology used for EGFR mutations, exons tested and mutations present/absent.

002
The presence of intra-tumoral neutrophils is an independent prognostic factor in early-stage non-small cell lung carcinomas

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Objective: Cancer-related inflammation has emerged as the seventh hallmark of cancer. In the tumor microenvironment, inflammatory cells, including neutrophils, influence almost every aspect of cancer initiation and progression, including tissue invasion and metastatic potential. However, the influence of intra-tumoral neutrophils in non-small cell lung carcinoma (NSCLC) progression is currently unknown. This study was conducted in order to correlate the number of tumor-associated neutrophils (TANs) and the prognostic of stage I NSCLC.
Method: Immunostainings on 320 stage I (120 T1N0 and 200 T2N0) NSCLC included on tissue microarrays were performed with anti-myeloperoxidase and anti-CD66b anti-

bodies and quantified using the Spotbrowser V7 software. Results were then correlated with overall survival (OS), disease-specific survival (DSS) and disease-free survival (DFS) of patients.
Results: The TANs ranged from 0 to 320 cells/mm^2 tumor tissue. The presence of TANs was associated with increasing tumor size (P=0.001). Number of TANs correlated with shorter OS (P=0.05), DSS (P=0.002) and DFS (P=0.05) of patients, independently of other prognostic factors.
Conclusion: The presence of TANs is a new independent prognostic factor for short disease-free, overall and cancer-specific survival in early stage NSCLC.

003

Are neutrophils new partners for circulating tumor cells migration? A preliminary study in 180 resectable non-small cell lung carcinoma patients using both the CellSearch method and the blood neutrophil count

V. Hofman, M. Ilie, C. Butori, C. Marquette, N. Vénissac, J. Mouroux, P. Hofman*
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Objective: Neutrophils within the tumor microenvironment, so-called tumor-associated neutrophils (TANs), facilitate angiogenesis, extracellular matrix breakdown and remodeling, and promote tumor cell motility. Recent studies reveal that direct communication between neutrophils and tumor cells leads to invasion and egress of tumor cells into the blood vessels. Moreover, cytokines present in blood circulation might physically link cancer cells to TANs, allowing them to travel together throughout the circulation. This study was conducted in order to correlate the presence and number of circulating tumor cells detected in resectable non-small cell lung carcinoma patients and the neutrophil blood counts evaluated at the same time.
Method: One hundred eighty consecutive patients who underwent surgery for NSCLC were included. CTCs have been detected and quantified using the CellSearch assay and the CellSearch Epithelial Cell Kit (Veridex). Results of CTC enumeration were expressed as the number of positive cells per 7.5 ml of blood. At the same time, blood neutrophils were counted and values were compared to the CTCs counts for each patient.
Results: Among NSCLC patients, 52% have CTCs preoperatively detected (range between 1 and 25 CTCs). Sixty-five of 180 patients have more than 10.000 neutrophils/mm^3. Among these patients, CTCs were found correlated with high levels of blood neutrophils in 75% of cases (p=0.05).
Conclusion: This preliminary study shows that CTCs (presence and number) are strongly associated with higher levels of blood neutrophils in NSCLC patients undergoing surgery. These results open new avenues in our understanding of CTC migration, outcome, and survival.

004

Mutations and copy number of EGFR in mixed type of pulmonary adenocarcinoma

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Objective: The EGFR genes act in the MAPK signaling pathway. Patients with non-small cell lung cancer (NSCLC) with EGFR mutations are more sensitive to treatment with tyrosine kinase inhibitors (TKIs). The different patterns recognized in adenocarcinomas were microdissected to identify mutations in EGFR and gene copy number in order to clarify their significance when selecting patients either to TKIs to be translated to small biopsies.
Method: Histological sections of 40 mixed-type adenocarcinomas from surgical specimens of the lung FFPE and were submitted to FISH by application of the probe LSI EGFR/CEP 7. Thirty-one samples were studied for EGFR exons 19 and 21 mutations by fragment analysis and by direct sequencing, respectively.
Results: FISH-positive was found in nine cases, two with amplification and one with high polysomy, by Cappuzzo's score; in one case, there were different results in the present patterns. In the 31 samples, 10 of 31 showed in-frame deletions and 4 of 20 L858R substitution in the EGFR. Only one mixed adenocarcinoma showed EGFR in-frame deletions in bronchioloalveolar pattern and Wt in acinar pattern.
Conclusion: In this set of mixed-type adenocarcinomas, the different histological patterns revealed to be inconsequent for EGFR determination of mutations and gene copy number, reinforcing the technical feasibility and reliability of small biopsies of lung cancer to determine personalized therapy.

005

Chromogranin A expression confers small cell lung cancer better prognosis

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Objective: Small cell lung cancer (SCLC) accounts for up to 13% of all newly diagnosed lung cancers. The prognostic value of six immunohistochemical markers (IHC) used in routine diagnosis, chromogranin A (Chromo A), cytokeratin 7 (CK7), thyroid transcription factor-1 (TTF-1), neural cell adhesion molecule/CD56, Ki-67 (MIB1) and high weight cytokeratin (LP34), was explored to enrich clinical data.

Method: A total of 100 cases (13 women, 87 men) were selected for this study to consider respective follow-up. Patients had histological diagnosis of SCLC, in accordance with the classical morphological parameters and IHC referred profile.
Results: The mean survival was 274 days (9 months) and median was 183 days (6 months). The survival mean for limited disease-SCLC was 482 days (16 months, with a 95% confidence interval of 10–22 months) and 182 days for extensive disease-SCLC (6 months, with a 95% confidence interval of 4–8 months). Significant prognostic meaning of Chromo A curves pointed to mean survival of approximately 11 months. Expression of Chromo A and CD56 had best prognosis survival mean of 726 days (24.2 months) against 234 days (7.8 months). The worst prognosis was seen with CD56 and Ki67—mean survival of 6 months against 11 months.
Conclusion: Chromo A isolated expression by neoplastic cells should be considered a prognostic factor of survival. Cases that express either Chromo A or CD56 have to be separated from cases expressing CD56 and Ki67 to delineate prognostic groups among SCLC patients, and therapy might be defined according to this profile.

006
Combined inhibition of mTOR and EGFR yields synergistic cytotoxic and biochemical effects in atypical lung carcinoid and large cell neuroendocrine lung carcinoma cells

Z. Bagó-Horváth, W. Sieghart, H. Hayden, D. Cejka, J. Werzowa, W. Berger, A. Lackner, M. Grusch, M. Filipits, K. Schmid*
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Objective: Epidermal growth factor receptor (EGFR) and mammalian target of rapamycin (mTOR) are crucial targets in cancer therapy that control cell survival and proliferation. Combined inhibition of both pathways yielded synergistic effects in several human cancer cell lines. However, the impact of EGFR and mTOR expression and inhibition in neuroendocrine lung tumors (excluding SCLC) is unclear.
Method: Cytotoxic effects of mTOR inhibitor everolimus and EGFR inhibitor erlotinib alone and in combination were tested using growth inhibition assays in NCI-H720 atypical lung carcinoid and SHP-77 large cell neuroendocrine lung carcinoma cells. Activation of EGFR and mTOR pathway members was analyzed by Western blotting. Cell cycle phase distribution was determined by FACS. To investigate the role of these pathways in neuroendocrine lung tumors, expression of the pathway constituents was assessed by immunohistochemistry in 110 tumor samples and correlated with survival of the patients.
Results: Combination of erlotinib and everolimus yielded highly synergistic combination effects in both cell lines. Downregulation of phosphorylated mTOR, p70-S6 kinase and Akt expression by everolimus was potentiated by the addition of erlotinib, although erlotinib alone did not affect the expression of these proteins substantially. Additionally, combined treatment showed effects on cell cycle regulation and phase distribution. Activation of both pathways could be demonstrated in all tumor entities investigated; however, no correlation with disease-free or overall survival of the patients was observed.
Conclusion: Due to these promising results, the effects of combined inhibition of mTOR by RAD001 and EGFR by erlotinib warrant further in vivo investigation in neuroendocrine lung tumors.

007
The role of epithelial–mesenchymal transition in malignant mesothelioma

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Objective: Malignant mesothelioma (MM) is the tumor arising from the lining cells of the serosal cavities, microscopically characterized by epithelioid and/or sarcomatoid features. This supports the hypothesis that MM could be a suitable model to study in vivo the epithelial–mesenchymal transition (EMT) process by which epithelial cells acquire mesenchymal shapes and properties but also invasive and metastatic abilities during tumor progression.
Method: One hundred nine formalin-fixed paraffin-embedded MM specimens (58 epithelioid, 26 sarcomatoid, 25 biphasic) from the Pathology Department of Padova University were analyzed by immunohistochemistry (IHC; E-cadherin, N-cadherin, β-catenin, cytokeratin 5/6, vimentin, αSMA, ZEB1/2, S100A4, MMP-2 and MMP-9), by qRT-PCR analysis (E-cadherin, N-cadherin, SNAI1/2, ZEB1/2, TWIST1, miR-205), and by in situ hybridization (miR-205). Results were evaluated by Student's *t* test and Kruskal–Wallis test.
Results: IHC showed that vimentin ($p=0.007$) and αSMA ($p=0.009$) expressions were higher in sarcomatoid than in epithelioid MM, and S100A4 ($p=0.046$) was higher in biphasic than in epithelioid MM. At qRT-PCR, E-cadherin ($p=0.001$ and $p=0.014$) was expressed at lower levels, while N-cadherin ($p=0.002$ and $p=0.002$) was at higher levels in sarcomatoid than in epithelioid/biphasic MM.

SNAI2 ($p=0.001$ and $p=0.001$) was higher in both sarcomatoid and biphasic compared to epithelioid MM. TWIST1 ($p=0.044$ and $p=0.011$) expression was higher only in biphasic cases.
Conclusion: MM can be considered a suitable model to study the EMT process in vivo. Indeed, EMT is involved in MM morphological spectrum of subtypes, but also plays a fundamental role in prognosis.

008

Pulmonary arterial hypertension in end-stage Idiopathic pulmonary fibrosis: a hypothetic role for viral infections

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Objective: Idiopathic pulmonary fibrosis (IPF) is a destructive lung disease of unknown aetiology, characterised by progressive fibrosis and inflammation. Pulmonary hypertension represents an important complication significantly influencing patient outcome. The contribution of viral infections in the development/progression of the disease is largely debated. The aim of the study was to investigate the presence of different viral genomes in native lungs with IPF and to correlate these findings with different clinical, functional hemodynamic and morphological parameters.
Method: Tissue samples from 52 native lungs of IPF patients requiring lung transplantation and from 20 controls were analysed by polymerase chain reaction to detect different viral genomes (Epstein–Barr virus; cytomegalovirus; human herpesviruses 6, 7, 8; adenovirus; rhinovirus; influenza viruses; parainfluenza viruses; parvovirus B19; metapneumovirus and respiratory syncytial virus). Morphological parameters (fibrosis extension, muscular artery wall thickness and inflammation) were quantified using morphometry.
Results: Higher frequency of virus-positive cases was detected in IPF patients than the control group (17/52 vs 1/20, $p=0.01$), and herpesviruses were the only detected genomes. Viral IPF cases showed increased values of mean pulmonary arterial pressure, mPAP ($p=0.05$), worse performance at the 6-m walking test ($p=0.008$), and were characterized by a higher thickening of muscular arteries ($p=0.03$). No significant correlations were found with other clinical and morphological data.
Conclusion: Higher values of mPAP in viral cases may prospect a role of herpesviruses in the development of pulmonary hypertension. A more extensive vessel remodelling may be related to a well-known vessel tropism of herpesviruses.

Friday, 3 September 2010, 17.00–18.30, Aula Duża A

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001

Spectrum of biopsy-proven renal diseases in adults: a 9-year review of nine regional renal databases in Poland

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Objective: This is a report on the epidemiology of kidney diseases in Poland based on kidney biopsy databases from nine nephropathological centers.
Method: All adult native kidney biopsy recognitions collected in the years 2002–2009 in Warsaw, Lodz, Cracow, Wroclaw, Bialystok, Bydgoszcz, Lublin, Poznan and Katowice were retrospectively analyzed for histological type of renal disease and demographic data. Biopsies were processed for light and immunofluorescence microscopy and electron microscopy in some cases.
Results: The total number of analyzed diagnostic biopsies was 5,560. Male-to-female ratio was 2,996:2,564; the mean age was 44.2 years. The most common recognition was glomerulopathy, with the most prevalent type being focal segmental glomerulosclerosis (FSGS, 17.9%), followed by IgA nephropathy (17.3%), membranous nephropathy (11.4%), membrano-proliferative glomerulonephritis (7.3%), mesangial proliferative glomerulonephritis (7.3%), extracapillary glomerulonephritis (6.1%), and minimal change disease (5.1%). Among secondary nephropathies, the most prevalent were lupus nephritis (8%) and renal amyloidosis (4.4%) of all diagnostic biopsies, respectively. Tubulointerstitial nephritis was reported in 1.9% of all renal biopsies.
Conclusion: Collection of data relating to renal biopsies in a national registry is a useful tool for nephrologists in that it meets one of the current challenges facing the clinical research enterprise. In Poland, the most common disease recognition upon kidney biopsy in adults is chronic glomerulonephritis with a domination of FSGS. Lupus nephritis predominates among secondary types of glomerulonephritis. This report constitutes the basis for the future Polish Registry of Renal Biopsies. (This work was supported by grant of Polish Ministry of Science and Higher Education NN402 088735)

002

Renal histopathology of patients with coexisting anti-glomerular basement membrane antibodies and antineutrophil cytoplasmic antibodies

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Objective: Antibodies against glomerular basement membrane (anti-GBM) and antineutrophil cytoplasmic antibodies (ANCA) are both associated with histopathologic features of extracapillary crescentic glomerulonephritis and may coexist in some patients.
Method: Of 37 patients with anti-GBM glomerulonephritis in kidney biopsy ($n=36$) or autopsy ($n=1$), examined from 1990 to 2009 at the Institute of Pathology Faculty of Medicine Ljubljana, 11 patients (29.7%) had in addition to anti-GBM antibody-positive ANCA, specific for myeloperoxidase. Renal biopsies were examined by light and immunofluorescence microscopy techniques.
Results: The patients comprised ten women and one man (mean age 64.1±7.2 years, age range 52–78 years). Immunofluorescence microscopy showed intense linear staining for IgG and C3 along glomerular and distal tubular basement membrane in all patients. By light microscopy, irregular diffuse or focal extracapillary crescentic glomerulonephritis was found in all patients, associated with necrosis of varying extent in nine. The percentage of crescents was 33% to 100%. Extraglomerular necrotizing vasculitis of small arteries was present in six patients, and in one, elastica destruction indicative of previous vasculitis was observed. An assessment of the age of histopathologic lesions showed lesions of varying age in all biopsies. Active lesions were predominant in four and chronic in three, while active and chronic lesions of equal distribution were seen in four biopsies.
Conclusion: All our patients with coexisting anti-GBM antibodies and ANCA were older and all had renal histopathologic lesions of varying age, active and chronic. These results confirm a hypothesis that anti-GBM antibody production may start after injury to the glomerular basement membrane by ANCA.

003

Renal amyloidosis secondary to Behçet's disease: clinicopathologic features of 10 cases

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Objective: Amyloidosis is a rare complication of Behçet's disease (BD). We describe ten patients with BD and renal amyloidosis.
Method: Kidney biopsy slides were culled from the archives and reviewed. Intensity and relative distribution of amyloid deposition in renal compartments were noted. Glomerular deposits were classified as hilar (pattern I), mesangial nodular (pattern II) and mesangiocapillary (pattern III). Chronic tubulointerstitial damage was graded from 0 to 3 (0%, 0–<25%, 25–<50% and ≥50%). Amyloid typing was performed by immunohistochemistry. Clinical information was gathered from hospital records and computer-based patient data system.
Results: All patients were male. Their age ranged from 27 to 56. Full-blown nephrotic syndrome was present in six cases. Duration of BD before diagnosis of amyloidosis differed from 24 to 192 months (mean 108), and mean follow-up period was 59.5 months. Immunohistochemical typing revealed AA amyloid in all ten. Four cases showed glomerular-dominant and two cases vascular-dominant amyloid deposition; the rest were co-dominant glomerular and vascular. The frequencies of glomerular amyloid patterns were 40% for hilar, 20% for mesangial nodular and 40% for mesangiocapillary. Hilar pattern was associated with vascular-dominant amyloid deposits. Severe proteinuria correlated with the presence of mesangiocapillary pattern. Grade III chronic tubulointerstitial injury was detected in three cases. Patients were treated with steroids and/or different immunosuppressives. Among them, two progressed to end-stage kidney failure, one showed remission, two were lost to follow-up, and rest had persistent proteinuria.
Conclusion: Renal amyloidosis in BD has a diverse pathology in terms of preferential location of amyloid deposition and its intensity. Patients follow variable clinical courses accordingly.

004

Acute and rejection and vascular remodeling on 180 post-cardiac transplant biopsies

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Objective: Acute and chronic rejection is the main cause of graft dysfunction post-cardiac transplantation. Assessment of chronic rejection (cardiac allograft vasculopathy) may prove difficult on biopsies.
Method: We reviewed 180 cardiac biopsies from 23 patients who underwent cardiac transplant between 2004 and 2008 in whom donor-specific antibodies were measured (5 women, 18 men; mean age at transplant, 39.7 years (range 17–69)). Diagnosis on explanted hearts included ischaemic heart disease ($n=10$), dilated cardiomyopathy ($n=9$), hypertrophic cardiomyopathy ($n=2$), congenital

heart disease (n=1), and AMP kinase deficiency cardiomyopathy (n=1).

Results: In total, 16 levels from 180 biopsies were reviewed (n=2,880), with a majority of low grade of rejection (according to the ISHLT grading system): grade 0R, 36.1%; grade 1R, 48.8%; grade 2R, 10%; grade 3, 0.5%. Other histological features assessed were subendocardial fibrosis (n=143, 79.4%) and interstitial fibrosis (n=126, 70%). Assessment of vascular remodelling was evaluated on all biopsies and was observed in 61 (33.8%). Intimal hyperplasia/thickening of intramural vessels with wall rigidity (n=39, 62.3%) and luminal narrowing (n=23, 37.7%) was recorded. These changes were observed as early as on the first biopsy, with marked variability in the time from transplant to their first appearance (from 7 to 834 days). Eight of these developed post-transplant circulation anti-donor HLA antibodies. The rejection grade on biopsies with vascular changes was 0R–1R in 81%.

Conclusion: Detection of donor-specific HLA antibodies as well as abnormal epimural vessel on biopsies might identify cardiac allograft vasculopathy and offer an opportunity for early clinical intervention and modification of immunosuppression.

005

Expression of K^+ channel subunits in different human blood vessels

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Objective: We investigate some agents (glibenclamide and resveratrol) that can prevent spasm of human blood vessels used as a graft by anti-vasoconstrictor effect of the K^+ channel opener. Therefore, it is very interesting to know the distribution and localisation of different K^+ channel subunits.

Method: In the present study, the cryostat sections of the human a. renalis, a. radialis, a. mammaria interna and v. saphena were used. Immunoperoxidase or immunofluorescent techniques were performed. For the analysis of expression of different K^+ channels, a panel of antibodies to intracellular epitopes of the C-terminal part produced in rabbits (Alomone Labs, Israel) was employed: anti-Kv1.2, anti-Kv1.3, anti-Kv1.6, anti-Kv2.1, anti-Kir6.1, anti-Kir6.2 and anti-Kca1.1. In some cases, double immunofluorescent technique with alpha-SMA antibody was applied.

Results: V. saphena expressed only Kir6.2 subunits on the smooth muscle cells of the wall. Internal a. mammaria showed expression of Kir6.1 and Kir6.2. A. radialis showed variable positivity with Kv1.2, Kv1.3, Kir6.1 and Kca1.1. Double immunofluorescent labeling with antibodies against Kv1.2 subunit and alpha-SMA revealed that Kv1.2 channel subunit is predominantly expressed in the perinuclear region of the myointimal cells and of the smooth muscle cells of the media of a. radialis. A. renalis predominantly expressed Kv1.3 channel in the endothelium. Interestingly, in few artery specimens with focal calcification or atherosclerotic plaque, we observed decreased expression of Kv1.2 channel subunit.

Conclusion: Expression of K^+ channel subunits is different in various human blood vessels.

Friday, 3 September 2010, 17.00–18.30, Sala Wystawowa B

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003

Locked nucleic acid-based PCR clamping method for the detection of KRAS mutations

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Objective: KRAS is an oncogene that is commonly mutated in various malignancies. Mutations in KRAS are associated with lack of response to anti-EGFR treatment in colon and lung carcinomas, and patients must undergo genetic testing to be eligible for treatment. Several genetic tests for KRAS mutation analysis exist, but most are either too expensive or lack the sensitivity to identify a mutation if the percentage of tumor cells in the sample is small. The purpose of the present study was to develop a novel KRAS mutation detection method that is both cost-effective and sensitive.

Method: We designed a locked nucleic acid (LNA)-containing probe that inhibits the amplification of wild-type KRAS, thereby giving an advantage to mutated KRAS in PCR reaction (PCR clamping). The sensitivity of this method was evaluated using serial dilutions of plasmids containing wild-type and mutated KRAS fragments. The method was further evaluated on 40 archived tissue samples of colon carcinoma and compared to direct sequencing and high resolution melting (HRM) methods.

Results: LNA-based probe successfully inhibited the amplification of wild-type KRAS. The method enabled the detection of as little as 1% mutated DNA in the sample analyzed. The method was superior to direct sequencing when the percentage of malignant cells in the sample was 20% or less. There was 100% correlation between the results of PCR clamping and HRM.

Conclusion: LNA-based PCR clamping method is a cost-effective and highly sensitive method for the detection of KRAS mutations.

004

Loss of p16 protein and hypermethylation of p16 gene in bronchiolar columnar cell dysplasia

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Objective: Bronchiolar columnar cell dysplasia (BCCD) has been proposed as a novel peripheral precancerous lesion of the lung adenocarcinoma. Hypermethylation of p16 gene occurs early during the development of squamous cell lung cancer. However, the role of gene hypermethylation during the development of adenocarcinoma remains unclear. The aim of our study was to estimate the expression of protein p16 and cellular marker for proliferation Ki-67 and to detect hypermethylation of p16 gene in BCCD.
Method: The study material consisted of specimens obtained from 145 patients surgically treated for non-small cell lung cancer in the Clinic of Thoracic Surgery, Medical University of Bialystok. BCCD was searched for in sections taken from uninvolved lung parenchyma. P16 and Ki-67 were evaluated by immunohistochemistry. Analysis of epigenetic changes was performed in 18 patients. Hypermethylation of p16 was examined by methylation-specific PCR.
Results: BCCD was identified in 27 (18.6%) patients. BCCD was observed in patients surgically treated for adenocarcinoma, squamous cell lung cancer and large cell lung cancer (23.7%, 17.6%, and 10.2%, respectively). Loss of p16 was found in 78.9% of BCCD; cytoplasmatic expression of p16 was identified in 21.1% of cases. Expression of Ki-67 indicated moderate mitotic activity of epithelium in BCCD. Hypermethylation of the p16 gene was found in 12.5% of BCCD. Methylation of p16 gene was absent in normal epithelial lung tissues.
Conclusion: Our study indicates that BCCD was seen in only 18.6% of patients with lung cancer. Loss of p16 protein and hypermethylation of p16 gene seen in BCCD points to the precancerous stage of this lesion.

005

Ewing's sarcoma cells promote osteoclast formation

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Objective: Ewing's sarcoma (ES) is a primary bone tumour associated with extensive bone destruction. To investigate the osteolysis in ES, we examined whether ES tumour cells directly resorb bone or whether they act to promote osteoclast formation and resorptive activity.
Method: Several ES cell lines + macrophage colony-stimulating factor (MCSF)-primed $CD14^+$ monocytes were cultured on coverslips and dentine with MCSF + receptor activator of nuclear factor kappa B ligand (RANKL). Cultures were examined for the osteoclast formation markers tartrate-resistant acid phosphatase (TRAP), CD51 and lacunar resorption. Release of organic matrix collagen fragments (CTX-1) was assayed by ELISA. Protein and RNA expression of osteoclastogenic factors in ES cells was investigated by Western blotting, immunohistochemistry and real-time PCR.
Results: Cultures of ES cell lines on dentine did not result in lacunar resorption or the release of CTX-1, but the formation of large $TRAP^+$ and $CD51^+$ osteoclast-like multinucleated cells capable of lacunar resorption was seen in ES monocyte co-cultures. Expression of the osteoclastogenic factors, MCSF and RANKL, was identified in ES tissue sections and ES cell lines.
Conclusion: Our results indicate that ES tumour cells do not directly resorb bone. ES cells express MCSF and RANKL, osteoclastogenic factors that are essential for osteoclast formation from mononuclear phagocytes. This RANKL-dependent mechanism is likely to contribute to the rapid, aggressive osteolysis of ES.

006

Analysis of a novel JK-1 gene expression in benign and malignant colorectal tumors

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Objective: A newly discovered gene, JK-1 (FAM134B), located in chromosome 5 p15.2 plays an important role in the molecular pathogenesis of esophageal cancer. This study aims at examining the role of this gene in colorectal tumors by analyzing the differences in gene mRNA expression in both benign and malignant colorectal tumors.
Method: The study was performed on 162 colorectal adenocarcinoma, 32 benign adenoma and 20 non-neoplastic colorectal tissues. Gene expression levels were determined using real-time PCR. The expression levels were calculated as a ratio of the Ct value for JK-1 to GAPDH and expressed as inverse ratio.
Results: The JK1 mRNA was detected in all colorectal tissues. The mean inverse ratio for JK-1 gene in adenoma samples was 0.929 ± 0.005, which was significantly higher than in non-tumor samples which was 0.901 ± 0.009 ($p = 0.005$). On the other hand, cancer samples had a mean inverse ratio of 0.875 ± 0.006, which was significantly lower than in non-tumor tissues ($p < 0.001$).

Conclusion: The under-expression of the gene in cancer suggests that loss of gene expression may promote tumor progression, that it may act as a tumor suppressor gene, or that it relates to certain genetic events in tumor pathogenesis. However, over-expression of JK-1 in adenoma samples may indicate that JK-1 has a functional role in dysplastic benign colon tumors, a function that is disrupted once the tumor progresses to a more invasive form.

Poster Sessions

Wednesday, 1 September 2010, Basement

PS-01 Poster Session Breast Pathology

0001

Subtyping estrogen receptor-positive breast carcinomas by routinely used immunohistochemical reactions

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Background: To subtype estrogen receptor (ER)-positive breast carcinomas by retrospectively analysing the results of the routinely used immunohistochemical (IH) reactions and compare the findings to the recorded conventional prognostic factors.
Method: Between 2000 and 2007, 1,415 invasive breast carcinomas were diagnosed histologically in the 2nd Department of Pathology, Semmelweis University Budapest. ER/PR, Ki67, Her2 status were recorded. Her2 score 2 cases were reevaluated by FISH. We evaluated the results in comparison with tumor type, grade, pTNM stage, vascular invasion. In 765 of 1,036 ER-positive breast cancer cases, we had complete set of data. Three groups were set: cases with $Her2^-$ and low Ki67 index (<20%) status (A, 504 samples), $Her2^-$ and high Ki67 index (≥20%) status (B, 165 samples), and $Her2^+$ status (C, 96 samples). We suggest that the latter two represent the genomic luminal B group of breast carcinomas. R 2.7.1 statistical programme was used. Value of $p \leq 0.05$ was taken as significant.
Results: Of the cases in group A, 68.7% were invasive ductal (IDC), whereas 18.8% were invasive lobular carcinomas (ILC). In groups B and C, IDC were diagnosed in 84% and 88% while ILC in 9.8% and 9.3%, respectively ($p=0.0002$). Forty-three percent of group A cases were grade 1, whereas in groups B and C, close to 40% of the tumors were grade 3; grade 1 tumors were found in 8% and 11.7%, respectively. In group A, 7.8% of tumors were grade 3 ($p<0.0001$). Vascular invasion was recorded in 28.3% of the cases in group A, while this was seen in 50.7% and 48.7% in the B and C groups, respectively ($p=0.0002$). Least pT1 tumors were present in group B (44.5%). pT1 stage was recorded in 62.3% and 63.8% of groups A and C, respectively ($p=0.0149$). pN0 was found in 61.6% of the cases in group A, while only 39.5% and 45.4% of the cases in groups B and C were pN0, respectively ($p=0.0360$).
Conclusion: According to the conventionally used prognostic factors, cases belonging to our group A of ER-positive breast carcinomas possess a favourable profile. Our groups B and C show similar prognostic features. We believe that routinely used protein profiling using a simple IH panel provides important information and may subdivide the group of ER-positive carcinomas, probably reflecting the genomic luminal subtypes (analysis of the follow-up data is in progress). Acknowledgements: ETT 29/2009 grant.

0002

Vimentin expression and immunophenotypic molecular classification of node-negative invasive ductal breast carcinomas

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Objective: The origin of vimentin expression in breast cancer tumor cells and its prognostic significance have been controversial. The purpose of this study was to assess the correlation between the expression of vimentin and immunophenotypic molecular classification of breast cancers.
Method: Tissue microarrays containing 305 invasive ductal breast carcinomas from axillary node-negative patients were tested by means of immunohistochemistry for the expression of vimentin, ER, PR, HER2, EGFR, CK 5/6, CK14, CK17, p 63, P-cadherin.
Results: A significant association was found between vimentin expression and immunophenotypic molecular classification ($p<0.00001$). Vimentin was expressed in 67% of basal-like, 17% of basal-like $HER2^+$, 14% of luminal B, 9% of luminal A, and 38% of unclassified carcinomas. Of 50 vimentin-positive tumors, 56% constituted basal-like, 24% luminal A, 8% basal-like $HER2^+$, 6% luminal B and 6% unclassified cancers. Sixty-four percent of triple-negative cancers expressed vimentin. Expression of vimentin was negatively associated with ER and PR ($p<0.00001$) and positively associated with CK5/6 ($p<0.001$), CK 14 and CK17 ($p<0.00001$), and nuclear p63 ($p<0.02$).

Conclusion: The results partially explain controversy in the literature concerning vimentin's prognostic significance and suggest three pathways of vimentin origin in cancer cells.

0003

Histopathologic, immunohistochemical and morphometric profiles of invasive breast carcinoma

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Objective: Invasive breast carcinoma represents are characterized by invasion of adjacent tissues and a marked tendency to metastasize to distant sites. In order to determine specific prognostics factors, we established histopathologic, immunohistochemical and morphometric profiles of these types of carcinoma.
Method: In our study, we investigate 120 cases diagnosed with invasive breast carcinoma in which we established microscopic characterization, immunohistochemical profiles (expression of proliferation markers, steroid receptors and Her2) and computer-assisted morphometric profiles by determining the mean values for nuclear area, cellular area and N/C ratio with Lucia Net Software. The distribution of markers and histological grade among types of carcinoma was statistically analyzed using the χ^2 test.
Results: The histological type of invasive carcinoma was: 83.5% ductal carcinoma, 9% mixed ductal and lobular carcinoma, and 7.5% lobular carcinoma. According to histological differentiation, 15% was G1, 54% was G2 and 31% was G3. Immunohistochemical expression of ER was positive in 93% and negative in 15%, for PR was positive in 85% and negative in 15% and the expression of Her2 was positive in 68% and negative in 32%. Ki67 was intensely positive in 47%, moderately positive in 43% and intensely positive in 16%. P53 was positive in 95% cases and negative in 5% of cases. Morphometric analysis revealed that mean nuclear area and N/C ratio increase are concordant with grading, but mean cytoplasmatic area has no characteristic variation.
Conclusion: The expressions of proliferation markers correlate with carcinoma grading. Poorly differentiated and triple-negative carcinoma (ER, PR and HER2) with intense expression of proliferation markers and increase mean nuclear area have negative prognosis.

0004

Profiling signalling networks in formalin-fixed and paraffin-embedded breast cancer tissues

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Objective: Mapping tumour cell protein networks in routinely processed clinical samples, like formalin-fixed and paraffin-embedded (FFPE) tissues, will be critical for realizing the promise of personalized molecular therapy as only a subset of patients will respond. Therefore, techniques being able to detect the entire spectrum of deregulated pathways in tumors before, during, and after treatment are required to assess success or failure of targeted therapies. The aim of our study was to monitor protein networks in FFPE breast cancer tissues with special emphasis on epidermal growth factor receptor 2 (HER2)-mediated signaling pathways to identify and validate new disease markers in order to predict response to current treatments.
Method: Using a recently developed technology for the extraction of full-length proteins from FFPE tissues, we analyzed molecules involved in HER2-related signaling by reverse phase protein microarray (RPPA) in a series of 106 FFPE breast cancer patients. To this end, we evaluated >50 commercial antibodies for specificity in lysates from FFPE breast cancer samples in Western blots and RPPA.
Results: We found HER3 (r's=0.581), the EGFR (r's=0.556) and the receptor of uPA (r's=0.585) correlating with HER2. However, we could not demonstrate any significant correlation between HER2 expression and clinicopathological parameters.
Conclusion: Our results uncover molecules linked to HER2 signaling and cancer progression. Thus, these finings provide possible new targets for cancer treatment and may assist in optimal patient selection.

0005

mTOR and S6Kp70 expression and prognostic role in human breast cancer and their relation with PI3K pathway alterations

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Objective: The PI3K/AKT/mTOR pathway has a fundamental role in signal transduction, and its alterations are of pathogenetic role in human cancer, including breast carcinomas (BC). Several drugs may interfere with the altered activation of this pathway, including the recently developed mTOR inhibitors. However, few data are available concerning mTOR levels in human breast cancer.
Method: The aim of the present study was to analyze the immunohistochemical expression of mTOR and p70S6K in a consecutive series of 165 BC, with long-term follow-up which had been studied for ER, PgR, Her2, p53 and MIB1 expression and for PI3K and AKT mutations.

Results: mTOR and p70S6K expression were positively associated ($p=0.002$), but were not related to other molecular markers with the exception of high p70S6K being associated with PI3K mutations ($p=0.047$). In the whole series of cases, mTOR and p70S6K are not associated with clinical outcome. However, high mTOR are associated with prolonged DFS and OS in node-positive cases, in St. Gallen high-risk group, and in patients treated with chemotherapy. High p70S6K expression was associated with prolonged DFS and OS in St. Gallen high-risk group.
Conclusion: Our data show that mTOR and p70S6K are promising biomarkers in BC whose level of expression may be of prognostic value.

0006

Proliferative activity in human breast cancer: assessment of Ki-67 automated evaluation and the influence of different Ki-67 equivalent antibodies

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Objective: Ki-67 labeling index (Ki-67LI) has an important clinical relevance in breast cancer. It is extremely important to standardize its evaluation.
Method: Three hundred fifteen consecutive breast cancer immunostained for Ki-67 (223 with SP6 (Labvision) and 92 with MM1 (Novocastra) antibodies) previously examined by an experienced pathologist have been reevaluated using Aperio Scanscope Xs.
Results: Mean human Ki-67LI values were 35.91±14.35% and 28.37±18.29%, respectively, for SP6 and MM1 antibodies; mean CAM Ki-67LI values were 30.97±19.19% and 22.12±18.36%, respectively, for SP6 and MM1. Human and CAM evaluation are statistically highly correlated (Pearson, 0.859, $p<0.0001$), although human LI are systematically higher. Cases have been subdivided into three groups based on tertile distribution; cutoffs varied depending on the antibody used and on the evaluation methods: For human evaluation using SP6 and MM1, they were ≤30, 31–42, ≥43, and ≤20, 21–40, ≥41 respectively; for CAM evaluation, they were ≤19, 20–37, ≥38 and ≤12, 13–24, ≥25.
Conclusion: Our study shows that (a) CAM can be easily adopted in routine; (b) human and CAM Ki-67LI are highly correlated; and (c) Ki-67LI grouping on the basis of tertiles obtained using different evaluation methods and different antibodies shows important differences in cutoff values, underscoring that it is not correct to adopt general cutoff values to subgroup cases without taking into consideration the evaluation methods and antibody used.

0007

The antigen Ki-67: marker of the tumoral proliferation in the invasive mammary cancer and the connection with the classical prognostic factors

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Objective: The Ki-67 antigen is a marker of the cell cycle and of the tumoral proliferation. Due to the immunohistochemical techniques, it can be easily emphasized. The Ki-67 antigen is largely used for the estimation of the proliferation coefficient of a mammary carcinoma. The Ki-67-positive cells percent is correlated with the aggressiveness parameters or with those of tumoral progression.
Method: The studied tissue fragments from the studied carcinomas were fixed in formaldehyde 10%, included in paraffin and immunohistochemically colored using the monoclonal antibody MIB1. The positivism for Ki-67 was shown by the red coloring of the proliferating cells' nucleuses using DAB as chromogen.
Results: Out of 72 studied mammary carcinomas, 52 (72.2%) overexpressed the Ki-67 antigen. We have observed a significant connection between the Ki-67 expression and the histopathologic degree of the mammary carcinomas; we did not see any connection between the Ki-67 and the patients' age, tumor size, histological type and the clinic stage of the disease.
Conclusion: In mammary tumors, the presence of the Ki-67 antigen is considered as an unfavorable prognosis factor by rapid tumor increment, invasion and metastasis marker.

0008

VEGFR-3, LYVE-1, Prox-1, and podoplanin immunohistochemical expression in breast cancer axillary lymph nodes

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Objective: Lymphangiogenesis occurrence and the biomechanisms in lymph nodes are still unknown. The immunohistochemical application of the usually known lymphatic markers VEGFR-3, LYVE-1, Prox-1, and Podoplanin was explored to study lymphatic vessels in tumour metastasis and non-metastatic lymph nodes of breast cancer cases to understand the metastization pathway.

Method: Lymph nodes without and with metastases from 29 cases of invasive breast cancer were selected. Serial sections were immunostained for VEGFR-3, LYVE-1, Prox-1, and Podoplanin using immunohistochemical protocols in accordance with the respective recommendations. The microanatomical distribution of each antibody in the lymph node, different cell type expression, adjacent mesenchymal structure positivity and neoplastic cell positivity were validated and reported as negative and positive (with the cutoff +, ++, +++ for the neoplastic cells).
Results: No markedly or obvious staining was obtained for lymphatic vessels. Positive staining of metastatic neoplastic cells, lymphoid cells of germinal centres and node pericapsular fatty tissue has been observed. Immunostained neoplastic cells revealed a predominant subcapsular and perihilar location.
Conclusion: The applied primary antibodies were unsuccessfully used as lymphatic markers. The spread of neoplastic cells expressing these markers may have acquired particular characteristics to develop lymphatic dissemination until reaching lymph nodes. Lymphangiogenesis in breast cancer continues without demonstration in lymph nodes, while the fatty tissue can be implicated in epithelial mesenchymal transition (EMT). This thesis turned up by the proximity of the positive neoplastic cells to the mesenchymal components of surrounding lymph node tissue as adipocytes were immunoreactive.

0009

Finding resistance biomarkers to trastuzumab in breast cancer

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Objective: There is a significant percentage of patients with metastatic breast cancer and HER2 receptor overexpression that do not respond to therapy directed with trastuzumab. In these patients, overall survival and disease-free time are significantly shorter. Several mechanisms that explain the resistance to therapy with trastuzumab have been reported. This paper aims to address the study of alterations in one of the signalling pathways of HER2-related trastuzumab in response to breast cancer in order to incorporate into the clinical routine of other predictive biomarkers of response to therapy.
Method: The study comprised 70 cases of metastatic breast carcinoma with HER2 overexpression (HercepTest 3+) or gene amplification (FISH). All patients were treated with trastuzumab. The assessment of PTEN and pAkt expression was based on staining intensity and percentage of stained cells.
Results: Our series showed a statistically significant association between a positive expression of PTEN and a reduced expression of pAkt ($p=0.034$). The positive expression of PTEN was associated with a better treatment response rate ($p=0.001$). It was also noted that the reduction of PTEN was associated with a shorter time of progression ($p=0.010$) and a lower overall survival ($p=0.063$). pAkt overexpression was associated with a lower overall survival ($p=0.081$).
Conclusion: Our study shows that immunohistochemical expression of PTEN and pAkt could be used as both prognostic and predictive biomarkers in breast cancer.

0010

Stromal–tumor cell interactions in breast cancer in young women

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Objective: Stromal components are attributed an important role in modulating tumor progression from noninvasive to invasive stages. The interrelations between stroma and tumoral cells are not elucidated and currently elicit a special interest among researchers. The aim of our study was to identify possible markers that could characterize the ways in which stromal components relate to tumor cells in breast cancer in young women (age < 35 years).
Method: Our study was performed on 30 ductal invasive breast carcinomas (age range = 23–35 years, $M=33$ years, SE=0.53) from the "Victor Babes" National Institute of Pathology. Formalin-fixed paraffin-embedded samples were analyzed immunohistochemically using an indirect bistadial technique performed with a polymer-based detection system with antibodies for MMP9, COX2, CerbB2, estrogen (ER) and progesterone (PR) receptors and for NF-kB. Statistical analysis was performed using the Student's *t* test from the Analysis Tool Pak of Microsoft-Excel 2003.
Results: We found significant statistical correlations between the immunohistochemical expressions of CerbB2, NF-kB and MMP9 in tumoral cells. CerbB2 strongly correlated with NF-kB and MMP9 in stromal cells (lymphocytes, fibroblasts), vascular structures and extracellular matrix, which varied proportionally with the histological grade. MMP9 was more expressed in stromal components, while NF-kB was more expressed in tumoral

cells. COX2 expression in tumoral cells increased with histological grade and correlated with PR and with COX2 positivity in lymphocytes and mast cells.
Conclusion: Our results indicate that MMP9, NF-kB and COX2 constitute reliable markers for stromal–tumoral cell intercommunication with possible implications in tumor progression, invasiveness and metastases.

0011

Comparing the prognostic value of proliferation markers Ki67, mitotic activity index, phosphohistone-3 and classical variables in breast cancer

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Objective: Proliferation factors have dominant prognostic value, but their independent value and reproducibility are largely unknown. The reproducibility of Ki67 was evaluated and its prognostic value compared with mitotic activity index (MAI), phosphohistone-3 (PPH3) and other variables.
Method: In 240 node-negative breast cancers, standardized immunohistochemistry was used for Ki-67 and PPH3 and a strictly formalized protocol for MAI assessment. Ki67% positive nuclei were determined by two pathologists (Ki67-1, Ki67-2), computerized interactive morphometry (CIM) and digital image analysis (DIA). Continuous variables were discretized according to established thresholds and receiver operating curve (ROC) analysis. Reproducibility, univariate and multivariate survival analysis were performed.
Results: One pathologist was well reproducible but not strongly prognostic; the other was strongly prognostic but not well reproducible. DIA-Ki67 and CIM-Ki67 both were reproducible ($r > 0.99$ and 0.94) and strongly prognostic (10-year survivals of 98% and 72%, 97% and 72%). The ROC-derived optimal thresholds differed both for the quantitative methods (6.45% for DIA, 10% for CIM) and the pathologists (4% and 13%). DIA-Ki67 with a threshold of 6.45 is the strongest multivariate prognosticator (10-year survivals of 98% and 72%), overshadowing other Ki67 thresholds and classical variables (tumor diameter, grade, ER, PR, Her2, cytokeratin 5/6). Only MAI (<3 versus ≥3) or PPH3 (<13 versus ≥13) were slightly stronger.
Conclusion: Proliferation factors MAI-3, PPH3-13 and Ki67-6.45 by digital image analysis are strong prognostic factors in node-negative breast cancer. Furthermore, Ki67 assessments by CIM or DIA are by far more reproducible than visual scoring.

0012

Study on relationship between expression of vascular endothelial growth factor (VEGF) and metastasis to axillary lymph nodes in invasive ductal carcinoma of breast

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Objective: Vascular endothelial growth factor (VEGF) has been shown to play a major role in tumor angiogenesis in many studies. This study aimed at evaluating the relationship between VEGF expression and lymph node metastasis in patients with invasive ductal carcinoma (IDC) of the breast.
Method: In a case–control setting, we evaluated specimens of IDC in patients with and without axillary lymph node metastasis. The immunohistochemistry staining was employed for evaluating the expression of VEGF in the specimens of both groups. The rate of positive specimens was then compared between the groups. The relationship between the VEGF expression and the size and grade of tumors were assessed.
Results: Eighty women with IDC of breast, 40 cases with axillary lymph node metastasis and 40 controls without any evidence of axillary lymph node involvement, were enrolled. There were 16 (40%) and 10 (25%) cases with positive expression of VEGF in the case and control groups, respectively ($p=0.152$). The mean size of tumor was significantly higher in patients with positive expression of VEGF (4.81±2.43 cm vs 3.64±1.54 cm, $p=0.034$).
Conclusion: The current study showed that there is no significant relation between the rate of expression of VEGF in cases of IDC of the breast and the axillary lymph node metastasis; however, there may be a relation between VEGF and the size of the tumor.

0013

Abnormal membrane cytoskeletal cross-linker ezrin cellular distribution is associated with clinicopathological features in invasive breast cancer

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Objective: Ezrin is member of the ERM (ezrin/radixin/moezin) protein family which acts as a membrane organizer and linker between the plasma membrane and cytoskeleton and plays a key role in the control of cell morphology, adhesion, polarity, motility and cell survival. The aim of the present study was to elucidate the clinical significance of

subcellular expression pattern of ezrin in patient with invasive breast cancer (BC).
Method: Immunohistochemistry for ezrin monoclonal antibody was performed on 311 paraffin-embedded specimens from patients with BC. Immunoreactivity was semiquantitatively scored according to Remmele immunoreactive score (IRS).
Results: Expression patterns of ezrin were divided into cytoplasmic (309, 99.36%), membranous (162, 52.1%), nuclear (44, 14.15%) and apical (16, 5.15%). Cytoplasmic pattern was the only one observed in 109 cases (35%) and mixed immunotopography in the others. There were significant positive associations between apical and membranous ezrin localization and favorable tumor characteristics and lack of lymph node metastases. Cytoplasmic ezrin staining was associated with adverse clinical outcome. Lack of apical ezrin was likely to present with bone metastases in the absence of visceral disease. Membranous ezrin localization was correlated with good prognosis both for CSOS (p=0.018) and DFS (p=0.007). A multivariate analysis demonstrated that membranous ezrin distribution is an independent prognostic factor (p=0.042).
Conclusion: The switch of ezrin localization from the apical and the membrane to the cytoplasm is correlated with dedifferentiation and adverse features in BC. Cellular ezrin distribution contributed to discriminating between patients with lymph node and distant metastases and those with better prognosis.

0014
Expression of EGFR in invasive ductal breast carcinomas

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Objective: Epidermal growth factor receptor (EGFR) is a tyrosine kinase growth factor receptor expressed in a variety of human cancers and related with poor prognosis. Recently, the astounding development of molecule-targeting drugs such as anti-EGFR-targeting drug has attracted considerable interest of oncologists. The objective of this study was to evaluate the expression of EGFR in breast carcinomas and its possible relationship with different clinical–pathological parameters.
Method: One hundred and seventeen breast carcinomas were included in this study. Using a standard immunohistochemical technique, the expression of EGFR, HER2, and hormone receptors was investigated. In addition, haematoxylin and eosin-stained sections of these tumors were studied for several morphological parameters.
Results: EGFR-positive expression was detected in nine tumors (7.69%). Expression of EGFR was positively associated with high histological grade (p=0.023), high-grade comedo-type necrosis (p=0.000), high degree of stroma lymphocyte infiltration (p=0.027), pushing tumor margin (p=0.027), and hormone receptor-negative HER2-negative status (triple-negative phenotype, p=0.026). No significant difference of EGFR expression according to age was recognized.
Conclusion: EGFR expression is more often present in the group of triple-negative breast carcinomas, and its expression is associated with features of poor prognosis. EGFR expression might be important as a potential target for molecular therapy in breast cancer.

0016
Morphometric parameters of nucleoli and nuclei of tumor cells from primary breast cancer with positive versus negative axillary lymph nodes

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Objective: The purpose of the study was to assess morphometric parameters of nuclei and nucleoli in invasive ductal breast carcinomas and to compare them with axillary lymph node status.
Method: Histologic sections from 150 tumors were stained with methyl green-pyronin Y and measurements were made using a computerized image analysis. The following seven parameters were evaluated: the nucleolar area, long-to-short nucleolar axis ratio, nucleolar shape parameter assessing the degree of nucleolar roundness, the nuclear area, long-to-short nuclear axis ratio, number of nucleoli in the nucleus and the percentage of the nuclear area occupied by nucleoli.
Results: A statistically significant association between a nuclear area, long-to-short nuclear and nucleolar axis ratio and Bloom–Richardson (B-R) histologic grade of breast tumors was found in relation to the axillary lymph node status. In B-R grade 1 tumors, the nuclear area was significantly larger in tumors with negative versus positive axillary lymph nodes (p<0.05). Long-to-short nuclear and nucleolar axis ratios were higher in B-R grade 2 tumors with negative versus positive lymph nodes (p<0.05), i.e. the shape of nuclei and nucleoli of tumor cells from node-negative cancers was more elliptic as compared to node-positive ones.
Conclusion: Morphometric parameters of nucleoli and nuclei of breast cancer cells may have prognostic value in invasive ductal breast carcinomas.

0019

Triple-negative breast cancer in the Romanian population—clinicomorphological and immunohistochemical phenotype

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Triple-negative (TN) breast tumors are described by immunohistochemical lack of expression of hormone receptors (ER and PR) and Her2/neu. This immunophenotype is associated with young age and increased biological aggressiveness, being described in the literature as an overlap with molecular basal-like subtype. The aim of our study was the illustration of the histopathological and, by the use of a wide range of biomarkers, the phenotypic features of TN tumors in our study group. Our study was multicentric, consisting of an extensive casuistry belonging to Victor Babes Institute, Bucharest, University Emergency Hospital of Bucharest, Emergency County Hospital of Craiova and spread over a period of 5 years (2005–2009). The study group consisted of 250 patients with ages ranging from 23 to 83 years (mean 52.64 years). The tumors were investigated using morphological analysis (gross and microscopic), the identification of the basal/myoepithelial phenotype, markers of cell proliferation, identification of gene abnormalities and markers of intracellular signaling pathways. The results were processed using digital image analysis, biostatistics and biomathematics. Better stratification of patients according to risk based on these markers may be useful in guiding therapy in TN breast cancers, the therapeutic decision including all the characteristics of a tumor.

0020

Quantitative protein network monitoring for uPA and PAI-1 in breast cancer

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Objective: The urokinase-type plasminogen activator (uPA) and its main inhibitor PAI-1 facilitate migration and invasion of cells in physiological and pathological contexts. Both factors are clinically applicable predictive markers in node-negative breast cancer patients used to stratify patients for adjuvant chemotherapy. Besides their classical functions within the plasminogen activation system, these factors are shown to play important roles in cell signaling, thus providing new possibilities to interfere with cancer progression and metastasis. Although such networks are well described in cell culture systems, their analysis in tissues was hindered because protein extraction and direct analysis was possible only from fresh or frozen material, which may be not available in routine laboratory setting.

Method: We extracted proteins from formalin-fixed paraffin-embedded tissues, being the main source of archived patient samples in pathology institutes worldwide, by a new protocol developed in our laboratory and analyzed the extracts for interactions of uPA and PAI-1 with molecules belonging to signaling cascades relevant in cancer progression in a collective of 206 breast cancer patients using protein microarrays.

Results: A substantial correlation of uPA with Erk and Stat3 expression was ascertained, while PAI-1 correlates with Akt activation and EGFR-related signaling. Statistical analysis of uPA and PAI-1 protein expression did not reveal a significant correlation of uPA or PAI-1 with staging, grading or age of the patients.

Conclusion: Network monitoring for uPA and PAI-1 in breast cancer reveals interactions with main signaling cascades, confirming the findings of cell culture experiments. Our results reveal possible mechanisms underlying cancer development, thus providing possible new targets for cancer therapy.

0021

Mammary-derived growth inhibitor (MDGI) and estrogen-induced gene 121 (EIG121): a transcriptional/posttranslational approach and potential implications for anti-EGFR therapy in invasive breast carcinomas

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Objective: EGFR is frequently overexpressed during malignant transformation and linked with resistance to cytotoxic/ hormonal drugs. Anti-EGFR therapies have shown clinical benefits in the treatment of colorectal, pulmonary, pancreatic and upper airway carcinomas. Conversely, no activating mutations have been clearly identified in invasive breast carcinomas (IBC). In an attempt to discover targeted modalities for new clinical trials, we explored EGFR mRNA levels of expression and EGFR: (1) nuclear translocation, (2) internalization with MDGI cytosolic protein and (3) lysosomal-mediated degradation by EIG121 protein.

Method: EGFR, MDGI and EIG121 mRNA expressions were investigated by quantitative RT-PCR in a series of 470 IBC. Immunohistochemistry was performed with a panel of anti-EGFR, estrogen receptor (ER), progesterone receptor

(PR), HER2, MDGI and EIG121 antibodies (Abs) in a series of 90 IBC.

Results: We observed (1) global low mRNA EGFR levels, (2) high EGFR mRNA levels (12%) in the triple-negative group (NNN), (3) EGFR protein hyperexpression (14%) in the NNN group, (4) no nuclear immunolocalization of EGFR, (5) positive correlation between EGFR mRNA and protein expression levels (0.0000049), and (6) high or normal MDGI mRNA levels (55%) and low EIG121 mRNA levels (38%).

Conclusion: Although EGFR expression is globally decreased in IBC, a targeted therapy may be justified by determining the expression levels of two EGFR pathway posttranslational regulatory proteins: MDGI (internalization) and EIG121 (degradation). Following our results, an anti-EGFR therapy seems indicated when (1) EIG121 level expression is low with normal or increased EGFR and (2) EGFR inhibitors preferred over Abs when MDGI is high.

0022

Bmi1 and Sox2—two stem cell markers identifying breast cancer patients with adverse prognosis

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Objective: PolycombGroup (PcG) protein Bmi1 (B-lymphoma Mo-MLV insertion region 1) is crucial in maintaining gene silencing over cell cycles in order to control stem cell regulation and cellular identity. Sox2—the SRY (sex-determining region Y)-box 2 high-mobility group (HMG) protein—is the key trigger of pluripotency in embryonal stem cells. Deregulation of these genes is an initial step towards tumourigenesis with clinical vital difference.

Method: In this study, we applied these markers to 86 invasive breast cancer patients and five controls from breast hypertrophy prepared as formalin-fixed, paraffin-embedded (FFPE) tissue blocks. Sections were immunohistochemically (IHC) stained, evaluated and compared to histopathological data. Also, six breast cancer cell lines (MCF-7, MDA-MB231, MDA-MB453, SK-BR3, SUM-159, T47-D) provided as FFPE cytoblocks were additionally analysed.

Results: IHC identified 32.94% $Sox2^+$ and 70.13% $Bmi1^+$ patients. $BMI1^+$ expression is correlated to intermediate grade, tumours <2 cm in size and ER^+/luminal/triple positive subtypes which possess the greatest benefit to common therapies. $SOX2^+$ cases revealed patients with poor prognosis because of high grade, bigger size and increased metastasis. Also, clear correlation with basal-like and absence in normal-like phenotype were observed. 96.3% belonged to invasive ductal carcinomas, a subtype of worse prognosis in contrast to invasive lobular carcinomas, mixed forms or other subtypes.

Conclusion: SOX2 and BMI1 oncogenes, despite their pivotal role in mammogenesis and breast homeostasis, act in different ways when overexpressed, resulting in breast tumours with various prognoses. Probably, they describe different types of stem cells coexisting in adult tissue or of different origin.

0023

Podoplanin expression in cancer-associated fibroblasts predicts poor outcome of patients with invasive ductal breast carcinoma

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Objective: Podoplanin (D2-40) was recently demonstrated to be upregulated in cancer and stromal cells and was associated with increased tumor invasiveness. The role of cancer-associated fibroblasts (CAF) in tumor growth is poorly understood; therefore, we analyzed podoplanin expression in CAFs of invasive ductal carcinoma of the breast.

Method: Studies were performed on archival tissue samples of benign lesions and invasive ductal carcinoma (IDC) of the breast which were stained for D2-40. Serial sections stained for vimentin and alpha-SMA were used to identify CAFs. Expression of D2-40 in CAFs, LVD, and LVI were assessed and correlated with well-known prognostic factors.

Results: No D2-40 expression in stromal cells was shown in benign lesions and few of IDCs. The extent of D2-40 expression in CAFs correlated with grade of malignancy, LVI, and tumor size. No correlation with nodal, distant organ metastasis and LVD was observed. D2-40 expression in CAF was associated with shorter survival time.

Conclusion: Our preliminary data indicate that podoplanin may be a marker for cancer-associated fibroblasts. The results of this study demonstrate also its usefulness as a prognostic factor because of strong association with poor patient outcome.

0025

Breast cancer in young women

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Objective: Breast cancer is one of the most common human neoplasms accounting for approximately one quarter of all cancers in females. In young women, the risk is low, but because of the density of breast tissue, by the time a lump can be felt, the cancer is often advanced. Fine needle aspiration of breast lesions is routinely performed in our hospital. We reviewed our archives and found the cases of breast cancer in women under 40 years old.
Method: Between January 2003 and March 2010, 850 fine needle aspirates in breast lesions were performed in our Institution. The aspiration was done using a 21-gauge needle. Conventional and ThinPrep smears were prepared. Estrogen and progesterone receptor studies were part of the routine workup of a breast cancer as well as other markers (c-erb-B2, p53, E-cadherin, GCDFP-15).
Results: One hundred and fifty-eight of the cases were carcinomas. Ten of these patients were under 40 years old (mean age 38.2). In eight of these patients, cytology correctly diagnosed seven ductal and a tubular carcinoma. In two cases, the cytologic diagnosis was suggestive of carcinoma, and the excisional biopsy revealed a lobular and a mixed carcinoma.
Conclusion: It is mandatory in young women that a breast lesion be identified using mainly ultrasound and fine needle aspiration, which are simple and well-tolerated diagnostic tools. However, mammogram may also be recommended for younger women when a family history of breast cancer or other risk factors exist.

0026

MGMT protein expression as a potential prognostic marker in breast cancer

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Objective: MGMT repairs DNA damages via alkylation by removing a methyl group from the O6 position of guanine, acting as tumor suppressor gene in normal cells. We evaluated the MGMT protein expression in breast tumors, correlating it with others to establish its prognostic value.
Method: Sixty-four cases of invasive breast carcinomas were evaluated by immunohistochemistry (IHC) for MGMT, estrogen and progesterone receptors (ER, PR), HER-2-neu, Ki-67, p53, p63, E-cadherin, cytokeratins 5 and CK14 in order to classify the cases into luminal and basal phenotypes. Fluorescent in situ hybridization (FISH) was performed in those cases considered 2+ to assess HER-2neu gene amplification status. qRT-PCR was performed in frozen tissue to evaluate mRNA expression of MGMT.
Results: Fourteen cases were triple-negative (21.8%) and, among those, seven cases were basal-like carcinomas (10.9%). Twenty-five cases (39%) were luminal-like type A, four cases were (6.25%) luminal-like type B, and one case (1.5%) was HER-2-like type. MGMT showed significant lower expression in the basal-like tumors when compared to both luminal-like types ($p=0.007$). Tumors with basal-like phenotype presented higher positivity for p53 and Ki-67 than the luminal types ($p=0.025$ and $p=0.003$, respectively). Conversely, positive p53 and highly positive Ki-67 tumors showed a significant lower expression of MGMT ($p=0.0184$ and $p=0.0081$, respectively).
Conclusion: MGMT assessment by IHC or molecular biology techniques may represent an important prognostic factor in breast cancer. [Supported by FAPESP and CNPq]

0027

Correlations between prognostic factors in breast cancer

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Objective: It is well known that high indexes of estrogen and progesterone receptors (ER and PR) in mammary carcinoma are indicative for good prognosis and predict good response to endocrine therapy. Other important prognostic factors in mammary carcinoma are the age of the patient and histological type of tumor.
Method: We performed a retrospective study analyzing a group of 137 cases of 35- to 67-year-old female patients with mammary carcinomas reported in our department between 2007 and 2009. The histopathologic types of the tumors were established based on microscopic evaluation in HE stain, and ER (SP1 neomarkers) and PR (SP2 neomarkers) indexes were evaluated based on immunohistochemical stains.
Results: Eighty percent to 100% ER and PR positivity was by far most frequent in invasive ductal and mixed (ductal and lobular) types of mammary carcinoma (92 cases, 67.15%); intermediate levels of positivity (ER 40–50% and PR 10–20%) were reported mainly in medullary carcinoma (34 cases, 24.81%); and low levels of positivity (hormonal receptors <10%) were present mostly in perimenopausal patients.
Conclusion: Invasive ductal and mixed mammary carcinoma associates high level of positivity for hormonal receptors, especially in young patients, while medullary carcinomas of the breast (histopathologic type with better prognosis than ductal carcinoma) have lower levels of positivity for ER and PR. Age also plays an important role,

a better response for young women with positivity to hormonal receptors. Note: Staniceanu and Gramada should be regarded as first authors with equal contribution.

0028

Endosalpingiosis in axillary lymph nodes: a possible pitfall in the evaluation of sentinel lymph nodes

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Objective: The occurrence of benign inclusions in axillary lymph nodes (LNs) is well documented. Heterotopic mammary glands and intracapsular nevi can mimic metastatic carcinoma and are frequently encountered in the evaluation of sentinel LNs for breast cancer. Benign Mullerian inclusions (endometriosis and endosalpingiosis) are often identified in pelvic and paraaortic LNs, but only rare reports have documented the involvement of supra-diaphragmatic LNs.
Method: We report our 2003–2010 experience with three patients found to have endosalpingiosis in axillary sentinel LNs obtained for staging of breast carcinoma. All patients were postmenopausal women, with age ranging between 65 and 75 years.
Results: Endosalpingiosis involved a single lymph node in one patient and two nodes in each of the other two; it was present in the lymph node capsule in all three cases, with few glands scattered within the lymph node parenchyma in two of the patients. The glands contained ciliated and intercalated peg cells, had no periglandular endometrial-type stroma and showed no atypia or mitotic activity. The epithelium demonstrated positive nuclear immunoreactivity for WT1 and PAX8 and was devoid of myoepithelium or basement membrane. Endosalpingiosis had been misinterpreted as metastatic carcinoma at another hospital in one of the three patients, with subsequent dissection of 19 additional benign axillary LNs.
Conclusion: We conclude that endosalpingiosis can involve axillary LNs and closely simulate metastatic mammary carcinoma. Morphologic identification of ciliated cells and peg cells is most helpful to recognize this benign inclusion, and positive immunoreactivity for WT1 and/or PAX8 can be used to support the diagnosis. Correct classification of endosalpingiosis in axillary LNs is critical for accurate staging and to prevent possible overtreatment.

0030

Pathological assessment of sentinel lymph node and progression of lymph node metastasis at primary breast cancer

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Objective: Sentinel lymph node (SLN) biopsy has become a common procedure for breast cancer patients. Generally, if there is SLN metastasis, we do axillary lymph node (ALN) dissection. But sometimes we experience a case in which their LN metastasis is limited in SLN. So, we investigated these questions. (1) What is the factor associated to the progression of LN metastasis? (2) How is the case that we can ignore ALN dissection, even if there is SLN metastasis? (3) What is the best way to assess SLN?
Method: We retrospectively examined 94 Japanese patients who had surgical treatment for primary breast cancer between 2005 and 2009. All of them received ALN dissection after SLN biopsy. We also compared how to make sections for assessing SLN.
Results: Thirty-eight cases had no metastasis (SLN(−)Ax(−), 40.4%), 30 had only SLN metastasis (SLN(+)Ax(−), 31.9%), 21 were SLN(+)Ax(+) (22.3%), and five were SLN(−)Ax(+) (5.3%). By univariate analysis, lymphatic and/or vascular involvement was significantly correlated with the progression of LN metastasis. The cancer nest's size of metastatic SLN had more than 1 mm in all of SLN(+)Ax(+) cases. To detect SLN metastasis, making sections <2.0 mm in thickness was better than making the largest section ($p < 0.0001$). There was no correlation between how to make sections and ALN metastasis.
Conclusion: Lymphatic and/or vascular involvement was significantly correlated with the progression of LN metastasis. There was no ALN metastasis if the size of cancer nest was <1 mm in metastatic SLN. Making sections <2.0 mm in thickness was a better way to detect SLN metastasis.

0031

Audit of sentinel node handling and reporting in Kent

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Objective: Sentinel lymph node biopsy (SLNB) is rapidly becoming the standard of care in staging the axilla for early-stage, clinically node-negative breast cancer. This audit reviews the laboratory handling and pathological reporting of sentinel nodes (SNs) across Kent and has been performed at the request of the Kent Cancer Network Breast Disease Oriented Group (DOG).
Method: A retrospective analysis of 20 consecutive breast SN cases from each of the five Kent hospitals was carried out. All reports and slides of positive SNs were reviewed. Detailed structured questionnaire-based enquiries to lead breast pathologists were made. Data were entered in Microsoft Excel and analysed in relation to the standards. All aspects of the pathology service were examined, including macroscopic data (number of SNs/case, dimensions, sampling and slicing details) and microscopic data

(performance of levels and immunohistochemistry, TNM staging and SNOMED coding).
Results: One site was unaware of radioactivity guidelines while another was unaware of national guidelines. Twenty-eight percent to 84% of SNs were bisected longitudinally and levels were sparingly used, except at 1 site. TNM staging and SNOMED coding in the reports were variable.
Conclusion: This audit demonstrates a substantial variation in SN handling and reporting practice across Kent. Since the quality of the pathology service in breast cancer is of paramount importance, it is imperative that a standardised approach is achieved in the handling and reporting of SNs. To achieve this, recommendations have been made and agreed upon, the success of which will be measured in a re-audit next year.

0032

Diagnostic value of automated Her2 evaluation in breast cancer: a study on 272 equivocal (score 2+) Her2 immunoreactive cases using an FDA-approved system

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Objective: Accurate immunohistochemical (IHC) Her2 evaluation is fundamental for the therapeutic selection of breast cancer (BC) patients.
Method: We analyzed a series of 292 equivocally (score2+) Her2 immunoreactive BC; all had been stained with Dako Herceptest, evaluated by an experienced pathologist and analyzed with FISH. The automatic Aperio categorization and the percentage of immunoreactive cells as evaluated by the computer (CPV) and by the pathologist (PPV) were recorded.
Results: CAA classified seven (2.4%) cases as negative (0), 136 (46.6%) as faintly positive (1+), 134 (40.5%) as moderately positive (2+) and 15 (5.1%) as strongly positive (3+). CCA classification is associated with Her2 amplification ($p<0.0001$). The mean CPV is 18.44% SD ± 19.00 (range 0.01–76.10). CPV and PPV are significantly associated ($p<0.001$) and have similar sensitivities and specificities in identifying Her2 FISH-amplified cases. CPV has very low inter-observer variation. The difference in CPV in amplified and non-amplified subgroups is statistically significant ($p<0.001$). ROC analysis indicates that CPV is good at separating FISH not amplified from amplified cases ($p<0.001$). The optimal cutoff value maximizing both sensitivity and specificity is 17.6% (sensitivity = 73.3%, specificity = 71.6%). Reducing the cutoff value to 0.67%, it is possible to reach the sensitivity of 100% with 16.2% specificity.
Conclusion: CCA Her2 IHC evaluation is feasible and reliable; however, automated classification is not satisfactory as some amplified cases might be erroneously clustered in the score 1+ group. Compared with traditional Her2 evaluation, CAA can reduce the number of cases unnecessarily submitted to FISH.

0033

HER2 gene status assessment on cytological specimens of breast carcinoma using FISH

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Objective: Assessment of HER2 status is mandatory for the treatment of patients with invasive breast cancer. According to ASCO/CAP recommendations, HER2 should be assessed on histological specimens with a validated immunohistochemical or FISH method. However, in some patients, histological material is not available and cytological specimens could be an alternative. The aim of our study was to optimise a FISH protocol for HER2 assessment on cytological samples.
Method: HER2 gene status was assessed using PathVysion HER2 probe (Abbott Molecular Inc.). FISH protocol was optimised on cytospines made from cultured cell lines with known HER2 gene status (SK-BR-3-positive, MDA-MB-453 borderline, MDA-MB-175-negative, MDA-MB-231-negative). The protocol was tested on 31 cases of primary or metastatic breast carcinoma with paired cytological and histological samples of the same tumour. FISH results obtained on paired samples were compared.
Results: In the four cell lines, the FISH protocol resulted in ratios that corresponded to the declared gene status. Analysis was successful in 27 (87.1%) cytospins and in 31 (100%) histological samples. Results were concordant in 26 of 27 (96.3%) cases (kappa 0.914). Among histological samples, 18 were negative, all also negative on cytospins, and nine were positive, of which eight were positive on cytospins. In the single discordant case (cytospin-negative/histology-positive), the analysis of the histological sample revealed intratumour heterogeneity of HER2.
Conclusion: FISH is a reliable method for the determination HER2 gene status on cytological specimens. However, FISH on cytospins is inferior to FISH on histology samples due to higher failure rate and absence of the morphologic features of the tumour.

0034

Application of image analysis in diagnostic assessment of HER2 gene status

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Objective: The demand for accurate HER2 gene status testing is increasing. Application of image analysis (IA) facilitates archiving and reporting and standardises analysis of FISH reaction. The aim of the study was to evaluate the accuracy of an IA system for the determination of HER2 gene status in breast carcinoma.
Method: HER2 gene status was assessed in 203 cases with FISH using PathVysion HER2 probe (Abbott Molecular Inc.). Ratio ≥ 2 indicated amplification of the gene. Each case was analysed three times: The first result was obtained by manual analysis (MA) of the slide. Then, the slides were scanned and analysed with ARIOL-50SL IA system (Genetix inc.): The second result was the raw IA (RIA) result, and the third result was obtained by brief manual inspection and, if needed, correction of the RIA (CIA) results. The two IA results were compared with the MA results.
Results: Of the 203 cases, 43 (21.2%) were positive with MA, 65 (32.0%) with RIA and 35 (17.2%) with CIA. MA and RIA results were concordant in 161 (79.3%) cases, indicating poor agreement (kappa 0.478) and the need for inspection and correction. MA and CIA results were concordant in 191 (94.1%) cases (kappa 0.810). Among 12 discordant cases, in 11, at least one of the results was borderline (MA 3, CIA 9; median ratio discrepancy 0.54), and in a single case, the results were clear-cut discrepant.
Conclusion: IA, if adjusted to the quality of FISH reaction, is a reliable method for the analysis of HER2 gene, but inspection of RIA results is recommended in most cases and a few cases still require MA.

0035

New automated approach for FISH analysis of HER-2 amplification in invasive breast carcinomas

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Objective: Computer-assisted digital image (DI) analysis has been used in the field of pathology to avoid human variability. This study shows a new automated method to evaluate HER-2 FISH amplification in composed DIs in a fast and easy manner.
Method: One hundred samples of invasive breast cancer were evaluated using pharmDxTMkit (DAKO). In each sample, three tumoral representative areas were selected to test the new methodology. All FISH signals are not in the same plane, so according to it, four DIs of representative areas were captured in different plains with FITC and also with Texas Red filters applying different focus settings. Definitive composite best-focus images were obtained for each filter using the "Extended Depth of field" tool of Image-Pro® Plus 5.0 software. The green channel from FITC and the red channel from Texas-Red images, captured as RGB colour images, were leveled by background subtraction using blurred large spectral low-pass-filtered images, as characteristic of non-homogeneity in light distribution. Resulting images were segmented using intensities threshold, which allows count FITC and Texas-Red signals in images. All the counted Texas Red and FITC signals were used to calculate the ratio for each sample manually and with the automated procedure, and both methodologies were compared.
Results: The k statistic was 0.935 with a $p<0.001$, showing a great agreement between the two methodologies.
Conclusion: This new automated method provides an accurate and practical alternative to the traditional manual determination of HER2- FISH amplification.

0036

New methodology to evaluate HER-2 FISH in invasive breast cancer

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Objective: In breast cancer, the efficiency of Trastuzumab (Herceptin®) therapy in unclear immunohistochemical scoring depends on the evaluation of HER-2 gene amplification using FISH. Traditional FISH evaluation is complicated and requires highly time-consuming work. This study presents a new methodology faster and easier than traditional based on the manual evaluation of HER-2 FISH using composed digital images.
Method: One hundred samples of invasive breast cancer were evaluated with FISH traditional method using pharmDxTMkit (DAKO). In each sample, three representative tumoral areas were selected to test the new methodology. An image of nuclei was captured with the DAPI filter at ×100 magnification. Without moving the section and applying different focus settings to cover the entire depth of the section, four consecutive images

were acquired with the FITC filter and then four with the Texas-Red filter. Then, using the "Extended-Depth of field" tool of Image-Pro® Plus 5.0 program, the four FITC images were superposed to obtain a new composite best-focus image with all the signals in the different plains. The same was done for the Texas-Red images. A global ratio was calculated using these composed images between all the Texas-Red and FITC signals, and it was compared with the ratio obtained with traditional evaluation.
Results: The *k* statistic was 0.968 with a $p<0.001$, showing a great agreement between the two methodologies.
Conclusion: The present work shows a new methodology to evaluate HER-2 FISH faster and easier. More evaluations should be made to show inter-laboratory agreement using this methodology.

0037

The value of the immunohistochemical panel: P53 and c-erbB2 in the prognostic appreciation of the mammary carcinomas

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Objective: Background: The study analyzes the positivism of the p53 protein and the oncoprotein c-erbB2 and their implication in the appreciation of prognostic invasive mammary carcinomas.
Method: In 60 invasive mammary carcinomas, we have realized the immunohistochemical determination of the p53 protein with the avidine–biotine complex method (ABC) using the DAKO p53 mouse monoclonal antibody (dilution 1:500). In 60 invasive mammary carcinomas, we have determined immunohistochemically the c-erbB2 oncoprotein with the DAKO rabbit polyclonal antibody, human anti-protein c-erbB2 (dilution 1:200). The tumoral tissues were fixed in 10% formaldehyde and included in paraffin.
Results: Out of 60 invasive mammary carcinomas, 21 (35%) have been p53-positive, and out of 60 carcinomas, 26 (43.3%) overexpressed the c-erbB2 oncoprotein. In the studied casuistic, we did not remark any connection between the p53 expression and the overexpression of c-erbB2.
Conclusion: The presently best-known mutant gene in invasive mammary cancer seems to be the p53 tumor suppressor gene. We did not remark any connection between the p53 protein expression and the tumors' sizes, patients' ages, the clinical stage of the disease and the overexpression of c-erbB2. The usage of the c-erbB2 status (HER-2/neu) in patients suffering invasive mammary carcinomas is a predictor of therapy response.

0038

Accuracy and correlative study in the diagnosis of breast lump by fine needle aspiration cytology and ultrasonography and diagnostic reliability of combined approach: a study on 222 patients

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Objective: A large number of patients in Bangladesh have been suffering from breast cancer. The present study is done to see the accuracy of FNAC and USG in the diagnosis of palpable breast lesions and to see whether combination of the methods increase the diagnostic yield.
Method: Two hundred twenty-two female patients with palpable breast lump were included in this study to see the preoperative diagnostic accuracy of ultrasonography (USG) and fine needle aspiration cytology (FNAC) and to see their comparative performance. Histopathology was done in 89 cases.
Results: USG showed 88.89% sensitivity, 96.74% specificity, 84.21% PPV, 97.80% NPV and 95.45% accuracy, and FNAC showed 97.22% sensitivity, 99.46% specificity, 97.22% PPV, 99.46% NPV and 99.095% accuracy. Ultrasonography was found to be less sensitive, specific and accurate in the diagnosis of breast lump, though there is a highly significant ($P<0.001$) correlation.
Conclusion: The study has shown a much higher performance of FNAC than other previous studies. Only FNAC is enough for preoperative diagnosis of palpable breast lump. USG can enhance the diagnosis of breast lump. Combined approach is necessary when the breast lump is not well defined or not palpable.

0039

Accuracy and correlative study in the diagnosis of breast lump by FNAC, mammography, ultrasonography and diagnostic reliability of combined approach

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Objective: A large number of patients in Bangladesh have been suffering from breast cancer. Because of existing social circumstances, female patients are hesitant to be examined by the physicians for breast lump, so report in advanced stage. The study was done to observe and compare the accuracy of FNAC, mammography and ultrasonography in the diagnosis of palpable breast lesion and to see whether combination of the methods increases the diagnostic yield.
Method: Two hundred twenty-two patients with breast lumps were included and mammography, ultrasonography,

FNAC and histopathology were done. Sensitivity, specificity and accuracy were evaluated.
Results: Thirty-six malignant cases were seen in 222 patients, mostly occurring in older age groups. Mammography and ultrasonography showed 82.76%, 88.89%, 97.22% sensitivity, 90.36%, 96.74%, 99.46% specificity and 88.39%, 95.45%, 99.095% accuracy, respectively.
Conclusion: Mammography and ultrasonography were found to be less sensitive, specific and accurate in the diagnosis of breast, lump though there is a highly significant correlation among them. However, the study has shown a much higher performance of FNAC than other previous studies.

0041
Differential diagnosis of chondroid differentiation in breast
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Objective: There are only a few entities in the breast which show a chondroid differentiation. The differential diagnosis varies from the harmless choristoma to the also benign pleomorphic adenoma, to the syringioma and phylloides tumour with uncertain dignity to the high malignant metaplastic carcinoma. The differentiation between these different neoplasias is essential for the patient because of the following therapeutical procedures and the prognostic outcome. The final diagnosis in a mammary punch biopsy may be difficult or impossible because of the small specimen and the heterogeneity of the tumour.
Method: Our specimen is taken from a 66-year-old female. With mammography, new polymorph calcifications were found in the left breast. The results were classified as BIRADS 4 and surgical excision was recommended. One month after mammography, a stereotactic wire-marked diagnostic excision was performed.
Results: In histological examination, a chondroid proliferation appears with a beginning metaplastic ossification and two small foci of spindle cells and myxoid appearance. No suspicion for malignancy was found in the whole specimen. A chondroid choristoma in the left breast was diagnosed. In mammographic control 6 months later, no calcifications were detectable. So the tumour was totally removed.
Conclusion: A choristoma of the breast is very rare. In literature, it is described as choristoma (Tavassoli 1999) or as true chondroma (Rosen and Oberman 1993). The secured demarcation between the differential diagnosis is only possible at the in whole removed and nearly totally examined tumour. A chondroid differentiation in a mammary punch biopsy should be classified in the category B3. A diagnostic excision should be always performed.

0042
Inflammatory myofibroblastic tumour of the nipple: case report of a pregnant patient
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Objective: Extrapulmonary inflammatory myofibroblastic tumours (IMT) are rarely seen; only few cases have been reported in the breast. This report presents the first case of IMT in the nipple and highlights the histological features in this unusual site. The 31-year-old pregnant woman developed a palpable mass at the upper half of the left nipple, which started after lactation and increased in size during the present second pregnancy.
Method: Conservative surgical excision was performed 24 weeks of pregnancy with an uneventful postoperative course. There was spontaneous regression after incomplete removal.
Results: The pathogenesis of IMT is controversial. Initially, it was considered an aberrant reactive and/or inflammatory process, but some aggressive features suggested a neoplastic origin with chromosomal rearrangement in 2p22–24 regions.
Conclusion: IMT can be considered to be clonal proliferation of myofibroblasts, a true neoplasm with low malignant potential.

0043
Primary epithelioid angiosarcoma of the breast in an old male patient—case report and review of the literature
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Objective: Vascular tumors of the breast are very uncommon, consisting of variants of hemangioma and angiosarcoma. Although angiosarcoma related to chronic lymphoedema is the most frequent, it only represents 0.05% of breast tumors. Usually, primary angiosarcoma involves young pregnant females, with only six cases occurring in males reported to date. Epithelioid angiosarcoma is very unusual; their presence in males has been reported only once before in a young patient.
Method: This is the second case report of an epithelioid angiosarcoma in a male breast but the first one of poorly differentiated type. It developed in a 62-year-old patient, unrelated to hormonal dysfunction or treatment.
Results: The tumor located in the breast parenchyma had a 7-cm diameter and solid firm multinodular grey colour

areas admixed with cystic spaces with hemorrhagic content. Microscopically, the tumor was composed of mainly polygonal epithelioid tumor cells with abundant eosinophilic or pale cytoplasm and pleomorphic nuclei, arranged in solid areas and admixed with few spindle cells. Less than 25% of the tumor was composed of more differentiated areas. The tumor cells in both solid and cystic areas were positive for CD31 and vimentin but negative for CD34, ER, PR, HMB-45, and S-100 protein. Pan-Ck was positive in both epithelioid and spindle tumor cells.
Conclusion: Immunohistochemistry is very useful in order to establish a correct diagnosis; factor VIII, CD31 and CD34 can be used singly or in combination, although they may be lost in high-grade tumors. Epithelial markers may be also positive in epithelioid angiosarcoma.

Wednesday, 1 September 2010, Basement

PS-02 Poster Session Dermatopathology

0045

The semiquantitative immunohistochemical evaluation of the lymphangiogenesis density at cutaneous melanoma and its correlation with the sentinel lymph node status

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Objective: Lymphangiogenesis is a predictor of the sentinel lymph node status at cutaneous malignant melanoma. The aim of our investigation was the immunohistochemical semiquantitative evaluation of the lymphangiogenesis density and its correlation with the sentinel lymph node status. The file comprises 30 patients with cutaneous melanoma, 15 of them having the positive sentinel lymph node. The results of the investigation are partially in harmony with another study.
Method: For this investigation, the files of 30 patients with cutaneous melanoma radically excised and the sentinel lymph nodes were extirpated. The lymphangiogenesis density was immunohistochemically evaluated using CD34 and D2-40 (podoplanin). The localization of the lymphangiogenesis in cutaneous melanomas was determined and the lymphatic vessels were counted in 1 mm^2. After that, we evaluated the correlation between the lymphangiogenesis localization and the invasion depth according to Clark, following the correlation of the lymhangiogenesis density with the sentinel lymph node status.
Results: Lymphangiogenesis was dominant in the peritumorous localization of cutaneous melanomas. The lymphangiogenesis density and its localization have no influence upon the lymph node status. The correlation of the depth invasion with the lymph node status was not confirmed.
Conclusion: The results of the lymphangiogenesis localization are according to other study papers. We did not confirm the correlation between the lymphangiogenesis density and the sentinel lymph node status. It is necessary to extend the set of investigated patients with cutaneous melanoma and to use the LYVE-1 antibody. The extension of the count of patients with cutaneous melanoma and using other antibodies such as LYVE-1 are necessary in consecutive evaluations.

0046

Role of Notch signaling into cutaneous melanoma development and progression

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Objective: Melanoma is a malignant tumor of the neural crest-derived cells named melanocytes. It is an aggressive neoplasm due to its high rate of proliferation and invasion. Disruptions in the cellular signaling pathways promote melanoma development and progression. Aberrant Notch activation has been related to a variety of human neoplasms. We investigate the potential involvement of Notch signaling in melanoma development and progression. For this purpose, we analyzed the expression of three essential components of the Notch pathway: Notch1, ADAM10 and ADAM17/TACE.
Method: Melanoma samples from seven patients were tested and compared with normal skin samples. Immunofluorescent staining was performed on 5-μm-thick cryosections. Primary antibodies were applied and incubated overnight at 4°C. To detect the primary immune reaction, we used FITC-labeled goat anti-mouse secondary antibodies. The samples were examined using light and UV microscopy. For the Western blot technique, the Western-Breeze kit was used.
Results: Notch1 expression in normal skin was detected in the epidermis and also in hair follicles, sweat glands, sebaceous glands and blood vessels. Melanoma cells showed moderate to high levels of expression of Notch1. ADAM10 expression was detected in the epidermis and also in hair follicles. In some melanoma samples, ADAM 10 over-expression was observed. TACE/ADAM17 expression was detected only in the epidermis. Western blot analysis confirmed the presence of these proteins in cytoplasmatic and nuclear lysates.
Conclusion: We observed that the Notch signaling pathway is activated in melanoma cells. Critical members of the Notch pathway are potential therapy targets.

0047
P-53, BCL-2 and KI-67 expression in amelanotic melanomas

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Objective: Amelanotic melanoma is a subtype of cutaneous melanoma characterized by little or no pigment on visual inspection; it represents 2–8% of all melanomas. The lack of pigment makes it a great mimic of other benign and malignant lesions, a fact that can mislead the correct diagnosis.
Method: The study included a group of 34 cases of amelanotic melanomas, aged 17–83 years. Most of the tumors were thinner than 3 mm Breslow and Clark levels I to IV. The diagnostic was formulated based on special coloration for the melanic pigment (Masson–Fontana) and using immunohistochemical techniques (HMB-45, vim, S-100). The simultaneous positivity of these markers has shown the melanic aspect of the tumor. Bcl-2, ki-67 and p-53 was performed by standard immunohistochemical staining using monoclonal antibodies.
Results: bcl-2 expression was positive in 94% cases; ki-67 expression was positive in most of the cases, values between 3% and 68%; and p-53 expression was positive in most of the cases, values between 2% and 42%.
Conclusion: Our frequency detection is not related with the size of the tumor, depth, ulceration or mitotic activity. Most lesions present expression of bcl-2 in 90% of the cases and were associated with good prognosis; p-53 expression is between 3% and 62% and was associated with poor survival. Although ki-67 expression is between 6% and 71%, it is not related with the prognosis. It was suggested that p-53 and bcl-2 expression could be useful in predicting the biological behaviour. Neoplastic embolisations were identified in two cases of the study group.

0048
Amelanotic lentigo maligna

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Objective: We present the case of a 64-year-old woman who went to the dermatology department with a 3-year history of a slowly enlarging, erythematous plaque with central atrophy on the left cheek.
Method: The pathologic exam revealed an atrophic epidermis and proliferation of atypical melanocytes along the dermo-epidermal junction with formation of nests and an associated chronic inflammatory infiltrate with breach of the basement membrane, indicating a vertical growth phase.
Results: After many sections, the appearances were felt to be consistent with lentigo maligna melanoma to Clark level 2.
Conclusion: Despite wide local excision, the treatment of choice in amelanotic lentigo maligna recurrence is often seen.

0050
Difficulties in clinical and histopathologic diagnosis between the spindle cell melanoma and Kaposi sarcoma at the lower limb

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Objective: Desmoplastic melanomas represent an extreme degree of fibroblastic or myofibroblastic metaplasia accompanied by abundant collagen synthesis. Those tumors are in general associated with high incidence of recurrence and metastasis and a poor prognosis.
Method: A patient P.C., male, 74 years old, presents at the clinical exam a right calcaneo-plantar ulcero-proliferative and multinodular lesion (2/1, 5/0, 5 cm), with a few months' course; there is local discomfort and functional impairment. Clinical diagnostic was that of Kaposi sarcoma and for the histopathology was sent a cutaneous specimen of 12/5/3 cm that contains the lesion. The specimens were examined using basic histological techniques and immunohistochemistry with antibodies anti-S100 protein, anti-CD34, anti-desmin, Melan A, HMB-45 and pan CK.
Results: Microscopically, it was identified as tumoral proliferation with spindle cells, uniform nucleus, some of them hyperchromatic, prominent nucleoli, some tumoral cells with brown melanic pigment and a rich mitotic activity. Immunohistochemical expression was intensely positive for S100 protein and Melan A and negative for HMB 45, desmin, CD34, pan CK, associated with focal lymphocytic inflammatory infiltrate. Tumoral proliferation includes adnexal structures of the skin and is ulcerated. The histopathological aspects lead to a desmoplastic melanoma. In spite of the large excision margins sidewise and in the depth, the tumor has reappeared locally after 4 months from excision and treatment, with inguinal ipsilateral metastasis.
Conclusion: The presented facts suggest a more close attention to the clinical and histopathological differential diagnosis of the tumoral cutaneous lesions in the lower limb extremities. This could influence the surgical therapeutic act.

0052

Metastatic potential of B16 melanoma in knockout (PAR-2$^{-/-}$) animals: pilot in vivo study

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Objective: Proteinase-activated receptor-2 (PAR-2) is a ubiquitous surface molecule. It belongs to the family of G protein-coupled receptors activated by the site-specific proteolysis of trypsin. Altered function of PAR-2 has been described in different malignant tumors. In the present study, we investigated the difference of the metastatic spread of melanoma B16 applied to knockout animals in comparison with C57Bl6 mice.
Method: Knockout mice B6.Cg-F2rl1tm1Mslb/J (PAR-2$^{-/-}$) and C57Bl6 controls were inoculated by established melanoma B16 tissue cell line subcutaneously. Fourteen days after inoculation, all primary tumors were removed and histopathologically analyzed. Onemonth after, animals of both groups started to die. After the autopsy, metastatic spread of the melanoma in different organs was evaluated in both groups.
Results: Our experiment confirmed the growth and metastatic spread in both murine groups. Removed tumors differed in volume; average weight was 0.62 g in PAR2$^{-/-}$ and 0.4 g in control animals. Metastatic spread was observed in both groups and reached 80% in PAR2$^{-/-}$ and 50% in control animals. Whether in control mice, only lung metastases were observed; local tumor recurrence and renal and lung metastases were observed in PAR2$^{-/-}$ mice.
Conclusion: The absence of functional PAR-2 could be an important factor influencing the growth and spread of melanoma in vivo probably due to the process of tumor cell migration, invasiveness and metastasis formation. However, further studies on larger animal groups need to be performed to confirm our results. Acknowledgement: Partly supported by IGA MZ NS/10423-3 and grant no. 309/09/P204 of the Grant Agency of Czech Republic.

0053

Identification of t(17;22)(q22;q13) (COL1A1/PDGFB) in dermatofibrosarcoma protuberans by fluorescence in situ hybridization in paraffin-embedded tissue microarrays. Clinical and histopathological correlation in 40 cases

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Objective: Dermatofibrosarcoma protuberans (DFSP) is genetically characterized by the translocation t(17;22)(q22;q13), resulting in PDGFB/COL1A1 fusion gene. The t(17;22) translocation leads to the activation of the PDGFB receptor that offers the possibility to use inhibitors, such as imatinib mesylate (Glivec®, Novartis), as a therapeutic alternative in locally advanced or metastatic cases. Fluorescence in situ hybridization (FISH) with specific probes allows a rapid detection of this gene. The objective of the study was to analyze the presence of the translocation t(17;22)(q22;q13) by FISH in paraffin-embedded tissue microarrays (TMA) of cases diagnosed as DFSP and dermatofibromas (DF).
Method: This is a multicentric retrospective study. Two tissue microarrays (TMA), including 40 DFSP and 20 DF, were evaluated. FISH analyses were performed using a dual-color, dual-fusion non-commercial probe. Clinical and histopathological features were systematically evaluated and association with FISH results was explored.
Results: Twenty-nine DFSP and 16 DF could be successfully evaluated. Twenty-five (86%) DFSP samples were positive for the translocation, which was not present in any DF samples. Two of the negative DFSP corresponded to more undifferentiated tumors, whereas one case exhibited overlap features with DF (this case was finally considered a deeper and more cellular variant of DF). The samples included in the study could show inter-individual variations in diagnostic criteria.
Conclusion: The COL1A1/PDGFB fusion gene is present in most of DFSP. The detection of the translocation by FISH is easier, more effective and less time-consuming than other molecular techniques and may have additional diagnostic and therapeutic interest in clinical practice.

0054

Early phase of Kaposi's sarcoma in a HIV-positive young woman

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Objective: AIDS-related Kaposi sarcoma is especially observed in young men with AIDS. This tumor is more rarely observed in an infected young woman and early phase is not easily diagnosed; it could be confused with inflammatory dermatosis or other skin lesions.
Method: Our paper is a case presentation.
Results: Case report: We present the case of a 20-year-old woman, diagnosed in 1998 with HIV infection, without antiretroviral treatment. Elevenyears later, she presented multiple skin erythematous plaques on the neck, trunk and lower limb. Serum level of T helper lymphocytes (CD4)

was 42/nmc. Histological exam of skin biopsies revealed in dermis a significant proliferation of irregular, small vascular channels, covered by a single layer of atypical endothelial cells. These channels were predominately arranged around preexistent mature vessels and adnexal structures. They were placed parallel to the epidermis and were marked by CD34 antibody. With this antibody, we observed that majority of these vessels were intermediary vessels whose proliferation could be suppressed by anti-angiogenic therapy. Based on clinical, histological and immunohistochemical aspects, diagnosis of Kaposi's sarcoma in early stage was made. Chemotherapy was initiated (HAART scheme) for 6 months. After this treatment, skin plaques have disappeared and serum level of CD4 increased at 146/nmc.
Conclusion: It is very important to recognize early phase of Kaposi's sarcoma and to initiate the treatment in order to improve prognosis of this lesion in HIV-infected and also immusdepressed patients.

0055

The evaluation of expression of chosen oncogenic markers in Merkell cell carcinoma with the usage of tissue microarray and immunohistochemical methods

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Objective: Merkel cell carcinoma (MCC) is a rare, aggressive primary neuroendocrine neoplasm of the skin. Its molecular development is not clearly defined.
Method: We undertook an immunohistochemical analysis of a series of 19 cases of MCC by tissue microarray method. The tissue microarray from MCC were analysed with antibodies to E-cadherin, CD73, matrix metalloproteinase type 2 (MMP2), endoglin (CD105), anaplastic lymphoma kinase 1 (ALK1), nestin, nm23 (NME1), CDX2, the cell cycle regulator proteins (P21, P53, P63), oncoproteins (Bcl-2, BRAF, RAS and TWIST1). We also examined expression of the potential anticancer drug targets, i.e. topoisomerase II.
Results: Depending on the type of the analysed protein, the nuclear (p21, p53, topoisomerase type II), the cytoplasmic (Bcl2, BRAF) and membranous-cytoplasmic (TWIST, N-RAS) pattern of expression was regarded as positive. The expression of TWIST, Bcl 2, N-RAS, BRAF, p21 and topoisomerase II was irregular and showed no mutual correlations. The remaining proteins were not revealed in MCC.
Conclusion: We conclude that expression of topoisomerase II and BRAF may indicate potential benefit from targeted therapy against these markers.

0056

Expression of nuclear ubiquitous casein and cyclin-dependent kinases substrate (NUCKS) does not correlate with the expression of histone H1.0 in cells of the basal cell carcinoma

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Objective: Nuclear ubiquitous casein and cyclin-dependent kinases substrate (NUCKS) is a 27-kDa chromosomal protein of uncertain function. Its amino acid composition and the structure of its DNA binding domain resemble that of high-mobility group A proteins. Since changes in the expression of HMGA are considered as markers of tumor progression, it is possible that similar changes in the expression of NUCKS could be useful in the diagnosis of malignant tumors.
Method: We used specific antibodies against NUCKS and linker histone H1.0 for immunohistochemistry on paraffin-embedded samples in basal cell carcinoma. We also used, Ki67 marker, to compare with the above two markers.
Results: We found a high expression of NUCKS in nuclei of BCC cells which exceeded the expression of well-known proliferation marker, Ki67. The expression of NUCKS in cancer cells did not correlate with the expression of H1.0. We also assessed the expression of NUCKS and H1.0 in inflammatory cells, i.e. lymphocytes and plasma cells. This expression was entirely found in single cells and remained very low in all cases.
Conclusion: With the present study and based on our previous experience, we would like to suggest NUCKS as a novel tumor biomarker in the immunohistochemical evaluation of formalin-fixed and paraffin-embedded BCC specimen.

0057

Bowen disease of the eyelids

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Objective: Bowen disease is a form of squamous cell carcinoma in situ. It is a distinct clinicopathologic entity of the skin and mucocutaneous junction.
Method: We present four cases of Bowen disease of the eyelids. Three patients (75%) were men and only one was a woman (25%). The mean age was 65 years. The average age at onset of the disease was 49 years.
Results: The appearance of the disease was a single or multiple erythematous, rounded to irregular, keratotic, nodular, pigmented plaques that appeared sharply demarcated from the surrounded unaffected skin. Low-power

microscopic features are hyperkeratosis, parakeratosis, a chronic inflammatory infiltrate in the upper corium and the appearance of crowding of atypical keratinocytes, with hyperchromatism. These changes are confined by an intact dermo-epidermal basement membrane
Conclusion: Surgical excision with complete removal may cure Bowen disease.

0058
Malignant chondroid syringoma of the scalp: a case report

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Objective: Chondroid syringoma, also known as mixed tumour of the skin, is an uncommon benign sweat gland tumour. Malignant chondroid syringoma is a very rare tumour. It may occur de novo or rarely develop in a chondroid syringoma.
Method: In contrast to the benign counterpart, which is common in the head and neck region, the malignant type occurs predominantly on the trunk and extremities.
Results: We report a case of malignant chondroid syringoma located on the scalp of a 77-year-old man.
Conclusion: The diagnosis of chondroid syringoma was made by histopathologic and immunohistochemical examination.

0059
P53 and Ki67 expression in skin epithelial tumors by immunohistochemical method

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Objective: Skin cancers are one of the most common human malignancies. Although the prognosis of these patients has improved in the last decades, there is still a need for novel treatment modalities and prevention from recurrence after the treatment of skin tumors. P53 is a tumor-inhibiting gene which is believed to be defective in many malignant situations. Ki67 is a non-histonic protein which mainly interferes with the proliferation and has many controlling effects during the cell cycle. This study aimed at evaluating P53 and Ki-67 expression in skin epithelial tumors by immunohistochemical method.
Method: In a descriptive setting, 50 biopsy samples—30 basal cell carcinomas (BCCs), ten squamous cell carcinomas (SCCs), eight keratoacanthomas (KAs) and two tricoepitheliomas (TEs)—were immunohistochemically evaluated for P53 and Ki67 expression during a 14-month period. The incidence and expression rate of these two variables were separately reported in each group of samples.
Results: The expression rate of P53 was 67.77%, 50.20% and null for BCCs, SCCs and KAs, respectively. For both TEs, it was 50%. The expression rate of Ki67 was 57.33%, 47.70%, 37.5% and zero for BCCs, SCCs, KAs and TEs, respectively.
Conclusion: This study showed that the incidence rate of P53 and Ki67-positive cells are very high in malignant epithelial tumors in the skin. The expression rate of these two variables is comparable with reports in the literature. Further studies with large sample size are recommended to be carried out for KA and TE samples.

0060
Extranodal Rosai–Dorfman disease: a case report

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Objective: Rosai–Dorfman disease, or sinus histiocytosis with massive lymphadenopathy, is a rare benign, proliferative disorder that involves primarily the lymph nodes, especially in the cervical region. In almost half of the cases, synchronous extranodal sites have been reported. Isolated cutaneous extranodal Rosai–Dorfman disease can occur in <20% of the cases. We present a case of extranodal sinus histiocytosis in the subcutis of the lumbar region.
Method: We report the case of a 56-year-old female who was diagnosed with breast cancer two2 years ago and underwent mastectomy and chemotherapy. In a follow-up examination, a subcutaneous nodule was found and was surgically removed.
Results: We received a well-circumscribed, white-yellow elastic nodule measuring 1.3 cm in maximal diameter, surrounded by fatty tissue. Histologically, the nodule consisted of solid nests of large cells with abundant eosinophilic cytoplasm and large "ground glass" nuclei with prominent nucleoli. Mitoses were rare and no atypia or pleiomorphism was observed. Immunohistochemically, the cells were positive for vimentin, S-100 protein and CD68 (KP1 and PGM1). Among these cells, scattered multinucleated giant cells and inflammatory infiltrates were also found.
Conclusion: It should be pointed out that Rosai–Dorfman disease is a non-neoplastic condition; thus, it does not require additional therapy. Surgical excision is the treatment of choice. Differential diagnosis includes other histiocytic lesions, such as histiocytosis X and epithelioid mesenchy-

mal neoplasm, mainly of neurogenic origin. The case of a metastatic melanoma should always be ruled out.

Wednesday, 1 September 2010, Basement

PS-03 Poster Session Nephropathology

0062

Membranous glomerulopathy with anti-neutrophil antibodies (ANCA)-associated crescentic glomerulonephritis. An unusual case of dual glomerulopathy

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Objective: The coexistence of anti-neutrophil crescentic glomerulonephritis with membranous glomerulopathy is very rare. Little is known about the clinical course, treatment and outcome of patients with concurrent coexistence of these nephropathies.
Method: We present the case of a 58-year-old man with rapidly progressive renal failure, anti-neutrophil perinuclear antibodies positivity, pulmonary infiltrates and vasculitis.
Results: The patient was admitted to the pulmonary department due to febrile state, weakness and weight loss. Physical examination revealed fine crackles over the left lung, leg edema, and vascular skin lesions on inferior extremities. Urinalysis showed proteinuria and hematuria with many RBC casts, and in a 24-h urine collection, the proteinuria of 2 g was observed. Serologic workup was positive for p-ANCA, negative for c-ANCA, ANA, and anti-GBM antibodies and showed normal levels of C3 and C4. HCV, HBV and HIV infection were excluded. In lab tests, the rapid increase of serum creatinine from 1.3 to 5.17 mg/dl over 2 weeks was observed. Chest CT revealed pulmonary infiltrates of left lung upper lobe. The patient underwent a kidney biopsy which showed necrotizing crescentic glomerulonephritis and membranous glomerulopathy. In light microscopy examination, glomeruli with necrotic lesions and cellular crescents were seen. Electron microscopy revealed epimembranous and intramembranous deposits. The patient was treated with steroids; cyclophosphamide and seven plasmapheresis were performed with improvement in renal function. On 1-year follow-up, his proteinuria had decreased to 300 mg/day and his creatinine had stabilized at 1.6 with eGFR of 56 ml/min.
Conclusion: In conclusion, this case illustrates membranous nephropathy superimposed with ANCA-associated crescentic and necrotizing glomerulonephritis. (Supported by grant of Polish Ministry of Science and Higher Education No. N402 088735).

Wednesday, 1 September 2010, Basement

PS-04 Poster Session Pulmonary Pathology

0066

AKT and PTEN in non-small cell lung carcinomas—acting together or apart?

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Objective: In the last years, the study of predictive and prognostic markers of non-small cell lung carcinomas (NSCLC) has been a continuous challenge. It is well known that the imbalance between AKT and PTEN expressions plays an important role in cell proliferation, angiogenesis and tumor cell invasiveness in various solid malignancies. The aim of our study was to analyze the AKT and PTEN expressions in primitive non-small cell lung carcinomas and, also, in their metastases.
Method: We have examined AKT and PTEN immunohistochemical expressions in 64 primitive non-small cell lung carcinomas with different stages and in ten metastatic non-small cell lung carcinomas: brain, adrenal glands and lungs.
Results: AKT expression was higher in metastatic than in primitive non-small cell lung carcinomas, while PTEN was over-expressed in 98% of all tumors. There were no obvious associations between tumor stage and AKT/PTEN expression. There were no correlations between PTEN over-expression and AKT expression.
Conclusion: AKT activation may play an important role in tumor cell invasiveness in non-small cell lung carcinomas, but PTEN suppressor gene may not be involved in AKT activation.

0067

miR-21 overexpression in pulmonary adenocarcinomas and squamous cell carcinomas

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Objective: The role of miRNAs in cancer is a rapidly emerging area of investigation. Expression profiling has

identified miRNA signatures in cancers that associate with the diagnosis, staging, progression, prognosis, and response to treatment. MiRNAs are ideal biomarkers in FFPE tissue because unlike mRNA, miRNA integrity is affected very little by formalin fixation. Previous studies have shown that miR-21 overexpression correlated with poor prognosis in NSCLC patients. In this study, we investigated the expression of mir-21 in primary carcinoma and metastasis and near non-tumor parenchyma.

Method: FFPE samples from surgical specimens and biopsies of seven pulmonary adenocarcinomas and five squamous cell carcinomas and respective metastasis together with normal lung tissue (alveolar and bronchial) from the same case; it was imperative to separate these well-characterized areas by laser-capture microdissection (LCM) prior to RNA analysis.

Results: The expression level of miR-21 by qRT-PCR was significantly higher in tumor tissues than in adjacent normal tissues ($p=0.005$). The overexpression in the metastasis samples compared to adjacent normal tissue was almost statistically significant ($p=0.051$).

Conclusion: miR-21 was overexpressed in tumor tissues relative to adjacent non-tumor tissues. We found an increase in miR-21 expression in primary carcinoma and metastasis in pulmonary adenocarcinomas when compared with miR-21 lower expression in squamous cell carcinoma. Despite the small sample studied, further investigation may indicate the therapeutic and prognostic relevance of this determination as previous studies suggest that miR-21 acts as an oncogene and has a role in tumorigenesis through the regulation of tumour suppressor genes.

0068

EGFR and KRAS mutations in mixed type of pulmonary adenocarcinoma

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Objective: The EGFR and KRAS genes act sequentially in the MAPK signaling pathway. The KRAS gene is located downstream in the transduction of signals transmitted from the transmembrane receptor EGFR to the nucleus through a series of intermediate genes. Patients with non-small cell lung cancer (NSCLC) with EGFR mutations are more sensitive to treatment with tyrosine kinase inhibitors (TKIs) without KRAS mutations. The different patterns recognized in adenocarcinomas were microdissected to identify mutations in both EGFR and KRAS in order to clarify their significance when selecting patients either to TKIs.

Method: Histological sections of 31 adenocarcinomas of the lung, FFPE, were selected to analyze EGFR exons 19 and 21 mutations and KRAS codons 12 and 13. DNA was extracted for polymerase chain reaction (PCR). Exon 19 was studied by fragment analysis; exon 21 and codons 12 and 13 were studied by direct sequencing.

Results: From the 31 samples studied, 10 of 31 showed in-frame deletions, 4 of 20 L858R substitution in the EGFR, and 5 of 31 point mutations in codon 12 of KRAS. In all cases, mutations were exclusive for EGFR and KRAS in alternative. Only one mixed adenocarcinoma showed EGFR in-frame deletions in bronchiolo-alveolar pattern and Wt in acinar pattern (KRAS Wt).

Conclusion: In this set of mixed-type adenocarcinomas, the different histological patterns revealed to be inconsequent for KRAS and EGFR determination of mutations, reinforcing the technical feasibility and reliability of small biopsies of lung cancer to determine personalized therapy.

0069

MLH1 and MSH2 methylation status in pulmonary adenocarcinomas and epidermoid carcinomas

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Objective: Five-year survival of lung cancer is 16% and in surgical stages increases to 50% as 14–16% comprise this group. DNA methylation emerged as potential cancer-specific biomarker, and although hypomethylated, DNA can become aberrant or hypermethylated in CpG-rich areas or near promoter region of tumour suppressor genes, inhibiting apoptosis and favouring angiogenesis, metastization and DNA miss repair, resulting silencing genes of cellular homeostasis and renewal.

Method: Methylation profile and possible silencing of DNA repair genes—MLH1 and MSH2—by methylation-specific PCR and protein expression, in tumoral tissue, preneoplastic lesions when available, respiratory epithelium and parenchyma with normal histological features were searched in 40 squamous cell carcinomas (SCC) and 40 adenocarcinomas in surgical TNM staging.

Results: IHC expression of MLH1 and MSH2 in SCC and adenocarcinoma, in hyperplasia, metaplasia and in normal cylindrical respiratory epithelium appeared reduced. The frequency of promoter hypermethylation of these DNA repair genes was elevated, with a higher prevalence of methylation of MLH1 in SCC. The differences are not so obvious for MSH2 promoter region hypermethylation. No statistical correlation was defined between the status of methylation, protein expression and the clinicopathological characteristics (age, gender, TNM stage).

Conclusion: This research made in surgical specimens may have future impact on the potential characterization of gene hypermethylation in small biopsies and preneoplastic lesions in assessing the prognosis/progression of lung cancer as it was developed in FFPE tissue. It was also demonstrated that methylation status and gene IHC expression have to be enrolled in different pathways.

0070

Nuclear magnetic resonance spectroscopy applied to small samples of bronchial–pulmonary carcinomas identifies metabolic profiles

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Objective: Routine diagnosis and prognostic methods may also be useful to add data to clinical information in advanced primary carcinomas if conveniently explored. The evaluation of the potential of ^{1}H NMR spectroscopy for providing biochemical information of the different histological types and for discriminating between tumour and pulmonary parenchyma as well as biochemical information about different histological types was tried as a preliminary approach.
Method: Small frozen samples of 24 pulmonary tumours were collected together with macroscopically normal parenchyma from surgical specimens before histological classification and mirror tissue was FFPE. Paired cases were directly analysed by high-resolution magic angle spinning (HRMAS) ^{1}H NMR spectroscopy (500 MHz). The spectral profiles obtained were subjected to multivariate analysis: principal component analysis (PCA) and partial least squares regression discriminant analysis (PLS-DA) with the predictive ability of the statistical models.
Results: Tumor and control tissues were clearly discriminated in the PLS-DA model with high level of sensitivity (95% of tumor samples correctly classified) and 100% specificity (no false positives). The metabolites giving rise to this separation were mainly lactate, GPC, PC, taurine, glutathione and UDP/UTP (elevated in tumors), and glucose, phosphoethanolamine (PE), acetate, lysine, methionine, glycine, myo- and scyllo-inositol (reduced in tumors compared to control tissues). Furthermore, PLS-DA allowed carcinoids to be discriminated from adenocarcinomas and epidermoid carcinomas.
Conclusion: This preliminary study raised the NMR profile between the different histological types of bronchial–pulmonary carcinomas through multivariate analysis and scaled metabolic profiles that may be delineated to improve clinical decisions in future.

0071

Correlation of the epithelial–mesenchymal transition markers with clinicopathologic features and the EGFR copy number status in non-small cell lung carcinomas

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Objective: The aim of this study was to investigate the expression of the epithelial–mesenchymal transition (EMT)-related factors (vimentin, E-cadherin, beta-catenin, twist1, slug, and snail) in non-small cell lung carcinoma (NSCLC). Association of these proteins with clinicopathologic parameters and EGFR gene amplification status was also analyzed.
Method: A total of 60 squamous cell carcinomas (SQ), 48 adenocarcinomas (AD), four sarcomatoid carcinomas, one large cell carcinoma, and one mucoepidermoid carcinoma was studied using immunohistochemistry for EMT markers and automated silver-enhanced in situ hybridization for the evaluation of the EGFR copy number.
Results: In a 114-sample NSCLC series, expression of vimentin was negatively associated with expression of E-cadherin (P=0.002) and positively correlated with expression of twist1 (P=0.022). Positivity of EGFR gene amplification was associated with a decreased expression of E-cadherin in NSCLC (P=0.037). In the 114 sample NSCLC series, 48 AD samples showed a positive correlation between vimentin and slug expression (P=0.034). On the other hand, a positive correlation between twist1 and vimentin expression was observed in 60 SQ samples from a total of 114 NSCLC samples (P=0.002). A positive correlation trend between tumor size and vimentin expression was identified for SQ (P=0.032). Expression of vimentin was significantly higher in AD than in SQ (P=0.027).
Conclusion: These results indicate that positive vimentin expression contributes to SQ tumor growth and that different expressions of the EMT phenotype in AD and SQ are implicated in different EMT pathways, resulting in new perspectives for therapeutic targeting of these tumor types.

0072

Prognostic significance of the expression of the RNA-binding protein HuR in stage I and II lung adenocarcinoma

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Objective: The RNA binding protein HuR can stabilize and/or regulate the translation of target mRNAs affecting

cellular responses. The expression of HuR is increased in several cancers, and cytoplasmic immunoreactivity was found to be closely related to poor outcomes.
Method: We analyzed, by quantitative immunohistochemistry, the expression of HuR in 54 lung adenocarcinoma of mixed histologic type from stage I and II patients. Moreover, we evaluated the level of HuR expressed both in the nucleus and cytoplasm of tumor cells (t-HuR) and evaluated the impact of nuclear/cytoplasmic ratio (N/C) on clinical outcome.
Results: Both t-HuR and N/C were not associated with age, tumor diameter and histopathological grading, whereas high t-HuR and low N/C were associated with lymph node involvement at presentation. Cox's regression analysis, using either t-HuR or N/C as continuous covariates, showed that high t-HuR and low N/C were associated with the risk of death and metastasis. The plots of the estimates, at 5-year follow-up, of metastasis-free and overall surviving as a function of t-HuR and N/C levels, showed that the increase of t-HuR and the decrease of N/C were associated with an increase of risk. In the multivariate analysis, both t-HuR and N/C retained an independent prognostic significance relative to metastasis-free and overall survival.
Conclusion: In conclusion, these data indicate that the over-expression of HuR in lung adenocarcinoma cells is an independent prognostic marker of a poor clinical outcome. Moreover, the cytoplasmic localization of HuR expression is more relevant than the nuclear one for the tumor cell behavior.

0074

Clinical features and prognostic implications of EML4-ALK fusion gene in resected non-small cell lung cancer

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Objective: The EML4-ALK fusion gene has been detected in 5–7% of non-small cell lung cancers. We evaluated the incidence, prognosis and the characteristics of ALK-rearranged non-small cell lung cancers and the optimal diagnostic modality to detect ALK rearrangements in routine clinical practice.
Method: Seven hundred seventy-nine surgical specimens of non-small cell lung cancer from two institutions were analyzed. To identify ALK rearrangements, immunohistochemistry and fluorescent in situ hybridization (FISH) examination were performed and compared. The clinicopathologic characteristics of tumors with and without ALK rearrangements were analyzed. EGFR and KRAS mutations were determined by DNA sequencing.
Results: We identified 49 (6.9%) non-small cell lung cancer with ALK rearrangements within our cohort by immunohistochemistry. Forty-three (87.8%) of the 49 EML4-ALK tumors were adenocarcinomas. ALK rearrangement was associated with never smoking ($p<0.001$) and female ($p < 0001$). Patients in the EML4-ALK fusion gene in adenocarcinoma had no effect on progression-free survival and overall survival. Immunohistochemical results were correlated with FISH results.
Conclusion: The patients most likely to harbor EML4-ALK are never smokers with adenocarcinoma. But EML4-ALK was not an independent prognostic factor. Immunohistochemical staining for ALK could be used as a screening method to detect ALK gene rearrangement.

0075

Primitive malignant lung tumors at the Emergency University Hospital Bucharest

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Objective: Lung cancer is one of the most common malignant tumors worldwide, with a growing incidence. In Romania, lung cancer has a prevalence of 8% and an incidence of 17%; in terms of deaths from cancer, it ranks first place in males and fourth place in females.
Method: We analyzed the malignant lung tumors from January 2006 to February 2009 using information obtained from observation sheets of Thoracic Surgery Clinic and the database of the Pathology Department of the University Emergency Hospital Bucharest. Distribution of cases was followed depending on age, environment, gender, living and working conditions (smoking, toxic, radiation), tumor stage and histological appearance.
Results: The analysis of these data shows that most lung tumors occurred in the fifth decade of age and is more common in men. Regarding histological type and age, the most common tumors were moderately differentiated squamous carcinomas (the decade IV, V, VI and VII). Four patients were hospitalized for another disease (inguinal hernia, uterine fibroma) and were subsequently transferred to the clinic for thoracic surgery for specialist treatment.
Conclusion: Physical examination, identifying the factors contributing to lung cancer appearance and its diagnosis in early stages, as well as an adequate communication between specialists, may contribute to the decrease of mortality as well as socioeconomical costs.

0076

Impact of the excision repair cross-complementation group 1 predictive biomarker on toxicity and quality of life in advanced non-small cell lung cancer patients randomized in a large, multicenter, cisplatin-based phase III trial

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Objective: We recently demonstrated that clinically applicable immunohistochemical evaluation of ERCC1-status by semiquantitative H-score is a promising predictive biomarker in advanced NSCLC receiving first-line, platinum-based chemotherapy (Vilmar et al. Ann Oncol, 23-03-2010, Epub ahead of print). However, current correlation of ERCC1 status with toxicity and quality of life (QOL) is unclear.
Method: To address this point, 443 patients with advanced NSCLC were enrolled in a multicenter phase III trial and randomized to triplet chemotherapy (cisplatin+paclitaxel+gemcitabine) or standard doublet regimen (cisplatin+vinorelbine). Immunohistochemistry for ERCC1 status was performed by *H* score (immunostaining intensity × % positive tumor cells) on bioptic material and then correlated with toxicity and patient-reported QOL.
Results: Two hundred sixty-four NSCLC samples were assessable/representative for ERCC1 status. Patients with ERCC1-negative tumours, especially those with adenocarcinomas, showed significantly improved outcome. However, the entire population of ERCC1-negative tumour patients displayed numerically more toxicity, reaching significance for leukopenia (P=0.015), nausea/vomiting (P=0.040) and neurotoxicity (P=0.037) in adenocarcinoma patients. Mean change in the entire population's QOL was −13.33 (ERCC1-negative, P=0.001) and −2.25 (ERCC1-positive, P=0.607) and in adenocarcinoma patients −14.86 (ERCC1-negative, P=0.006) and 0 (ERCC1-positive).
Conclusion: QOL significantly deteriorated in patients with otherwise survival-favourable ERCC1-negative NSCLC, especially adenocarcinomas, possibly due to increased toxicity. Thus, careful patient selection and prospective validation of ERCC1's predictive value to prove a true survival benefit before clinical implementation are needed.

0077

Multiple pulmonary macronodular lesions in patients with primary lung carcinoma

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Objective: The neoplastic multiple pulmonary macronodular lesions (MPML) predominantly result from lung metastases of primary extrapulmonary localisation of the tumor—kidney, colon, prostate, etc. Some types of lung carcinomas are with similar radiothoracic presentation in multiple cases. Goal: To evaluate patients with primary lung cancer presenting with MPML as first manifestation.
Method: Materials: 16 patients with MPML were studied for the period 2002–2009. A primary extrathoracic localisation of the tumour was searched in all cases, but such was not found. Methods: medical history, physical status, chest radiography (lesions with diameter >3 mm were evaluated as macronodular), computed tomography (CT), fiberoptic bronchoscopy (FBS) with transbronchial lung biopsy (TBLB), morphological methods: routine staining with hematoxylin–eosin, periodic acid Schiff (PAS), Alcian blue; immunohistochemical methods: thyroid transcription factor-1 (TTF-1), Ki-67, cytokeratin AE1/AE3, EMA, carcinoembryonic antigen (CEA).
Results: From the studied patients, six were males and ten were females, with age varying between 26 and 70 years. MPML were established on the chest radiography and thorax CT in all patients. In 15 of them, the morphological diagnosis was proven by FBS and TBLB and in one with thoracotomy and lung excision. From the studied 16 patients, 12 were with diffuse bronchioloalveolar carcinoma (BAC). It is interesting to note that in three cases, adenoid cystic carcinoma (ACC) was found and in one squamous cell carcinoma (SCC). The immunohistochemical studies confirmed the primary lung localisation of the tumours.
Conclusion: The primary lung carcinoma may present initially with MPML, and in this case, it is predominantly diffuse BAC or ACC and, rarely, SCC.

0078

Increased number of lymphoid follicles in emphysematous patients with α1-antitrypsin (AAT) deficiency

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Objective: Lung damage in emphysema has been traditionally attributed to an imbalanced proteases/antiproteases, especially in α1-antitrypsin deficiency (AATD). However, activation of lymphocytes with organisation in lymphoid follicles (LFs) seems to play a crucial role in disease pathogenesis. The aim of this study was to quantify the number and distribution of LFs in native lungs from patients transplanted for emphysema.

Method: We compared two groups of patients with either AATD (n=9) or normal levels (AATN, n=27). Bronchiolar, perivascular and parenchymal LFs were evaluated on sections immunostained with anti-CD20.Results were expressed as LF number per square centimeter of examined tissue and, for bronchiolar LFs, as percentage of airways with LFs.
Results: LFs, almost absent in normal lungs from donors, were frequent in subjects with severe emphysema both with and without AATD. LF total number was increased in AATD patients as compared to AATN, and this was mainly due to a higher number of parenchymal LFs (median, range: 6, 2–12 vs 2, 0–6 LFs/cm^2, p=0.0002). Perivascular and bronchiolar LFs were numerically increased in patients with AATD (5, 0–24 vs 3, 0–8 LFs/cm^2; 33, 20–100 vs 20, 0–60%), but did not reach the levels of statistical significance.
Conclusion: LF number is increased in patients with end-stage emphysema, particularly in those with AAT deficiency. These results extend our knowledge of disease pathogenesis, suggesting that lymphocyte activation is a crucial event even in AATD-related emphysema, and the excessive lung damage observed in these patients may trigger the persistence of the immune response, possibly with autoimmune mechanisms.

0079
Pulmonary lymphomatoid granulomatosis (LYG): a case report

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Objective: (LYG) is a rare angiocentric lymphoproliferative process predominantly affecting the lung. The diagnosis of this condition is often difficult as the physical signs, history, chest X-ray, and routine laboratory investigations are usually nonspecific. Nevertheless, it is important to establish a tissue diagnosis as this lymphoproliferative disorder can be refractory to treatment and even progress to overt lymphoma.
Method: One case of pulmonary LYG in a 52-year-old Nigerian man of Ibo extraction treated in our centre in 2001 and followed up for a year is presented. The difficulty in making diagnosis is highlighted and treatment modality discussed.
Results: Our patient received two courses of cyclophosphamide and prednisolone before defaulting. There was evidence of some remission, even though incomplete, following the first course of chemotherapy (Fig. 4). However, his representation a year later with evidence of relapse was a pointer to the ongoing process of the lesion. Whether this case had progressed to lymphoma was difficult to say as he declined a readmission and further investigation.
Conclusion: A subset of angiocentric lymphoproliferative process including LYG shows clinical response to cytotoxic chemotherapy and steroid when commenced early, as is partly true here. Lymphomatoid granulomatosis should be considered in long-standing nodular pulmonary lesion and pleural effusion of uncertain aetiology.

0082
Immunohistochemical study about pathogenesis of acute respiratory distress syndrome

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Objective: Despite modern treatments, the prognosis of acute respiratory distress syndrome (ARDS) remains unfavorable and its pathogenesis is not explained enough. In few recent studies, the pathogenetic and prognostic role of vascular endothelial growth factor (VEGF) and its receptors was investigated in ARDS, but the obtained results were controversial.
Method: We have compared the immunohistochemical expression of CD68, VEGF-A and its receptors (VEGFR1and VEGFR2) in normal lung (n=10) and also in early and late ARDS (within 48 h, respectively, after 4 days). Cases with ARDS were necroptic cases (n=50), and also lung biopsies (n=12). LabVision antibodies were used. Serum level of VEGF was also determined.
Results: In normal lung, all antibodies marked the endothelial and alveolar cells and also macrophages. In early ARDS, the intensity of VEGF decreased in both endothelial cells and alveolocytes, but increased in hyaline membranes (HM) after 2–3 days. VEGR1 marked the preserved alveolar cells and VEGFR2 marked alveolar macrophages. HM were negative for both receptors. In late ARDS, down-regulation was more prominent for all antibodies. Serum level of VEGF was up-regulated at the same time with decreasing VEGF intensity in lung cells, and also with HM appearance. All dyed patients presented HM, independently by phases of ARDS. CD68 marked alveolar macrophages and sometimes HM.
Conclusion: The formation of HM in ARDS showed an unfavorable prognosis. Strong intensity of VEGF and the inconstant presence of CD68 in HM proved that both destroyed alveolar cells and macrophages are responsible for VEGF production, but the increased serum level of VEGF also proved its serum extravasations in HM.

0084

Optimalization of flow cytometric assessment of TNF 945 in alveolar macrophages using multiparametric analysis

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Objective: Alveolar macrophages (AMs) contribute to the pathogenesis of different lung diseases by producing pro-inflammatory cytokines. Within the frame of the project, which is mainly concerned with cytokine expression in bronchoalveolar lavage fluid (BALF) of patients with idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease, we optimized the flow cytometric (FC) examination of TNFα expression in AMs. The aim was to determine spontaneous TNFα production, the production during the culture and after stimulation with lipopolysaccharide. Furthermore, we focused on antibody selection since AMs displayed high level of autofluorescence.
Method: We examined BALF obtained from control patients. TNFα production was assessed immediately after BALF delivery, after 5-h stimulation with lipopolysaccharide (monensin, protein transport inhibitor was added after 2 h of incubation) and also after incubation with monensin only. Simultaneously, various concentrations of lipopolysaccharide were tested (0.5, 1 and 2 μg/ml). FC detection of AMs was simplified using multiparametric analysis, a combination of optical parameters along with the expression of surface markers CD14 and CD45.
Results: Concentration of 1 μg/ml of LPS was found to be optimal for AMs activation, although all tested concentrations are suitable. The high background autofluorescence of AMs can be efficiently reduced by the use of antibody conjugated with long-wave emitting fluorochrome, e.g. APC.
Conclusion: Optimalized FC protocol enables us to analyze TNFα production of AMs in BALF obtained from the patients. Supported by IGA MZ NS/10423-3 and Research Project MZO FNM 2005/6704.

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PS-05 Poster Session Hematopathology

0087

Chronic lymphoproliferative diseases associated with chronic hepatitic viral infections

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Objective: Romania remains an area with increased incidence of hepatitic viruses (8.5 per 100,000 for HBV in 2004). Our aim was to analyze the distribution of chronic lymphoproliferative diseases (CLPD) in patients with chronic HBV, HCV, HDV infections.
Method: We studied 28 extramedullar biopsies and 29 bone marrow biopsies from 29 patients diagnosed with chronic viral hepatitis (HVC 44.8%, HBV 34.5%, HBV+HDV 17.2%, and HCV+HBV 3.5%) and suspected of CLPD.
Results: We identified 26 cases (89.6%) of B cell non-Hodgkin lymphomas (including diffuse large B cell lymphoma, marginal zone lymphoma, B cell chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma grade 1, mantle cell lymphoma), two cases (6.9%) with peripheral T cell lymphoma unspecified and one case (3.5%) of mixed cellularity Hodgkin lymphoma. If the percentage of diffuse large B cell lymphomas (34.4%) was compared to that of patients without chronic hepatitic viral infections, we noted an increased percentage of patients with marginal zone lymphoma. Diffuse large B cell lymphoma was associated with all types of chronic hepatitic viral infections, whereas marginal zone lymphoma was more frequently associated with HCV.
Conclusion: Chronic hepatitic viral infections associate more frequently with certain subtypes of CLPD, especially marginal cell lymphoma; at the same time, chronic hepatitic involvement poses serious therapeutic challenges.

0088

Significance of bone marrow fibrosis in monitoring multiple myeloma

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Objective: The aim of the present study was to contribute to the understanding of the importance of evaluating bone marrow biopsy (BMB) by computer-assisted digital image analysis (CIA). Moreover, the intention was to investigate the frequency of marrow fibrosis in multiple myeloma (MM) and to correlate it with plasma cell infiltration during the follow-up of patients.
Method: Bone marrow biopsy from 31 patients diagnosed with MM were analyzed at the time of diagnosis and reevaluated after the completion of the therapy. The percentage of plasma cells was estimated on immunohistochemically stained paraffin sections with anti-CD138 and Ig light chains by Alphelys Spot Browser 2 integrated system. The fibrosis was analyzed according to the European consensus on grading bone marrow fibrosis.

Results: Plasma cell percentage at the time of diagnosis ranged from 3% to 97% (median 70%) and in follow-up from 5% to 100% (median 34%). Increased fibrosis, which appeared to be restricted to areas of severe plasma cell infiltration, was seen in 26% of BMB at first diagnosis. In a follow-up study, the percentage of plasma cell infiltration decreased in 16 of 31, increased in 8 of 31 and remained nearly equal in 7 of 31 MM patients. All patients with disease progression after first-line therapy had increased fibrosis in BMB. The degree of fibrosis correlated with plasma cell percentage, clinical stage and response to therapy.
Conclusion: In all cases of MM, a BMB is essential at diagnosis as it helps identify a subset of myeloma patients with increased marrow fibrosis and response to therapy.

0090

CD 20 expression in B cell lymphomas

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Objective: The role of CD20 expression in the deciding for the treatment of B cell lymphoma patients with rituximab has not been well established yet. The aim of our study was to determine the CD20 antigen expression in B cell lymphomas and compare it to reactive lymphocytic proliferations.
Method: Cytological samples of 65 diffuse large B cell lymphomas (DLBCL), 56 follicular lymphomas (FL), 31 chronic lymphocytic leukaemias (CLL), 34 mantle cell lymphomas (MCL), 18 marginal zone lymphomas (MZL), 15 B cell lymphomas unclassified and 70 reactive lymphocytic proliferations (RLP) were analysed for CD20 expression by quantitative four-color flow cytometric measurements using FACSCalibur flow cytometer (BD Biosciences).
Results: The median CD20 expression in RLP was 50,657 MESF (range 14,175–251,757) and 64,936 MESF (range 2,737–679,577) in B cell lymphomas. When compared to RLP, CD20 expression was significantly higher in DLBCL ($p<0.01$) and FL ($p<0.001$), but significantly lower in CLL ($p<0.0001$). On the contrary, when CD20 expressions in DVBL, FL, MLC, MZL, KLL and B cell lymphomas unclassified were compared, it was found to be significantly lower ($p<0.001$) only in CLL and did not significantly differ from other lymphoma types ($p=$NS).
Conclusion: CD20 expression seems very similar in most histological types of mature B cell lymphomas. However, its range was very broad, varying from very low or even null to very high. Therefore, further studies should be carried out to establish at which level of CD 20 expression could rituximab be used as an effective treatment.

0091

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL): a challenging case of histologic transformation to large B cell lymphoma

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Objective: Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare distinct histopathologic entity with a favorable prognosis despite a tendency for recurrence. Histologic transformation (HT) to diffuse large B cell Lymphoma (DLBCL) occurs in <10% of cases.
Method: A 16-year-old boy presented with a 12-month history of left inguinal and iliac lymphadenopathies. Disease staging including CT, ultrasonography, biopsies of nasopharynx, tonsils, bone marrow and myelogram was negative. TEP-CT was positive. A surgical inguinal lymph node excision was performed.
Results: The 4.5 × 2 × 1-cm lymph node had a nodular architecture. The nodules were surrounded by thin collagen bands and composed of predominantly large tumor cells of centroblastic morphology and occasional cells with Reed Sternberg-like features within a background of small lymphocytes with admixed histiocytes, but without eosinophils or plasma cells. Tumor cells were strongly positive for CD20, CD79a, IgD and BCL6, focally positive for EMA and expressed B cell transcription factors BOB1 and OCT2. CD30 was also expressed, but CD15 and MUM1 were negative. The background was composed predominantly of small $CD3^+$ $PD1^+$ T cells. No follicular dendritic cell network or EBV was detected. This observation raised the problem of differential diagnosis between NLPHL with increased number of large cells and large B cell lymphoma. The latter was favored based on morphologic and immunophenotypic features. A chemotherapy according to LMB 2003 protocol led to the regression of lymphadenopathies with TEP-CT negativity.
Conclusion: Diagnosing HT of NLPHL may be challenging. Further studies are needed to understand the molecular pathways implicated in this transformation.

0092

Lymphoma: an overview over an eleven-year period

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Objective: Lymphoma is the commonest haematological malignancy in the developed world. We undertook an audit of all laboratory reports from the Pathology Department of Beaumont Hospital that confirmed or suspected a diagnosis of lymphoma over an 11-year period, assessing the following: incidence of lymphoma by age at presentation and gender, site of presentation, type of lymphoma diagnosed, number of cases utilising immunohistochemistry, number of cases where bone marrow staging was performed and the results.
Method: Six hundred forty-seven cases were retrieved from the laboratory database where the histology report provided a definitive diagnosis of lymphoma or strongly suspicious for lymphoma. Seventy-seven were diagnosed as Hodgkin's lymphoma and 519 diagnosed as non-Hodgkin's lymphoma, with 51 cases strongly suspicious for lymphoma.
Results: The study showed an average age of 58.08 years at presentation, with incidence of lymphoma increasing with age and peaking at an age range of 61–70 years, reducing thereafter. The highest incidence of Hodgkin's lymphoma occurred in the age ranges 16–20 and 31–35 years. Incidence of lymphoma was similar in both genders. B cell lymphoma had the highest incidence, followed by Hodgkin's lymphoma. Three hundred thirty-one cases had a nodal site of presentation, 240 were extranodal, with the remaining from uncertain site (site labelled, for example, mediastinal mass). Brain was the most common extranodal site with 65 cases. Immunohistochemistry was performed in 92% of cases. Six percent of cases had bone marrow involvement.
Conclusion: The incidence of lymphoma for 11 years in Beaumont hospital is mostly consistent with the epidemiology established by the WHO.

0093

MMP9 immunohistochemical expression by Hodgkin–Reed–Sternberg cells as a prognostic marker in young patients with classical Hodgkin lymphoma

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Objective: To identify differences in gene regulation according to the status of Epstein–Barr virus (EBV) infection in classical Hodgkin lymphoma (cHL), we compared the expression profiles of three EBV-negative (L-428, L-1236, KM-H2) and one EBV-positive (L-591) Hodgkin lymphoma (HL) cell lines. We observed that 756 genes are significantly up- or downregulated in the EBV-negative cell lines as compared to the EBV-positive cell line.
Method: For four of the differentially expressed genes (caspase-1, caveolin-1, CCL20, and MMP9), immunohistochemical validation was performed in a tissue microarray (TMA) containing 148 cHL cases and the results compared to EBV infection status and patient outcome.
Results: Only CCL20 expression by Hodgkin–Reed–Sternberg (H-RS) cells was associated with EBV infection ($p<0.0001$). On the other hand, caspase-1 and MMP9 expression by H-RS cells significantly associated with lower disease-specific survival rates in patients between 15 and 45 years old, and the expression of MMP9 by neoplastic cells emerged as an independent factor of unfavourable prognosis.
Conclusion: These results suggest the ability of H-RS cells to explore different signaling pathways, regulating different genes according to EBV infection status. Of these, CCL20 protein expression was shown to be specifically associated with EBV infection in cHL cases. We also observed the expression of other proteins by H-RS cells, of which MMP9 might be promising as an independent prognostic factor for this group of patients. (Supported by FAPESP and CNPq).

0094

ERK2 and BCL2 proteins in myeloproliferative neoplasms

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Objective: MPNs consist of polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF). Though a number of clinical and diagnostic factors have been determined, the current classification in MPNs is still not satisfactory. All MPNs are characterized by JAK2 tyrosine kinase (V617F) mutation related to the deregulation of intracellular signalling pathways, e.g. RAS-ERK and PI3K-AKT, while BCL2 negatively regulates the PI3K-AKT pathway. The aim of our study was to attempt to indentify the presence and distribution of ERK2 and BCL2 expression as factors which might distinguish each MPN.
Method: Expressions of ERK2 and BCL2 were investigated (immunohistochemistry with EnVision and DAB) on trephine samples in PV, ET and PMF patients. Anti-ERK2 antibodies were directed to the epitope corresponding to a site near the C-terminal of these molecules. BCL2 antibodies bind an epitope located between amino acids 1–205 of human BCL2. Finally, sections were studied under light microscope and evaluated using a semiquantitative method.
Results: There were similarities and differences in terms of ERK2 expression among three MPN disorders. There was a widespread occurrence of anti-ERK2 in the cytoplasm as well as in the nucleus. Observed differences concern the levels of expression of this antigen in the nucleus. The

highest level was detected in PV, lower in ET and the lowest in PMF patients. Antigen binding BCL2 antibody is scarcely represented only in the cytoplasm of MPNs. A relatively high expression of BCL2 was detected only in ET.
Conclusion: Our preliminary results indicate that the expression and distribution of ERK2 and BCL2 could be suitable diagnostic discriminators of MPN.

0095

Bone marrow morphology of patients with polycythemia vera and its relation to JAK2 mutations

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Objective: Polycythemia vera (PV) is diagnosed in the presence of polyglobuly and JAK2 (JAK2V617F, resp. exon 12) mutations. Representing a "minor diagnostic criterion", bone marrow (BM) biopsy confirms PV and allows its distinction from other myeloproliferative neoplasms (MPN) and reactive conditions. In our study, we focused our attention on the impact of JAK2 mutations on BM morphology.
Method: Our series includes 64 patients with BM morphology supporting PV. JAK2 mutations were analysed by allele-specific PCR and exon 12 mutations by sequence analysis. BM morphological analysis was focused on features pivotal for MPN (cellularity, megakaryocyte / mgk/morphology and fibrosis).
Results: JAK2V617F mutation was present in 60 patients (31x heterozygous and 29x homozygous forms). Two cases showed exon 12 mutations and two were unmutated. In the majority of cases, BM showed characteristic PV morphology (hypercellularity, trilinear myeloproliferation, pleomorphic mgk morphology without dysplasias), however, often with inextensive atypical BM features (mgk dysplasias, slight reticulin fibrosis). Their presence was not related to either the heterozygous or homozygous form of JAK2V617F. In spite of the absence of some PV hallmarks (mgk morphology, trilinear myeloproliferation), the BM morphology of exon 12 cases supported the PV diagnosis.
Conclusion: Although BM morphology might be influenced by disease progression, therapy or other factors, its evaluation represents a reliable contribution to PV diagnosis. The missing identification of distinct associations of JAK2 mutation with BM morphological patterns seems to be related to insufficient data on causal MPN and/or other associated mutations. Supported by grant Vega No. 1/034/10 and Centrum of excellence at CUJFM (IMTS Code 26220120016).

0096

Consecutive intestinal biopsies for accurate therapy adjustment in patients with post-allogeneic stem cell transplantation and persistent diarrhea

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Objective: There is a wide overlap of symptoms and signs among gastrointestinal (GI) graft versus host disease (GVHD) and other GI diseases (infections, drug toxicity, etc.). The diagnosis is based upon histologic and endoscopic findings. Accurate diagnosis is important as it determines a specific treatment and contributes significantly to the morbidity and mortality of post-allogeneic stem cell transplant (SCT) patients. Persistence of GI symptoms despite initial treatment is frequent, and ulterior histologic evaluation may be helpful to address therapy.
Method: We performed a retrospective study of 29 patients with persistent diarrhea despite treatment based on the first biopsy diagnosis in order to determine the diagnostic value of serial GI biopsy evaluation in SCT patients. Simultaneous microbiologic analyses of stools and CMV antigenemia monitoring were performed.
Results: Of the 29 first rectosigmoid biopsies, 18 (62%) were diagnosed as GVHD, two (7%) as GVHD with CMV infection, four (14%) as non-CMV infection, and five (17%) as normal or unspecific. Second GI biopsies were diagnostic of active GVHD in six (17%) cases, GVHD with CMV infection in four (14%), regenerative changes post-GVHD in eight (28%), CMV infection in three (10%), and normal or unspecific in eight (28%). In 21 out of 29 (72%) patients, the histologic findings of the second biopsy were different compared to the first biopsy, leading to a therapy change in 20 (69%) patients.
Conclusion: Our results showed a reliable diagnostic value for serial GI histologic evaluation that could impact on the therapeutic decision in patients with persistent diarrhea after allo-SCT.

0097

Thymoma with subtotal loss of keratin expression: a potential diagnostic pitfall

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Objective: The differential diagnosis between thymoma and T lymphoblastic lymphoma poses major difficulties in small needle biopsies since both entities contain immature and highly proliferating T cells. One essential diagnostic

criterion favouring thymoma is the demonstration of increased numbers of cytokeratin-positive epithelial cells. We describe a series of 7 type B2/B3 thymomas with substantially reduced cytokeratin expression.
Method: Eight thymoma cases were analyzed for the expression of various cytokeratins and other epithelial markers (panCK AE1/3, CK19, CK5/6, Cam5.2, CK5/14, p63, beta-catenin, E-cadherin). As controls, 44 type B2 and B3 thymomas and 23 thymic carcinomas arranged on a multi-tissue histoarray were analyzed.
Results: With regard to cytokeratin expression, three B2 thymomas were completely negative for panCK, CK19, CK5/6 and Cam5.2. With regard to the other epithelial markers, seven of seven cases showed a strong nuclear expression of p63 and strong membranous staining for E-cadherin and beta-catenin. Analysis of control cases on a multi-tissue array showed the expression of panCK, CK19, CK5/6, Cam5.2 and CK5/14 in 100%, 88%, 80%, 60% and 91%, respectively. The other epithelial markers p63, E-cadherin and beta-catenin were found in 87%, 68% and 50%.
Conclusion: Loss of cytokeratin expression in type B2/B3 thymomas is a potential diagnostic pitfall in the differential diagnosis with T lymphoblastic lymphoma and can be expected in 10–40% of cases. Interestingly, thymomas with loss of keratin expression seem to upregulate E-cadherin and beta-catenin.

0098

An unusual uterine tumour with signet ring cell features

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Objective: Lymphomas with signet ring cell features are rare, as is uterine dissemination of lymphomas. We report an exceptional case of a uterine tumor combining these two characteristics.
Method: A 61-year-old female was diagnosed in 2004 with localized nodal grade 2 follicular lymphoma (stage IA). She received local radiation therapy, experienced total remission, and did well until 2009 when a systematic CT scan evidenced a pelvic anterior–lateral mass. Total enlarged hysterectomy was performed.
Results: The anterior uterine wall contained a 4.8-cm fish flesh well-delineated mass corresponding to a mostly diffuse and focally nodular proliferation of medium to large cells with extensive signet ring cell changes. Tumor cells were CD20-, CD10-, Bcl2-, and Bcl6-positive with a low proliferation rate (<10–15%); CD21 underlined a focal follicular architecture. The vacuoles were PAS-negative and did not stain for immunoglobulin; ultrastructural analysis revealed nonspecific degenerative vacuoles. No lymph nodes were identified isolated from the surgical specimen. The tumor was considered as a secondary localization of the systemic follicular lymphoma, though no signet ring cells were evidenced in the cervical lymph node biopsy (reviewed). Follow-up showed retroperitoneal tissue infiltration (PET-CT) and normal medullar biopsy. She recently started R-CHOP chemotherapy.
Conclusion: This case illustrates both an unusual site of dissemination and challenging cytological characteristics in a follicular lymphoma. The signet ring cell changes challenged the adequate classification of this lymphoma as either a large B cell or a follicular B cell lymphoma.

Wednesday, 1 September 2010, Basement

PS-06 Poster Session Other Topics

0099

The use of genes in the study of cellular micrometastasis in experimental tumorigenesis

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Objective: Tumorigenesis is a highly complex systemic process. Until now, there is no experimental evidence concerning what happens in the whole organism and in distant sites after the application of tumor cells. Aim: We attempted to monitor what happens in the spleen when oncocells are applied to different sites.
Method: On a rat experimental model, we applied BP6 fibrosarcoma cell line with an incorporated gene for the green fluorescent protein GFP. Oncocells were applied either intraperitoneally (IP) or subcutaneously (SC). After 4 weeks, histological slides were prepared from spleen samples.
Results: Rats with IP oncocells developed multiple solid tumors in the peritoneum; those with SC oncocells developed a solid tumor. Histological sections of the spleen in both groups showed the presence of polymorphic fluorescent cells in the periarterial lymphatic sheath or the lymphatic follicles. Similar fluorescence was detected in the subcapsular area. Samples from the absolute control group showed no fluorescent activity.
Conclusion: We identified fluorescent polymorphic cells as BP6 cells even without the typical formation of a sarcoma metastasis. Our observation of a similar picture of the spleen in both IP and SC injected oncocells indicates a high level of regularity in the tumor cell behavior, disregarding the surrounding microenvironment and suggests a potential role of the spleen in actively attracting tumor cells, either to attack them or to process a form of information that will modulate further body–tumor interactions. Our model is

suitable for the study of oncocell spread from the primary site to the whole organism.

0100

Notch1 inhibition in pancreatic ductal adenocarcinoma with a receptor-specific blocking antibody diminishes anchorage-independent growth but not growth rate

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Objective: Upregulation of the embryonic Notch signaling pathway has been observed in several different malignancies, among which is pancreatic ductal adenocarcinoma (PDAC). For lack of a receptor-specific blocking antibody, less specific methods were used to suppress Notch signaling in earlier research. With use of a receptor-specific monoclonal blocking antibody against the Notch1 receptor (N1-AB), we determined the exact effects of Notch1 receptor inhibition on different PDAC cell lines.
Method: After confirming active Notch1 signaling, N1-AB activity and specificity were tested with immunofluorescence and qRT-PCR. Cells were treated with N1-AB and different assays were performed to establish the effects of Notch1 receptor inhibition on growth rate and colony forming potential. In vivo experiments were performed: Mice were injected with treated or untreated cells subcutaneously and tumor growth was monitored.
Results: Treatment with N1-AB did not influence the growth rate of the cells. Colony forming potential did decline upon treatment with Notch1-AB, although this decline was only twofold. In vivo experiments did not show any differences between the untreated and treated group.
Conclusion: Although Notch1 receptor inhibition did diminish anchorage-independent growth in PDAC, this effect was much less profound than previously reported. No effect on growth rate was observed upon treatment with N1-AB, which conflicts with the general statement that Notch1 receptor inhibition results in a decline in growth rate. Notch1 should still be looked upon as a potential treatment target, but further determination of the different components of the Notch pathway and their role in tumorigenesis seems advisable.

0105

Pathomorphology of the suprarenal in the short-term lateral electrical surface stimulation of the paravertebral muscles in rabbits

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Objective: Due to adverse side effects, efforts are made to reduce the time of daily lateral electrical surface stimulation (LESS) therapy in the treatment of idiopathic scoliosis in children and adolescents.
Method: Studies were carried out on ten male rabbits, New Zealand purebred, aged 3.5 months, with b.m. of 2,000–2,200 g. Animals were divided into two groups ($n=5$): (1) short-term LESS was applied for 2 h daily during 3 months and (2) control group without LESS, but with other experimental parameters as in group 1. Stimulation was performed with SCOL-2 (ELMECH). Clinical, macroscopic and microscopic observations were performed.
Results: Similar growth of b.m. was observed in the rabbits from both groups during the first 2 months of the experiment. In month 3, a slightly smaller increase was observed in the animals from group 1 than in those from group 2. The mean mass of the suprarenal glands in the stimulated rabbits was 0.4253 ± 0.0033 g, while those from the group 2 were 0.2981 ± 0.0087 g. A microscopic examination revealed hypertrophy of zona fasciculata with visible overgrowth of glandular cells in rabbits from group 1.
Conclusion: The results of clinical observations as well as morphological lesions indicate the presence of adaptive stress in rabbits stimulated with short-term LESS.

0106

Is basal cell carcinoma an aggressive cancer?

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Objective: Basal cell carcinoma (BCC) is considered a skin cancer with long evolution and low metastatic risk.
Method: We selected for presentation three cases with BCC operated between 1995 and 2010, with a special history and evolution.
Results: The first case (42-year-old female) developed a small occipital tumour, incompletely explored and excised in another surgical department. We performed ten serial excisions (soft tissue and invaded bone) during 12 years (1996–2008) and used complex reconstructive techniques (local/muscular/free omentum flaps). The second case (65-year-old male) had a neglected frontal tumour invading the orbital region; during a period of 5 years, we performed three excisions and reconstructed the region with skin graft and temporal flap, trying to save the eye. Finally, we had to excise the invaded eyeball and we used frontal flap for reconstruction (2003). The third case (63-year-old female) had a small BCC on the left buttock, which was excised in correct limits and reconstructed with a skin graft (1995).

Five years later, a new ulcer appeared. The patient neglected the tumour during a period of 10 years. The BCC progressively invaded with a buttock, the upper third of posterior left thigh and the left gluteus maximus muscle, inducing severely altered general status and anemia. We performed a wide excision (including the muscle) and we reconstructed with a fascio-cutaneous flap and skin grafts (2010).

Conclusion: BCC can be a cancer with a high local aggressivity if the patient or the surgeon underestimates it.

0107

Basal cell carcinoma of the eyelids and periorbital region

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Objective: Eyelids represent a special location of the basal cell carcinoma (BCC) due to the proximity of the eyeball. The aim of this study was to review the clinical and histopathologic features and outcomes of eyelid basal cell carcinomas.

Method: The clinical records and histopathologic specimens of 426 patients with eyelid basal cell carcinomas were reviewed and analyzed retrospectively. The main outcome measures are clinical characteristics, lesion size, histologic subtypes, severity of peritumorous inflammation, recurrence rate and prognostic features.

Results: The most common histologic subtypes were infiltrative, nodular, and basosquamous basal cell carcinomas. Of the patients, 31.5% were previously recurrent. Recurrent basal cell carcinomas were larger, with longer duration of lesion. Basosquamous basal cell carcinomas were more likely to have prior recurrences, larger lesion size, and the highest rate of orbital invasion. Perineural invasion was most frequent in morpheaform and basosquamous subtypes. Peritumorous inflammation differed between subtypes and was highest in the superficial subtype. The recurrence rate was 6.2%.

Conclusion: The outcomes were worse than previously reported due to the delay in treatment. The prognostic factors associated with secondary orbital invasion are previous recurrences, aggressive histologic subtypes, longer duration of lesion and larger lesion size.

0108

Histological assessment of small bowel hypoperfusion lesions in the pig

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Objective: Authors propose the use of a quantitative morphological assessment for helping in studies concerning intestinal hypoperfusion. The method was applied to small intestine mucosa stained with standard hematoxylin and eosin in pigs that underwent severe hypotension due to acute hemorrhage.

Method: Six large white pigs underwent total intravenous anesthesia with propofol and remifentanil. Arterial blood (25 ml/kg) was passively removed from the femoral artery over 20 min. Volume was replaced using Ringer lactate in group 1 and hydroxyethyl starch 130/0.4 in group 2, with a delay of 20 min after bleeding. One hour after the volume replacement, pigs were euthanized and small intestine samples were taken for histopathological examination. Parameters were classified using two specific scales (Chiu 1970; Çetin 1995; Kaplan 2007). Mucosal loss (ML) percentage and crypt/interstitium ratio were obtained (Faleiros 2001).

Results: Inflammatory infiltrate was present in all animals, varying from grade 2 to grade 3. Hydropic cellular degeneration and epithelial detachment were more pronounced in duodenum and more noticeable in group 1. In group 1, ML percentage was 2.18±0.46% in duodenum, 0.62±1.07% in jejunum and 0.45±0.77% in ileum. In group 2, ML percentage was 0.75±1.30 in duodenum and 0±0% in the other intestinal segments. In the whole small intestine was 1.08±0.78% in group 1 and 0.25±0.35% in group 2. Crypt/interstitium ratio did not present significant differences between the groups.

Conclusion: Quantitative morphological assessment may be useful in quantifying the degree of mucosal lost in small intestine stained with hematoxylin and eosin from pigs submitted to acute severe bleeding.

0110

Vasculitides associated with malignancies

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Objective: Vasculitis shows various kinds of physical symptoms. ANCA-related vasculitis and autoimmune disease are famous for its cause. Malignancies are also related to vasculitides. In this paper, we present two rare malignancies causing vasculitides.

Method: Case1: The patient was a 78-year-old male who had vasculitis syndrome. Computed tomography revealed aortic thrombus and narrowing of the renal arteries, suggesting polyarteritis nodosa. Case2: An 86-year-old male was diagnosed as malignant lymphoma. Acute renal failure with gross hematuria developed. MPO-ANCA was positive.

Results: Case1: The tumor in the thoracic aorta was found at autopsy. Histologically, the tumor consisted of isolated atypical cells, suggesting sarcoma. Immunohistochemically, tumor cells were positive for CD31. The same atypical cells were seen in the intrarenal arteries as emboli accompanied with vasculitides. Case2: Autopsy revealed lymphoplasmacytic lymphoma (LPL) involving kidneys. And more, amyloid deposition in the lungs and MPO-ANCA-associated crescentic glomerulonephritis, i.e. MPA, were noted.
Conclusion: Aortic angiosarcoma is an extremely rare lesion, and it is known that the tumor often causes distal emboli and presents vascilitis syndrome. The second case was diagnosed as LPL, MPA and amyloidosis. MPA is known to occur in some patients with malignancy. It has been reported that some malignancies produce vasculitides-inducing factors. In our cases, such factors produced by malignant cells might induce vasculitides.

0113

Monoclonal antibodies in the diagnostic of spinal metastases

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The skeletal system is the third most frequent seat of metastases, and metastatic tumours are the most common bone malignancies. The diagnostic workup of spinal metastases begins with the identification of the primary neoplastic site. The aim of the study was to determine the utility of monoclonal antibodies in the diagnostic workup of spinal metastases.
Materials and methods: This is a retrospective analysis of 203 patients whose histopathologic examination confirmed the presence of neoplastic foci in the spine. Samples of metastatic tumours of 57 patients whose primary tumour sites had not been identified were subjected to an immunohistochemical analysis based on monoclonal antibodies and assays for antigens associated with tumours most often producing bony metastases.
Results: The monoclonal antibodies and assays were shown to be useful aids for the identification of the histology and location of the primary tumour in patients in whom routine histological assessments had failed to determine the histological type of tumour.
Conclusions: The length of survival of patients with malignant spinal metastases is influenced by the type of neoplasm and locally radical surgery combined with palliative radiation therapy. In many cases, effective immunohistochemical workup can contribute to halting the progression of the tumour by enabling qualification for appropriate surgical and oncological treatment.

0114

Origine and frequency of pulmonary bone fragment embolism: an autopsy study

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Objective: Pulmonary bone fragment embolism (PBFE) is a rare event observed at autopsy. Pulmonary bone marrow embolism (BME) is more common, but both types of embolisms are considered to have little clinical relevance. The aim of this study was to analyze the frequency of PBFE and BME and correlate these events with clinical findings.
Method: In an autopsy cohort, the frequency of embolic particles of PBME and BME was evaluated by reanalyzing all pulmonary H&E- and Elastin-stained (EvG) sections.
Results: In a total of 1,002 autopsies, 5 (0.5%) PBFE and 29 (2.9%) BMEs were detected. Compared to BME, PBFE were significantly more common in patients with osteomyelitis [2 of 8 (25%) versus 0 of 29, $p<0.05$). In contrast, BME was more common in patients with costal fractures (16 of 29 versus 1 of 8, $p<0.05$), but no patient showed both types of embolism. In addition, PBEF and BME were observed in patients with bone metastasis and orthopaedic devices. The highest density (30.5 particles per square centimeter) was observed in a patient who died immediately following femoral medullary nailing, presenting with the clinical picture of pulmonary thromboembolism.
Conclusion: In the autopsy cohort analyzed, an incidence of 5‰ for PBFE was considerably higher as previously reported and PBEF showed a strong correlation with osteomyelitis, and the application of orthopaedic devices and extensive PBFE may lead to death. BME are correlated with rib fracture, but no association between PBFE and BME was observed, suggesting a different pathogenetic mechanism.

0115

Local flaps used for post-tumoral facial defects

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Objective: The reconstruction of facial soft tissue defects can be difficult because we should regain the shape and the function of the region. The facial esthetic units should be respected too, and we cannot neglect the oncological limits of excision.
Method: We are reporting 63 patients with facial tumors operated in the last 5 years (2005–2010), 71.4% (45) aged over 50 years and 66.7% (42) males. They needed excision

for basal cell carcinoma (BCC 55.6% (35), squamous cell carcinoma (SCC) 33.4% (21), and malignant melanoma (MM) 11% (7)).

Results: We performed correct oncological excision in 84% of the cases; ten patients with BCC needed re-excision. In the same surgical stage, we reconstructed the defect using local flaps for 50 patients (79.3%), local flaps and skin grafts for six patients and skin grafts only in the seven cases with MM. The main techniques used were: frontal or temporal flaps for the orbital /nasal regions, naso-genian or fan flaps for the lips and cervico-facial flaps for the cheek. When necessary, full-thickness skin grafts taken from pre- or retroauricular donor zones were performed (13 cases). After surgery, all the patients were examined by the oncologist; only 15 patients diagnosed with squamous cell carcinoma or malignant melanoma accepted the excision of the local regional lymph nodes.

Conclusion: Correct tumoural excision in the face is difficult to perform. The skin graft is the best reconstruction technique from the oncological point of view, but to respect the aesthetic and functional rules, we have to perform flaps.

0117

Indian ink vs tissue marking dye: a quantitative comparison of two widely used macroscopical staining tool

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Objective: The evaluation of the surgical margins is a major concern in surgical pathology and marking of surgical margins with substances; Indian ink and tissue marking dyes are widely used. As there is no systematic study comparing tissue marking dyes and Indian ink, the most common substances used for the purpose, this study was conducted to compare the two.

Method: Unit price, penetration into the tissue, the spreading area of one drop of dye on tissue paper, brightness under the microscope and the intensity of colors on image analysis program were compared for each of the five colors of Rotring's Indian ink and Thermo-Shandon's tissue marking dyes applied on reduction mammoplasty specimens.

Results: Indian ink seemed to be slightly less smudgy than tissue marking dye. Indian ink tended to remain on the surface and showed less tissue penetration compared to tissue marking dye with respect to all colors, except yellow. Blue color of Thermo–Shandon and yellow color of both Rotring and Thermo–Shandon had the deepest penetration in fatty tissues. Red color of Rotring was lost nearly totally after processing. Rotring has revealed comparable results to Thermo-Shandon; even the black and green colors of Rotring were more intense. On the other hand, Thermo–Shandon was better when red, blue and yellow colors are concerned.

Conclusion: Rotring's Indian ink is proven to be just as effective as Thermo-Shandon's tissue marking dye and bares majority of the characteristics of a perfect staining substance, which are easily applied, quickly fixed, durable and cheap, contain no potential contaminants, is work-safe, would not smudge/stain surrounding tissues, and look bright under the microscope without obscuring the view.

0118

Reestablishing surgical pathology service in Kumasi, Ghana

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Objective: In 2005, Ghana had five active pathologists. At Komfo Anokye Teaching Hospital (KATH) in Kumasi, all histology stopped in April 2004. Reasons were a broken microtome, low budget priority, and the single pathologist overloaded with teaching and forensic autopsy commitments. The department lost accreditation to train pathologists. The head of KATH oncology department met the author during a conference and asked for help.

Method: At a planning visit to KATH, a comprehensive plan was worked out. KATH administration acquired basic new equipment. Two KATH technicians trained at the University Hospital of North Norway (UNN) for 3 months. On their return, slides were produced at KATH and sent to UNN for diagnosis. Two young doctors from KATH were trained in pathology at UNN for 4 years. Double-headed microscopes were provided from Norway. Financial support was given by both hospitals and the Norwegian Government (NORAD).

Results: Attracted by the ongoing activity, a senior histotechnician and two Ghanaian pathologists returned to KATH from Accra and Scotland. The two doctors trained at UNN returned to KATH in March 2010 as specialists in pathology. Accreditation to train pathologists at KATH is expected to be regained by mid-2010.

Conclusion: Important success factors have been: appreciation of the importance of pathology by KATH and their initiative to ask for help, personal contact and commitment, support from UNN and NORAD, a comprehensive plan for sustainable development with attention to the needs and wishes of the receiving institution, doctors for training selected by receiving hospital.

0119

The insight into the history of anatomopathological museums in Europe

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Objective: During the development of anatomy since the fifteenth century, a phenomenon of anatomical museums appeared.
Method: We present a short history of this phenomenon in Europe and give insight into the form of acquisition and exhibition of the preparations.
Results: The prototypes were "curiosities of nature" collections with one of the first founded by Aldrovandi in Bologna (sixteenth century). The next famous and focused on the human body were the collections of F. Ruysch in the Netherlands (seventeenth century). He was performing autopsies in public and after that presented preserved parts or whole bodies and osteological preparation sets as artistic dioramas in a special exhibition house. His collection, sold to Peter the Great, created the foundations for Kunstkamera in Sankt Petersburg. In the eighteenth century, teachers in surgical and anatomical schools (beginning of anatomopathology) realized the educational power of such collections. Museums like the one by H. Fragonard in Paris, France and Hunterian in Glasgow were established. The next big collection—Narrenturm in Vienna—had many exhibits obtained from Algemeine Krankenhaus. The first typical anatomopathological museum was created by R. Virchow in Berlin (nineteenth century). Virchow's motto was "no day without a preparation". In Polish universities, L. Bierkowski (Cracow) and A. Bielkiewicz (Vilnius) founded such museums. That time in all Europe, anatomopathological collections became popular, gathering preparations: osteological, wet (alcohol, formaldehyde, other preservatives), dry and mummified. The fall of phenomenon started in the second half of the twentieth century. Nowadays, we face the era of digitalisation of images but, on the other hand, collections of "plastinated bodies". The ethical and legal aspects become more important.

0120

Importance of autopsy in present medical care system

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Objective: Autopsy is an important part of pathologic activity representing the final diagnostic method both in the medical and philosophical views. Autopsy has wide educative opportunities both for trainees and specialists and represents the best internal quality control of medical care.
Method: We analyzed 367 autopsies performed in our department during 2006–2008, evaluating the concordance between clinical and postmortem diagnoses.
Results: Complete concordance was present in 50.14% of the cases, partial concordance in 41.96% and discordance in 7.90%; these proportions were established irrespective of the admission duration, except three cases of patients dead on arrival. Main problematic diagnosis was bronchopneumonia (either clinically overestimated 4.36% or underestimated 8.99%). The second in frequency as clinically unrecognized diagnosis was pulmonary vascular thrombotic occlusion, either primary or secondary (thromboembolism)—8.17%. Further discordance was due to cerebral lesions (7.08%) either as presence recognition (3.27%) or lesional type (inappropriate labeling of ischemic lesion as hemorrhage or vice versa 3.81%). Also, cancer and tuberculosis were not identified as causes of death prior autopsy (5.45% and 4.63%, respectively). Unspecific inflammation (acute pyelonephritis, endocarditis, leptomeningitis, encephalitis, sigmoid diverticulitis, phlegmonous cholecystitis, etc.) were identified postmortem (7.63%). Incidental lesions unrelated to death were identified in some cases, most often renal serous cysts and liver hamartomas, but also Peutz Jeghers polyposis, aortic aneurism and syphilitic aortitis.
Conclusion: Special attention should be given to interdisciplinary consultation in our hospital, especially for pulmonary pathology, in order to increase clinico-necroptic concordance. Postmortem surprises reemphasize the importance of autopsy as an irreplaceable link in the process of medical care optimization.

Wednesday, 1 September 2010, Basement

PS-07 Poster Session Cardiovascular Pathology

0122

Immunohistochemical heterogeneity of the non-myocytic cells in different stages of heart development

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Objective: The heart is the earliest organ to function. Cardiac development and function depend on a complex of

factors with dynamical interactions, factors related to the cell populations and the extracellular matrix. Our purpose was to investigate the non-myocytic cell population of the human heart in different regions and stages of development.
Method: We analyzed 36 formalin-fixed paraffin-embedded cardiac tissues (22 adult, 12 fetal and 2 embryonic hearts) from our institute archives. We applied an indirect bistadial immunohistochemical (IHC) method using a Dako EnvisionTM+Dual Link System-HRP and antibodies against vimentin, desmin, smooth muscle actin (SMA), CD34.
Results: The embryonic heart morphology varies rapidly and intensely with age. In fetal myocardia, we noted a direct correlation between CD34 positivity in vessels and fibroblasts ($r=0.72$, $p=0.01$) and a direct but statistically insignificant correlation between vimentin expression in vessels and fibroblasts ($r=0.82$, $p=0.4$). In adult myocardia with post-ischemic remodelling, vimentin expression was increased compared to fetuses, in non-myocytic cells, especially fibroblasts ($p<0.001$), and in capillaries ($p=0.003$). As opposed to vimentin, desmin expression increases progressively from embryonic to adult life.
Conclusion: Embryonic and fetal non-myocytic cells show an IHC polymorphism. Fibroblasts form the numerically dominant population in adult, fetal and embryonic myocardia and, generally, express SMA. Vimentin is expressed in a variable number of elongated cells, indicating that fibroblasts are generated constantly. Myocardial vascular structures show a marked CD34 expression in all vessels, including capillaries. This study was supported by CNCSIS project I.D. 1388/2009.

0123
Aortic stenosis and aortic arch anomalies found at autopsy in newborn infants

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Objective: We performed 244 autopsies of congenital heart malformations (CHM) in the period between 2001 and 2009, out of which 49 cases (30 males and 19 females) of newborn infants with stenosis of atresia of aortic ostium and aortic arch (AA) were found. The aortic stenosis varied from vaguely expressed to completely interrupted AA. In most of the cases, the infants died in the first months of their life.
Results: There were 28 infants with aortic stenosis or atresia in the region of the ostium associated with hypoplasia of the left heart; four cases were with isolated valvular stenosis, three cases with bisemilunar valvula and one with supravalvular stenosis. There were 14 cases with a narrowing of the aortic arch with coarctation of the aorta. There was tubular hypoplasia of the AA in three cases, and in other three cases, we found atresia with complete interruption of the aortic arch, four with larger number of branches on the AA and three cases with common arterial trunk. Myocardium of the left ventricle showed hypertrophy due to hypertension. Diffuse endocardial fibroelastosis was found in four cases with left heart hypoplasia. The latter was histologically detected on slides stained for elastic fibers according to Van Gieson–Orcein technique.
Conclusion: The presented aortic anomalies were associated with other heart malformations in complex cardiopathies or were combined with anomalies of other organs and systems in 15.5% of the cases.

0124
Heart transplantation: forensic contribution to the cause of death investigation

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Objective: Cardiac-transplanted patients receive broad medical/hospital care. The authors intend to highlight the importance of legal medicine in this setting.
Method: A 21-year-old black female, heart-transplanted in 2001 due to dilated cardiopathy of valvular cause, was submitted to a medico-legal autopsy in 2007 after dying unexpectedly at home.
Results: Macroscopic examination revealed the absence of traumatic lesions and the presence of cardiomegaly, three-electrode pacemaker inserted in the right ventricle, anomalously coloured myocardium, lung oedema and congestion, chronic stasis liver and cerebral oedema. Microscopic examination showed lesions of severe cellular acute cardiac rejection (grade 3R) in a heart with graft vascular disease.
Conclusion: The medico-legal autopsy allowed the confirmation of a death of natural cause (1); the exclusion of infection and neoplasia—frequent and severe causes of morbidity in the heart-transplanted population (2); the documentation of graft vascular disease, which is related to the time span after transplantation (3); and the diagnosis and characterization of the acute rejection (4) as adequate cause of unexpected death, without prodromic signs and symptoms. Finally, it allowed the correlation of acute rejection and the suspension of immunosuppressive therapy by the patient—data obtained through the family (5). All these conclusions may provide information about death causes of the heart-transplanted population dying outside the hospital, which will complement that of transplanted inpatient deaths.

0125

Sudden adult cardiac death and Ebstein disease

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Objective: Sudden death is a possible major complication of congenital cardiac malformations.
Method: The authors report the case of a 44-year-old male that unexpectedly died at work while performing a minor effort task. He was apparently healthy, apart from the occurrence of very rare "epileptic attacks". A medico-legal autopsy was performed.
Results: The autopsy—in the setting of a forensic postmortem examination—revealed heart malformations that are consistent with Ebstein's disease of the tricuspid valve, associated (as seldom may occur) to mitral valve dysplasia and patent foramen ovale.
Conclusion: The relevance of this case lies upon three facts: (1) the rarity of the pathological entity, (2) the survival of the victim until the adult age without surgical intervention and (3) the fact that the symptomatic announcing event was sudden and unexpected death.

0126

Evaluation of myocardial morphological changes and plasma cTnI concentration in rats treated with doxorubicin and tirapazamine

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Objective: The clinical use of doxorubicin (DOX) is limited by dose-related cardiotoxicity. One-electron redox cycling of the quinone moiety has long been known to form reactive oxygen species. Tirapazamine is a relatively new cytostatic compound most efficient in areas of hypoxia, however under aerobic conditions causing significant oxidative stress. Aim: The aim of this study was to assess morphological changes in the myocardium of rats treated with doxorubicin and tirapazamine and evaluate changes in plasma level of a cardiac damage marker, cTnI.
Method: Rats were weakly treated i.p. with 1.8 mg/kg b.w. of doxorubicin and 5 or 10 mg/kg b. w. of tirapazamine for 6 weeks. Control group was given saline. Samples of myocardium and plasma were taken a week after last dose. Tissue samples were stained with hematoxylin and eosin, van Gieson method and Selye method and examined under light microscope.
Results: Myocardium of rats receiving a combination of doxorubicin and lower dose of tirapazamine exhibited similar changes as rats administered with doxorubicin solely. Animals treated with doxorubicin and higher dose of tirapazamine displayed vacuolization of sarcoplasm, wavy cardiomyocytes and fuxin-positive areas in Selye method as well as signs of minor interstitial fibrosis. All described alterations in this group were accompanied by significantly higher levels of plasma cTnI.
Conclusion: Both drugs caused cardiac changes in rats, including necrosis, mononuclear infiltrations and minor fibrosis. Combination of doxorubicin and higher dose (10 mg/kg) of tirapazamine seems to have a synergic effect on changes in myocardium.

0127

Cardiomyopathy significantly increases the amount of insoluble advanced glycation end products in cardiocytes, especially in the presence of diabetes mellitus

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Objective: Disturbed glucose metabolism, particularly in diabetes, is an important factor leading to the formation of advanced glycation end products (AGE). The amount of AGE and its distribution in the cardiocytes of a diabetic myocardium and in cardiopathies is not yet well understood. Studies were performed to assess AGE deposits in cardiocytes in chronic heart failure.
Method: The diabetic groups consisted of 14 autopsy cases with diagnosed diabetes, 14 hearts explanted during transplantation with ischemic cardiopathy and diabetes, and eight hearts from dilated cardiopathy with diabetes. The two non-diabetic groups comprised hearts explanted from non-diabetic subjects: 67 with ischemic cardiopathy and 47 with dilated cardiopathy. The control group consisted of samples from 20 healthy heart donors. Immunohistochemical localization of AGE was applied, and a semiquantitative scale was used.
Results: Positive staining was present in both cardiopathic groups with diabetes (100% of cases), then gradually decreased to 71% in diabetes, nearly 50% in cardiomyopathy, and to 15% in the control group. Negative staining seemed to be characteristic of control group and non-diabetics, whereas granular and diffuse staining pattern (mixed pattern) was most frequent in diabetic groups. The semiquantitative results supported an increased AGE accumulation in cases with cardiomyopathy and in diabetes. AGE staining showed no significant correlation with patient age, BMI or diabetes duration.

Conclusion: The amount of AGE increases significantly in failing and diabetic heart cardiocytes and possesses a typical staining pattern.

0128

Deposition of advanced glycation end products (AGE) in myocardial vasculature is enhanced by both heart failure and diabetes

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Objective: Disturbed glucose metabolism, particularly in diabetes, is an important but not the sole factor leading to advanced glycation end product (AGE) formation. AGE amount and its distribution in myocardial tissues and components in cardiopathies with and without diabetes are not well documented. This study was performed to assess AGE deposits in unchanged myocardial vessels in chronic heart failure.
Method: The following groups were established: 14 hearts with ischemic cardiopathy and diabetes; eight hearts from dilated cardiopathy with diabetes; 67 with ischemic cardiopathy; 47 with dilated cardiopathy, and 14 autopsy cases with diagnosed diabetes. The control group consisted of samples from 20 heart donors. Immunohistochemical localization of AGE was applied. The semiquantitative scale served to assess reaction intensity in arteries, arterioles, capillaries, venules and veins.
Results: Both types of cardiomyopathy increased AGE accumulation in myocardial veins more than in arteries. The coexistence of diabetes increased AGE significantly in arterioles and capillaries. The type of cardiopathy did not change AGE accumulation in myocardial vessels.
Conclusion: The coexistence of both chronic heart failure and diabetes intensified AGE pathology. Both cardiomyopathy and diabetes change susceptibility on myocardial vasculature to glycation; however, chronic heart failure increases AGE deposition mostly in veins, while diabetes predisposes arterioles to AGE accumulation.

0129

Advanced glycation end products in cardiocytes are joined with lipofuscin granules

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Objective: Disturbed glucose metabolism, particularly in diabetes, is an important factor leading to the formation of advanced glycation end products (AGE). The other intracytoplasmic garbage, lipofuscin (Lf), could theoretically also be subjected to glycation. The relation between these compounds is not yet well understood. Studies were performed for elucidating the relationship between AGE and Lf in insufficient human heart cardiocytes.
Method: The study encompassed four groups: one diabetic, two non-diabetics and one control. The diabetic group consisted of 14 autopsy cases with diagnosed diabetes; 14 hearts explanted during transplantation with ischemic cardiopathy and diabetes; and eight hearts from dilated cardiopathy with diabetes. The two non-diabetic groups comprised hearts explanted from non-diabetic subjects: 67 with ischemic cardiopathy and 47 with dilated cardiopathy. The control group consisted of samples from 20 heart donors. Immunohistochemical localization of AGE was applied. The coexistence of lipofuscin inside AGE was studied by Lf autofluorescence in AGE-positive slides.
Results: Lf granules inside AGE deposits were present in all studied groups with varying frequency, but the differences were non-significant. Lf granules joined significantly with dispersed patterns of AGE, i.e. diffuse and mixed, whereas inside granular pattern, Lf granules were found rarely.
Conclusion: We demonstrate that AGE deposits are constituted not only of glycated proteins but also partly from Lf. The frequency of this phenomenon is dependent on the grade of AGE dispersion, but not on the presence of diabetes or heart insufficiency, or both.

0130

Hereditary cardiac amyloidosis associated with transthyretin mutation

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Objective: Amyloidosis is a group of diseases characterized by amyloid deposition in various tissues. Hereditary forms of transthyretin (TTR) amyloidosis, previously regarded as rare diseases, related to the mutation in the gene encoding the serum protein TTR. The inheritance is autosomal dominant. The accumulation is seldom significant, except within the heart where the consequences of deposition are heart block, arrhythmias and sudden death.
Results: A 53-year-old man presented in 2003 with atrial fibrillation and heart failure. Echocardiography was characteristic of non-obstructive hypertrophic cardiomyopathy. In 2007, he suffered myocardial infarction. After episodes

of abdominal pains, CT was done and showed thickening of the rectum. Biopsy revealed TTR amyloid deposition. Scintigraphy showed amyloid deposition in the heart, bones, lungs, kidneys, urinary bladder and intestine. Molecular analysis confirmed amyloidosis of TTR type, with C425C-T mutation in TTR gene. In 2009, he had liver transplantation and died 2 days later. At autopsy, TTR deposition was found in almost all examined organs and was the most abundant in the heart. The heart was enlarged, weighted 1,170 g, and had very thickened walls. Left ventricle measured up to 2.6 cm and right up to 1.3 cm. Microscopically massive deposition of amyloid, positive in Thioflavin-T, Congo red and TTR staining, was confirmed.

Conclusion: Cardiac amyloidosis should be considered in elderly patients presenting with cardiac failure and/or arrhythmia, particularly if resistant to conventional treatment. More than 80 TTR mutations have been associated with amyloidosis, usually presenting with peripheral and autonomic neuropathy and/or cardiomyopathy. Early liver transplantation seems to be curative.

0133

Benign primary hemangioma of the heart: a case report

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Objective: Hemangiomas are benign endothelial tumors commonly affecting the skin. In the heart, those tumors are extremely rare, accounting for 2% to 3% of all benign cardiac tumors. The paper describes a case of hemangioma of the heart in a 38-year-old male who suffered from tachycardia.

Method: Echocardiography and a computed tomography scan of the chest revealed a large tumor mass arising from the left atrium of the heart measuring 10×8×6 cm, attached to the right atrium, surrounding the superior vena cava and extending to the inferior vena cava. The patient underwent open heart surgery for complete resection of the tumor. Grossly, the tumor was firm and spongy. It was a reddish brown-colored mass bleeding extensively.

Results: Histologically, the tumor was composed of vascular channels of varying size. The channels were lined with endothelial cells. Immunohistochemical staining for vimentin, CD43 and CD31 was positive in most tumor cells.

Conclusion: Cardiac hemangiomas usually arise from the anterior wall of the right ventricle and the lateral wall of the left ventricle. The location and size of the presented tumor is very rare and uncommon. Although cardiac hemangiomas are often asymptomatic, the main symptoms include exertional dyspnea, arrhythmias and signs of right heart failure. In the presented case, the patient was admitted to the hospital for arrhythmia. After the surgery, the patient made an uneventful recovery and remained asymptomatic during follow-up. The prognosis of hemangioma of the heart is variable because the tumor may grow or spontaneously involute. Surgery is curative for most patients.

0134

Morphologic aspects in a case of malignant fibrous histiocytoma

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Objective: They occur at any age, but mostly affect the middle-aged and the elderly. Malignant fibrous histiocytoma occurs more frequently in men. This presentation has revealed the rare occurrence of this tumor affecting a 22-year-old woman in an uncommon intracardiac, right ventricle localization.

Method: The surgical intervention consisted of tumor excision through the right atrium and tricuspid section. Post-operator evolution was favorable. The gross anatomy was a 5/4/3-cm tumor, with relatively incapsulated, hard, yellow with red and grey translucent zones. The cross-section revealed a solid aspect and white/grey/pink-colored zones. The histological slides were colored with HE, VG, Trichrome Masson, Gomori, Sudan IV and IHC.

Results: Malignant fibrous histiocytoma (MFH) is generally a high-grade pleomorphic tumor. The IHC reveals VIM diffuse positivity in the tumor; MNF 116 negativity in the tumor cells; S100 zonal low positivity in the tumor; ACT positivity in rare tumor cells dispersed; MYOGENIN negativity in tumor cells; Myo D1 negativity in tumor cells; MITF negativity in tumor cells; CALPONIN negativity in tumor cells; CD34 negativity in tumor cells, positive in vases; MDM2 negativity in tumor cells; P53 positivity 3–5% in tumor cells; and Ki67 positivity, 25% in the tumor. The IHC sustains the diagnosis of HFM of storiform-pleomorphic type (undifferentiated pleomorphic sarcoma).

Conclusion: The case described above is a very rare tumor and the only case of this type that we have met in our clinic in the last 10 years. The prognosis depends on their grade: High-grade tumors have a poor prognosis with about 20% 5-year survival rate.

Thursday, 2 September 2010, Basement

PS-08 Poster Session Soft Tissue and Bone Pathology

0137

A case report of osteolytic bone tumour

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Objective: Adamantinoma is a primary low-grade malignant bone tumor making up <1% of all bone cancers. It predominantly arises in bone in subcutaneous location, mostly in the middle portion of the tibia (80–85%). It is a locally aggressive tumor and is extremely low-growing, with the potential to metastasize.
Method: We present the case of a 32-year-old male with a slow-growing, painful tibial tumor. Excisional biopsy was performed and the tissue has been evaluated by haematoxylin-eosin and van Gieson stains.
Results: Microscopic examination of the tissue revealed islands of epithelial cells with tubular and basaloid pattern in a fibrous stroma.
Conclusion: Adamantinoma is a rare bone tumor and can cause significant morbidity. CT and MRI are not specific in the differentiation of this tumor from other conditions; a variety of tumors and tumor-like lesions can mimic adamantinoma, but histological examination is key to its identification. It is important to recognize this rare bone tumor because adequate treatment will result in excellent prognosis.

0143

Extraskeletal myxoid chondrosarcoma—rare histopathologic variant of myxoid soft tissue tumors: case report

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Objective: Myxoid soft tissue tumors are mesenchymal neoplasia with abundant myxoid stroma with biochemical composition of sulphated and non-sulphated glycosaminoglycans. This tumor stromal appearance is similar irrespective of tumor histopathologic type.
Method: We present the case of a 64-year-old woman with a deep-seated tumor located in the posterior region of her left calf.
Results: The tumor grew during 1 year from a relatively small size (2–3 cm) to a 10-cm mass. Magnetic resonance imaging (MRI) revealed a subfascial tumor with heterogenic signal of solid and liquid areas and perivascular growth (sarcomatous MRI characteristics). Conservative surgical excision of the lesion was decided. The tumor appeared partially well-delimitated; some difficulty occurred during profound dissection of the lesion. Macroscopic appearance revealed a 10.5/7.5/5 cm polycyclic tumor covered by whitish capsule, yellow-whitish on the cut surface. Microscopic examination showed multinodular proliferation of round/ovoid tumor cells with eosinophilic cytoplasm, forming groups, chords and strands in a myxoid stroma with rare capillary blood vessels. Hemorrhagic and necrotic areas were noted. Immunohistochemical markers revealed positivity for vimentin and S100 protein and negativity for cytokeratins, EMA, SMA, HHF35, desmin, CD34, calponin, caldesmon, GFAP. Based on the histopathologic and immunophenotypic appearance of the tumor, several entities were excluded (metastatic mucinous carcinoma, cellular myxoma, myoepithelioma, chordoma/parachordoma, myxoid liposarcoma, low-grade fibromyxoid sarcoma, and myxofibrosarcoma); our final diagnostic was grade 2 extraskeletal myxoid chondrosarcoma.
Conclusion: Extraskeletal myxoid chondrosarcoma is a rare and difficult but worthy diagnosis with a major impact on the patient's outcome. Note: Zurac and Socoliuc should be regarded as first authors with equal contribution.

0144

Primary pulmonary synovial sarcoma PNET-like type: a case report

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Objective: Synovial sarcoma (SS) is probably the most common sarcoma in the lung. Histologically, SSs are subdivided into biphasic and monophasic forms. Biphasic type can be characterized by distinct epithelial (sometimes glandular) and spindle cell components in varying proportions. Monophasic type can be subdivided into monophasic fibrous type and monophasic epithelial type. The poorly differentiated cell type is a rarely histological variant of SS. The aim of this study was to describe the histopathological and immunohistochemical features of this rare cell type of SS.

Method: We studied the case of a 62-year-old woman with SS of the right lung. The tumor was removed surgically. Paraffin-embedded tissue was stained with hematoxylin–eosin. We used immunohistochemical markers such as CD99, bcl-2, CD34, SMA, vimentin, WT1 and Ber-EP4.
Results: The tumor size was 7×5×3 cm. Histologically, the tumor consisted of small, round and ovoid cells with pale nuclei; the cytoplasm was sparse. These cells were packed into dense sheets. An epithelial cell component was also present. The epithelial cells had ovoid nuclei and abundant cytoplasm. These cells formed glandular structures, sometimes with epithelial mucin. We performed immunohistochemistry for vimentin (diffusely positive), SMA (negative), CD99 (negative), CD34 (negative), WT1 (negative), bcl-2 (negative) and Ber-EP4 (positive in the epithelial cells).
Conclusion: Although the tumor cells revealed only vimentin positivity, our diagnosis of the tumor was poorly differentiated synovial sarcoma, small cell variant (PNET-like). The recognition of these cell types of SS is important to differentiate them from other malignant mesenchymal neoplasms in the lung.

0145

Unusual localization of primary synovial sarcoma: case report

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Objective: A 41-year-old male patient suffered from acute pulmonary embolism. During his hospitalisation, abdominal computed tomography revealed a renal tumor of 5 cm associated with vena cava thrombosis. This renal tumor was not fixed in PET scan, contrary to a pulmonary lesion revealed by a radiologic exam.
Method: The patient underwent a radical nephrectomy with vena cava thrombectomy. Histologically, this infiltrating, highly aggressive tumor originating from the kidney parenchyma and involving the pelvic–calyceal system and renal vessels was poorly differentiated, characterized by solid sheets or fascicles consisting of spindle atypical cells and by a little cystic epithelial component difficult to appreciate. Immunohistochemical features: $CD99^{+}$, $CD56^{+}$, $BCL2^{+}$, $pS100^{-}$, $CD34^{-}$, smooth muscle $actin^{-}$, $chromogranine^{-}$. The epithelial component was EMA^{+}, $CKae1/3^{+}$ and some scattered fusiform cells as well. The cytogenetic study and molecular biology analysis of the frozen materiel demonstrated the translocation t(X;18) and presence of SYT–SSX fusion gene transcripts.
Results: The above findings help exclude other diagnostics. The molecular genetic study demonstrated the clonality of this biphasic synovial sarcoma. SSX2 involvement is probably associated with poor prognosis and aggressive clinical behaviour of the tumor, but it requires further investigations.
Conclusion: In our case, the patient showed tumoral pulmonary embolism associated with a pulmonary tumoral lesion requiring a surgical intervention. In this presentation, we discuss some clinical and histopathologic findings useful for the differential diagnosis and medical care of patients with this tumor.

0146

Diagnostic value of SYT–SSX detected by fluorescence in situ hybridization for synovial sarcoma

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Objective: Synovial sarcoma (SS) accounts for up to 10% of soft tissue sarcomas, can occur in any part of the body, and mainly in adolescents and young adults. A specific chromosomal translocation of t(X;18)(p11.2;q11.2) can be detected in over 95% of synovial sarcomas. This study aimed to evaluate the diagnostic value of SYT–SSX detected by fluorescence in situ hybridization (FISH) for synovial sarcoma (SS) in known and potential cases.
Method: Eighteen cases were selected among small biopsies and surgical specimens diagnosed as SS or potential SS at the Hospital of the University of Coimbra between 2000 and 2009 after a review by a pathologist to select cases with variable patterns of cellularity. Interphase FISH was carried out in FFPE using a LSI SYT (18q11.2) dual-color break-apart probe.
Results: Patients with these tumors presented ages from 15 until 54, the mean age being 30 years, and the tumors occurred in 8 women and 11 men. SYT–SSX fusion was detected in 83% (15/18) of known SS; 17% (3/18) failed to show SS18 rearrangement and were classified as negative on account of only 2% of cells showing unequivocal break-apart signals. One case was not interpretable by FISH and failed to show neoplastic cells due to absent/weak fluorescent signals.
Conclusion: From an economic point of view, FISH is a method of first choice as it allows microscopic control of a true positive result. Using this approach, 80% of SS can be diagnosed by FISH only and 20% would need to be confirmed by RT-CR. (unpaired fluorescent signals in a break-apart assay).

0147

Myxofibrosarcoma with local recurrence

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Objective: Myxofibrosarcoma may occur anywhere in the body, mostly arising in the extremities and is one of the most frequent soft tissue tumours in elderly patients.
Method: We present the clinicopathologic features of 11 myxofibrosarcomas. These include five low-grade, four intermediate grade and two high grade. Six were primary tumours and five were local recurrences.
Results: Histologic examination discloses all degrees of differentiation, from slowly-growing tumour that closely resemble cellular fibromatosis to a highly cellular neoplasm dominated by architectural disarray, pleomorphism, frequent mitoses and areas of necrosis.
Conclusion: Local recurrences showed an increase in grade compared to their primary lesions. Thus, increase in grade parallels the increase in malignant potential.

0148

Malignant giant cell tumor of the tendon sheath distal phalanx: case report

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Objective: Malignant giant cell tumors of the tendon sheath are very rare. Some cases have a poor outcome. The others, despite the histologically malignant features, have a good prognosis if wide surgical excision ablates the tumor completely. The tumor exhibited histological similarities to a benign giant cell tumor of the tendon sheath.
Method: Differential diagnosis of these tumors from other soft tissue sarcomas may be difficult. Pathologic diagnosis made by histologic features; nodular pattern, presence of a combination of spindle fibroblast-like cells and histiocyte-like cells, foci of xanthomatous change, four to five atypically mitosis in one high power field.
Results: These findings were consistent with a giant cell tumor of the tendon sheath.
Conclusion: A case of malignant giant cell tumor of the tendon sheath at the right-hand fourth digits phalanx, which developed in a 58-year-old Turkish woman, is described.

0149

Paratesticular desmoplastic small round cell tumor: a challenging diagnosis

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Objective: Desmoplastic small round cell tumor (DSRCT) is a rare malignant serosa-related tumor involving mostly the pelvic and abdominal cavities, but very uncommon in the paratesticular region. DSRCT affects young adults, mainly men, and is classically associated with a bad prognosis.
Method: We report the case of a 25-year-old man admitted to the hospital for a progressive enlargement of the right hemiscrotum with a 5-month history. A preoperative diagnosis of testicular tuberculosis was set and consecutively a right high orchiectomy was performed. At macroscopy, the testicular parenchyma showed no abnormal areas, but the tunica vaginalis was irregular and diffusely enlarged by a whitish to gray firm tumoral mass. Microscopically, the tumor consisted of nests of small, round to spindle cells, with scant cytoplasm, surrounded by a mild desmoplastic stroma. An immunohistochemical study was performed for the differential diagnosis.
Results: Immunohistochemically, the tumor cells were positive for AE1/AE3 and NSE and negative for LCA, chromogranin, synaptophysin and CD56 antibodies. Positivity and a very peculiar staining aspect were noticed with Desmin antibody: perinuclear and dot-like, suggesting a DSRCT. Fluorescence in situ hybridization (FISH) was accomplished, demonstrating EWSR1 rearrangement, which in this immunophenotypical context pleads a diagnosis of DSRCT.
Conclusion: The awareness and the knowledge of DSRCT in the paratesticular area are needed for a correct diagnosis. The differential diagnosis of this tumor with other malignancies involving this region is mandatory as it has a different clinical management.

0150

Comparison of prognosis of gastrointestinal stromal tumors depending on a primary site and a type of mutation

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Objective: Majority of primary GISTs originate from the stomach (G) or small bowel (SB). The aim of the study was

the analysis of the pathologic and molecular factors influencing the patients' prognosis after resection of primary GISTs, deriving from the stomach and intestinum, omentum (NG).
Method: In our department, a group of registered prospectively 546 primary GISTs CD117(+) comprising 303 G-GISTs (55.5%) and 243 NG-GISTs (44.5%) was analyzed; molecular examination was also made using PCR. Surgeons from our Institute compared our results, observing disease-free survival (DFS).
Results: Median size of G-GISTs as compared to NG-GISTs was significantly lower (5 vs. 8.5 cm,) and G-GISTs were characterized by lower mitotic activity (median, 3/50 vs. 5/50 HPF). In G-GIST, the most common detected mutations were exon 11 KIT (57%) and exon 18 PDGFRA, but in NG-GIST exon 11 and 9 KIT. Prognosis of G-GISTs was significantly better as compared to NG-GIST: 5-year DFS rate, 69% vs. 43%. In NG-GISTs, the following factors showed negative impact on DFS based on analysis: mitotic index >5/50 HPF, primary tumor size >5 cm, pathological subtype: spindle. The high prognostic significance of AJCC staging system classification in G- and NG-GISTs was confirmed also in multivariate analysis.
Conclusion: Reliability of AJCC risk classification after resection of primary GIST was confirmed. Patients with primary GIST, originating from the stomach, have better prognosis as compared with GISTs located in other places. In both groups, primary tumor size and mitotic activity are still the most important prognostic factors.

0151
Malignant peripheral nerve sheath tumor: report of three cases with EGFR overexpression

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Objective: Malignant peripheral nerve sheath tumors (MPNST) are highly aggressive sarcomas which may be encountered either sporadically or in the context of the inherited tumor syndrome, neurofibromatosis type 1 (NF1). Epidermal growth factor receptor (EGFR) overexpression has been reported in MPNST, which has been implicated in their pathogenesis. We present three cases that have different pathogenetic origins.
Method: The first patient was a 67-year-old woman who received radiotherapy to the axillary region following radical mastectomy for breast cancer 20 years ago. She presented with a mass in the axillary region that has been growing during the last 1.5 years. Second case was a 25-year-old man who was admitted with a 6.5-cm mass in the right anterior femoral region. The tumor was low-grade and consisted of cartilaginous heterologous component. Third case was a 39-year-old man with congenital café-au-lait spots and soft tissue swellings all over his body. He was admitted for soft tissue swellings in the left axilla and in the neck region 4 and 3 years ago, respectively. Both of the lesions were diagnosed as plexiform neurofibroma. Currently, the patient attended with 10-cm mass in the gluteal region. Pathologic diagnosis was consistent with MPNST.
Results: The first and the third cases showed a higher EGFR expression than the second case.
Conclusion: We aimed to describe the morphological features and discuss the clinical background and different pathogenetic origins of these three cases with MPNST. The role of EGFR overexpression was also evaluated in this case series.

0154
Sclerosing variant of retroperitoneal PEComa

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Objective: PEComas are a heterogeneous group of mesenchymal neoplasms characterised by the presence of epithelioid cells in a perivascular distribution (perivascular epithelioid cells, PEComas) and exhibiting the expression of both muscle and melanocytic markers. PEComas generally show a benign course, but cases with an unfavourable outcome have been reported. There are currently no established pathological criteria for malignancy.
Method: We report the case of a 66-year-old woman presenting with an 8-cm retroperitoneal mass contiguous to the left kidney. Light and electron microscopic analyses, along with immunohistochemical studies, were performed.
Results: Histologically, the tumour consisted of small clusters and trabeculae of monomorphous epithelioid cells within an abundant sclerotic and hyalinised stroma. A radial arrangement of the neoplastic cells around small/medium-sized vascular channels was occasionally observed. Mitoses were absent and the proliferation index was <1%. Neoplastic cells showed immunoreactivity for smooth muscle actin and HMB-45. On electron microscopy, neoplastic cells contained a variable number of dense granules, whereas bundles of microfilaments were occasionally identified in the peripheral cytoplasm. A diagnosis of sclerosing PEComa was established.
Conclusion: A sclerosing variant of PEComa has recently been described. Although rarely encountered, the incidence of this tumour should not be underestimated, particularly when the retroperitoneum is affected and the patient is a

middle-aged woman. Further studies are needed to better clarify the histogenesis and biological behaviour of this uncommon entity.

0155

Osteoclast-like giant cells in pelvic leiomyosarcoma: characteristic immunophenotype and possible histogenetic origin

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Objective: Leiomyosarcoma of soft tissues with osteoclast-like giant cells (OLGCs) is a rare neoplasm. The nature of OLGCs is controversial since some investigators hypothesize that they derive from the neoplastic smooth muscle cells, while others believe that they derive from tissue-associated macrophages (TAMs). The aim of the present study was to determine the immunophenotype of OLGCs and to compare it with that of the malignant smooth muscle cells in a case of pelvic leiomyosarcoma.
Method: In the Pathology Department, we received a neoplastic mass arising at the broad ligament, measuring 6.5 × 6.5 × 5.3 cm, from a 61-year-old woman. The neoplasm was fixed in neutral-buffered formalin and representative sections were taken. Immunohistochemical staining for vimentin, desmin, SMA, HHF-35, myoglobulin, MyoD1, CD68, KP1, PGM1, CD14 and CD45RO was performed.
Results: Microscopic examination revealed the presence of a highly cellular neoplasm, with areas of necrosis. The neoplastic cells were spindle, with pleomorphic nuclei and abundant atypical mitoses. Many OLGCs were also noted. Immunohistochemistry showed that the malignant smooth muscle cells expressed vimentin, desmin, SMA, myoglobulin, MyoD1 and HHF-35, while they were negative for CD68, KP1, PGM1, CD14 and CD45RO. The OLGCs reacted strongly to CD68, KP1, PGM1 and CD45RO and were negative for all other markers. The diagnosis was giant cell-rich leiomyosarcoma.
Conclusion: The immunophenotype of OLGCs is similar to that of cells of the mononuclear phagocyte series and differs from that of malignant smooth muscle cells. Our findings indicate that the OLGCs derive from TAMs, possibly as a host reaction to neoplastic cells.

0156

An audit of the rate of soft tissue sarcoma presentation in Beaumont Hospital, Dublin, over a ten-year period

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Objective: We examined the incidence of soft tissue sarcoma diagnosis over a 10-year period. The incidence of soft tissue sarcomas in Ireland is unknown, and we set out to compare our rates to internationally published figures and examine the epidemiology of these cases. Data recorded: gender, age, site, grade, size and type, primary v recurring, margins, immunohistochemistry and consultant care.
Method: All cases reported as 'sarcoma' from 1999 to 2009 were compiled. Cases without a definite diagnosis of sarcoma were excluded.
Results: The cases reviewed belonged to 28 different subtypes of sarcoma, with leiomyosarcoma of greatest incidence (16%). The most affected age at presentation was 15–64. Gender distribution was almost equal (M/F 53:47). The majority (51%) of cases were located in the trunk. Ten commonly used immunohistochemical markers were identified, with vimentin being the most common (34%). Over 50 surgeons were responsible for the care of these sarcomas. Less than half the cases were graded at diagnosis. Of those graded, 61% were high grade, 13% intermediate grade and 25% low grade. The size at presentation ranged from 1 to 32 cm (mean 5.6 cm).
Conclusion: Soft tissue sarcomas are a widely diverse group of uncommon tumours. We confirmed that our incidence rates are comparable with those published in the USA, with leiomyosarcoma being the most common subtype. Of note is that over 50 surgeons were involved in the management of these cases over 10 years. This would suggest a need for a nationalized specialist centre with surgeons experienced in the treatment of these rare tumours.

0157

Composite ganglioneuroblastoma in adult

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Objective: Ganglioneuroblastoma (GNB) is a tumour arising from nerve tissue which is intermediate between benign ganglioneuroma and malignant neuroblastoma. It is thought that this tumour arises from a single cellular clone and that the morphologically distinct components of ganglioneuroblastoma represent cells in different stages of differentiation. It is an extremely rare neoplasm of adults. It usually occurs in young children with equal frequency in either sex.
Method: We present the case of a retroperitoneal ganglioneuroblastoma in a 47-year-old man.
Results: Macroscopy: well-encapsuled tumoral mass measuring 16 cm in its large axis. It was yellow in color and has areas of hemorrhage, necrosis and cystic degeneration. Microscopy: The tumor was composed of two distinct

components. In the dominant component, there were neural cells with spindle-shaped nuclei and ganglion cells with large acidophilic cytoplasms in between them. In the second component, tumoral cells were small and have narrow cytoplasms and round, dark staining nuclei. Immunohistochemical analysis showed that tumor cells were strongly positive of NSE and focal positive staining of chromogranin A and synaptophysin. Protein 100 is positive on the level of the intricate spindle-shaped cells to the proliferation.
Conclusion: Ganglioneuroblastoma is a rare childhood tumor and rarely appears in adults. In adults, this tumour is generally discovered by accident or by compression. Ganglioneuroblastoma can look like a neuroblastoma in a partial ganglioneuroma stroma. In adults, prognosis depends on surgical margin resection.

0158

Calcifying aponeurotic fibroma: a case report

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Objective: Calcifying aponeurotic fibroma is a rare soft tissue tumor of children and adolescents. These lesions are presented as slow-growing painless masses that usually affect the distal part of the extremities. They are locally aggressive tumors that infiltrate the surrounding muscle and fascia. Recurrence rate is over 50%, but malign transformation is very rare. We report a 14-year-old boy with calcifying aponeurotic fibroma.
Method: We present a 14-year-old boy with non-movable firm mass on his leg. It was represented as a 2 × 1.5-cm diameter mass which surrounded the peroneal tendon with an infiltrating contour on X-ray and MRI. Fibroblastic proliferation areas, dense collagenous stroma, calcification and osteoid matrix were observed histopathologically. The tumor infiltrated peripheral muscle tissues and surrounding vessels and nerves. There were no mitotic figures and evidence of atypia. Overall, histopathologic and radiologic features exhibited the same characteristics of calcifying aponeurotic fibroma.
Conclusion: Calcifying aponeurotic fibroma is a locally aggressive tumor with a high rate of recurrence. Especially in young patients, those under 5 years of age had higher risk of recurrences. Very few cases of malignant transformation have been reported. Surgical total excision is suggested if ever possible and is the mainstay of the treatment approach. However, it is not possible in most of the cases because of its locally infiltrative behaviour. Differential diagnoses include aggressive fibromatosis, nodular fasciit, fibrosarcomas, monophasic synovial sarcoma and some other malignant spindle cell tumors. Surgical management should be conservative; excision and re-excision, if necessary, are preferable to radical or mutilating surgical procedures to maintain the function of the extremity.

0159

Elastofibroma dorsi: case report. Clinicopathological findings

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Objective: Elastofibroma is a tumor-like lesion of soft tissue characterized by a large number of coarse, enlarged elastic fibers and fatty tissue. Its usual location, in the scapular area, deep in the latissimus dorsi and rhomboid muscles, led to the name "elastofibroma dorsi". Most of the elastofibromas are usually asymptomatic, unilateral and occur mainly in middle-aged and elderly women. Clinically, these lesions may simulate an aggressive soft tissue tumor.
Method: Design: A 56-year-old woman presented with a slow-growing subcutaneous mass in the right lateral chest wall. CT scan revealed an ill-defined solid mass, lying deep within the latissimus dorsi. The diagnosis of elastofibroma was suspected and the patient underwent a simple excision of the mass. The surgical specimen consisted of a poorly circumscribed mass, measuring 6×4×3 cm, with gray-white cut surface and elastic consistence. The sections were examined with H+E and elastic stains and followed by immunohistochemical study for a-SMA, CD34, and H-Caldesmon.
Results: The lesion was composed of a mixture of collagenous stroma, few spindle cells, mature adipose cells and large coarse eosinophilic elastic fibers, which stain with Verhoeff-elastica and Masson-Trichrome. The spindle cells were positive for CD34 and negative for a-SMA and H-Caldesmon. The microscope findings confirmed the preoperative diagnosis.
Conclusion: Elastofibroma is an unusual benign lesion cured by simple excision. The irregular elastic fibers may be the result of abnormal elastogenesis. The familial occurrence supports a genetic predisposition to this lesion. The most important differential diagnosis consideration is desmoid fibromatosis.

0160

Evaluation of the prognostic value of TOP2a and survivine protein expression in sarcomas

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Objective: Soft part sarcoma (SPS) represents a difficult working area, either for the pathologist and the oncologist. Prognostic factors are limited in these tumors, and the search for clinically relevant molecular markers is relatively incipient. We have proposed the present study based on previous results by our group on differential gene expression of soft tissue tumors, which showed significant overexpression of the TOP2a gene in sarcomas. As TOP2a is coded by a gene sited in chromosome 17, we have also included HER-2-neu. Survivine was also included as previous studies have shown a significant correlation with survival in SPS. Our aim was to evaluate immunohistochemical protein expression of the three genes and also assess the gene copy numbers of TOP2a and HER-2-neu by FISH analysis in SPS in order to verify the existence of prognostic value on these parameters.
Method: Paraffin-embedded tissues from 274patients with SPS were included in the study.
Results: Analysis of multiple parameters showed that combined immunohistochemical expression of TOP2a and survivine were significantly associated with overall survival among sarcomas. This association was also valid when only high-grade sarcomas were analysed. There was no correlation between gene amplification and protein expression of TOP2a, or with HER-2-neu.
Conclusion: The present results indicate that combined immunohistochemical study of TOP2a and survivine may emerge as a relevant prognostic factor for clinical usage. (Supported by FAPESP and CNPq).

0162
Disease amelioration of K/BxN mouse model of spontaneous chronic polyarthritis by monoclonal anti-CD8 therapy

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Objective: The inflammatory amelioration of affected joints was characterized by AA-PAS and Herovici methods in a K/BxN mouse model of spontaneous chronic arthritis that shares many similarities with rheumatoid arthritis (RA) and was used to study the potential of $CD8^+$ T cell depletion with monoclonal antibodies (mAb) to stop and reverse experimental arthritis progression.
Method: Five groups of mice (including control) were treated with specific anti-CD8 mAb (YTS105 and YTS169.4) with and without thymectomy. $CD8^+$ T cells from the blood and articular infiltrate of K/BxN mice were characterized for cell surface phenotypic markers and for cytokine production. Affected joints were submitted to routine decalcification and paraffin inclusion.
Results: Mice receiving anti-CD8 mAb improved the arthritis score 5 days after treatment. Recovery of the $CD8^+$ T cells was associated with a new increase of the arthritis score after 20 days. Thymectomized and antiCD8 mAb-treated mice improved arthritis score permanently. Anti-CD8 mAb-treated animals normalized the serological levels of TNF-a, IFN-, IL-6 and IL-5, and histological analysis showed an absence of inflammatory infiltrate.
Conclusion: To the best of our knowledge, it was shown for the first time that K/BxN mice activated and effector memory $CD8^+$ T cells are present in the peripheral blood and joints and that they play an important role in arthritis maintenance since treatment with specific anti-CD8 mAbs significantly improved disease signs, supported by histological observations. These results document that $CD8^+$ T cells should be regarded as major players in the K/BxN model of experimental arthritis alongside $CD4^+$ T cells and B cells.

Thursday, 2 September 2010, Basement

PS-09 Poster Session Gynaecological Pathology

0169
Prognostic factors in carcinoma of the vulva

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Objective: Early invasive squamous cell carcinoma of the vulva has emerged as a controversial issue in recent literature. Reports illustrating metastatic disease in the inguinal lymph nodes have conflicted with other reports suggesting local treatment only.
Method: The clinical and pathologic characteristics of squamous cell carcinoma of the vulva in 14 women treated by vulvectomy were evaluated in relation to inguinal node status and survival. Tumor diameter, depth of invasion, clinical node status, vascular invasion and pattern of invasion were all individually correlated with the pathologic status of the inguinal nodes.
Conclusion: When evaluated in combination, the clinical diameter of the lesion was the most important predictor of survival; depth of invasion and vascular invasion contributed additional information.

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The prognostic factors of granulosa cell tumour of the ovary

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Objective: Granulosa cell tumours of the ovary are rare malignancies with a relatively favorable prognosis. These tumours have preponderance for local spread and extremely late recurrence. The aim of this study was to investigate the pathologic features related to disease recurrence in adult-type ovarian granulosa cell tumors.
Method: Four patients with granulosa cell tumours were retrospectively reviewed, and we analysed clinical and pathological characteristics.
Results: The most common presenting symptom was abnormal uterine bleeding (74.5%). Mean age was 49.7 years. The median follow-up was 87 months. According to univariate analysis, there were only two significant factors for overall survival: stage and presence of residual disease. The 5-year survival rate was 61% and median survival after recurrence was 18 months.
Conclusion: Despite the small number of patients, the study showed that stage and macroscopic residual disease are significant prognostic factors.

0171

PECOMA (perivascular epithelioid cell tumor)-like epithelioid endometrial stromal sarcoma in uterus: case report

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Objective: The World Health Organization recently recognized a family of neoplasms showing at least partial morphological or immunohistochemical evidences of a putative perivascular epithelioid cell (PEC) differentiation. It is characterized by an epithelioid morphology, clear to eosinophilic granular cytoplasm, perivascular location and co-expression of smooth muscle and melanocytic markers. Malignant perivascular epithelioid cell tumour (PECOMA) is an extremely rare mesenchymal neoplasm, although only 14 cases of uterine PECOMA have been described.
Method: A 71-year-old woman presented with postmenopausal bleeding. The diagnosis was mixed Mullerian tumoral proliferation in the probe cüretage material. Later on, total abdominal hysterectomy and bilateral salpingo-oopherectomy was performed. Macroscopically, the specimen had a 6.5 × 3 × 1-cm polypoid mass in the uterine cavity. Microscopically, there was a tumoral proliferation of swollen epithelioid cells that formed sex cord-like tubular formations and showed trabecular and insular patterns. One-layered columnar cells laid the endometrial glands peripherally of this proliferation. The neoplastic cells had mild hyperchromatism and pleomorphism and mitosis. There was no necrosis. Ki-67 proliferation index was 2–3%; the neoplastic cells were positive for vimentin, estrogen receptor, progesterone receptor, smooth muscle actin and Bcl-2, but negative for pancytokeratin, CD34, HMB-45 and alpha-inhibin immunohistochemically.
Results: By the help of these findings, we reported the case as 'PECOMA-like epithelioid-type endometrial stromal sarcoma', though HMB-45 was negative, but the morphological findings were similar.
Conclusion: We present the case here because of its rareness and to discuss it with the description of additional cases; more insight into their behavior and predictive morphological parameters may be achieved.

0172

Intestinal-type adenocarcinoma in a background of mature cystic teratoma of the ovary: case report

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Objective: Mature cystic teratoma of the ovary, frequently called a dermoid cyst, is the most common of all ovarian neoplasms and represents the majority of benign ovarian neoplasms in women <30 years of age. Malignant transformation of mature cystic teratoma of the ovary occurs in approximately 2% of all cases. Patients found to have malignant transformation in a mature cystic teratoma are likely to be postmenopausal. Adenocarcinoma has been reported to occur in 6.8% of cases of mature cystic teratoma.
Method: The operation of total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed on a postmenopausal woman of 55 years. In the macroscopical sections of the ovary, there were multiple cysts which had 3.5 cm of greatest diameter. There were sebum-like material and hair in some of these cysts. Microscopically, there was a malignant proliferation of epithelial type that formed tubular, cribriform and polyadenoid formations. The cells of this proliferation were large and pleomorphic and had a high mitotic index. There was necrosis widely. Columnar epithelium was lying in the cystic spaces. There were lipid-

laden macrophages and histiocytic multinuclear cells, lymphoplasmositer inflamatuar cell infiltration around them. There were hair follicles focally. Immunohistochemically, tumor cells were positive for cytokeratin 7 and CDX-2 diffusely and negative for cytokeratin 20.
Results: The diagnosis was intestinal-type adenocarcinoma in a background of cystic teratoma. Endoscopically and colonoscopically, there was no tumor in intestines.
Conclusion: Although gastrointestinal epithelium is found in mature cystic teratoma, intestinal adenocarcinoma is uncommon in the ovary. Because of its rareness, we present the case here.

0176

Expression of lymphangiogenesis-associated markers VEGF-D, D2-40 and Prox-1 in human cervical squamous cell carcinoma

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Objective: Cervical carcinoma metastasises mainly via the lymphatics. Attempts to correlate the expression of various lymphatic markers with lymph node metastasis have provided contradictory results. We investigated the expression of lymphangiogenesis-associated molecules in human cervical squamous cell carcinoma (SCC).
Method: The expression of VEGF-D, Prox-1 and D2-40 were evaluated by immunohistochemistry in paraffin-embedded tissue samples from 72 cases of invasive cervical SCC. Correlations with tumour grade, FIGO stage, lymph node metastases, lymphatic emboli and inflammation were also examined.
Results: Cytoplasmic and/or nuclear VEGF-D expression in the tumor cells was found in 63 of 72 (87%) cases and correlated significantly with LN metastasis ($p=0.003$), lymphatic emboli ($p=0.017$) and FIGO stage ($p=0.023$). D2-40 and Prox-1 were expressed in the lymphatic endothelium, while in 35 of 72 (%) cases, nuclear staining for Prox-1 in the tumor cells was also observed. There was a significant correlation between D2-40 peritumoral lymph vessel density (LVD) and the presence of lymphatic emboli ($p=0.009$) as well as between both intratumoral and peritumoral Prox-1 LVD and the degree of inflammation ($p<0.001$). A significant correlation of VEGF-D expression with peritumoral D2-40 and Prox-1 LVD ($r=0.281$, $p=0.017$ and $r=0.258$, $p=0.03$, respectively) was also noticed.
Conclusion: Our results indicate that VEGF-D seems to be implicated in lymphangiogenesis and in the progression of cervical SCC, and D2-40 may be a useful diagnostic tool in the assessment of lymphatic emboli. Inflammation also probably plays a critical role in promoting lymphangiogenesis.

0177

Immunohistochemical expression of Mena, E-cadherin, Ki67 and p16 antibodies in cervical intraepithelial neoplasia and carcinomas

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Objective: Some studies revealed that E-cadherin, which plays an important role in the intercellular adhesion of epithelial cells, presented alterations in cervical lesions, but clinical significance has not yet been understood. On the other hand, Mena (mammalian Ena) expression, one of the actin-binding proteins which belong to the Ena/VASP family (enabled/vasodilator stimulated phosphoprtotein), was not studied yet in cervical intraepithelial neoplasia (CIN) or cervical carcinomas (CC).
Method: We have analyzed in biopsic cervix specimens the immunohistochemical expression of Mena, E-cadherin, Ki67 and p16 antibodies in 30 cases with CIN (1, 2 and 3) and ten cervical carcinomas. All antibodies were provided by LabVision.
Results: We observed that E-cadherin intensity decreased from CIN to CC, but did not present statistical differences between different grades of CIN. Mena was not expressed in normal cervical epithelium, but its expression was increased at the same time with increasing grade of CIN, and significant up-regulation upon transition to invasive carcinoma was observed. In cases with CIN1/CIN2 and immunophenotype $Ki67^{-}/p16^{-}$, Mena was not expressed and E-cadherin intensity had higher intensity than in cases $Ki67^{+}/p16^{+}$.
Conclusion: Correlation between Mena, E-cadherin and also Ki67 and p16 immunoexpression could be an important prognostic factor in cervical lesions and could help the pathologist define more accurately the grade of CIN and to determine the risk for malignant transformation.

0178

Distribution of human papillomavirus genotype and significance of expression of p16 and bcl-2 in patients with cervical squamous intraepithelial neoplasia from Korean women

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Objective: Human papillomavirus (HPV) is considered to be most important agent in cervical carcinogenesis and is also associated with precancerous lesion. Prevalence and distribution of HPV genotype varies geographically. Early detection of cervical intraepithelial neoplasia (CIN) results in the prevention of progression to cancer. This study investigates the distribution pattern of HPV and the expression of p16 and bcl-2 in CIN among Korean women.
Method: We selected 177 samples of CIN (CINI 25, CINII 14, CINIII 98) and 40 squamous cell carcinoma obtained by punch biopsy or conization. All of them had HPV genotyping in routine Pap smear using DNA chip. The tissue specimens were immunohistochemically stained for p16 and bcl-2.
Results: HPV DNA was detected in 92% of the specimens. The most prevalent HPV type was HPV 53, including multiple infection in CIN I, while HPV 16 was the most common type in CIN II and III. HPV 58 was the second most common type in CIN III. Multiple infection was detected in 26.3% of the sample. P16 expression was observed in all of CIN lesions and was significantly correlated with HPV infection. There was a positive correlation between the expression of bcl-2 and HPV 16 observed in CIN III.
Conclusion: The distribution of HPV genotype in Korean women disclosed a somewhat different pattern from that of western countries by showing a higher prevalence of HPV 58, 56 and 52. Immunoexpression of p16 was intimately associated with HPV infection and was a highly sensitive marker for CIN. Bcl-2 had correlation with HPV 16-associated CIN III.

0179

P 16 as a diagnostic marker of cervical intraepithelial neoplasia in cervical biopsies

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Objective: The protein p16(Ink4a) is overexpressed in cervical lesions associated with high-risk human papillomavirus (HPV) subtypes 16 and 18, but not in low-risk HPV subtypes 6 and 11 or non-HPV-associated cervical lesions. Distinction between low- and high-grade cervical lesions is very important for the management of patients with cervical intraepithelial neoplasia. In this study, we wanted to determine the usefulness of p16(Ink4a) expression in biopsy specimens with indistinct initial histologic diagnosis (CIN 1-2).
Method: We applied immunostaining for p16(Ink4a) in 47 consecutive cervical biopsy specimens with equivocal initial histologic diagnosis (CIN 1-2) during the period between January 1, 2009 and December 31, 2009.
Results: Diffuse positive expression in the lower third of epithelium was found in 21% (10/47) of cervical biopsies; in 47% (22/47), the positive expression extended to two thirds of the epithelium, suggesting the diagnosis of CIN 2. Fifteen biopsy specimens (32%) were negative or only focally positive for p16(Ink4a), indicating the diagnosis of immature squamous metaplasia.
Conclusion: The data support the routine use of p16(Ink4a) immunohistochemical evaluation of cervical biopsy specimens for better discrimination of negative, low-grade and high-grade cervical lesions.

0180

Cervical adenocarcinomas concomitant with CIN or squamous cell carcinoma lack gastric phenotype

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Objective: Uterine cervical adenocarcinoma with gastric phenotype was recently proposed and shows an aggressive clinical course. The aim of the study was to examine whether the gastric phenotype is present in cervical adenocarcinoma in situ (AIS) or invasive adenocarcinoma (AC) concomitant with cervical intraepithelial neoplasia (CIN) or invasive squamous cell carcinoma (SC).
Method: Fifteen cases of cervical glandular neoplasms concomitant with CIN or SC were retrieved from KCH files and subclassified by AIS+CIN3 (n=8), AC+CIN1-2 (n=2), AC+CIN3 (n=3), AC+SC (n=2). Of those, AC and SC were independently present and the so-called adenosquamous carcinoma was excluded. In addition, no cases of minimal deviation adenocarcinoma were observed. We evaluated the presence of gastric morphology based on the criteria previously described by Kojima et al. (AJSP 2007). In immunohistochemistry, the staining of MUC6 and HIK1083 was performed and scored as negative; <10%, focal; 10% to <30%, diffuse; 30% or more.
Results: All 15 cases lack gastric morphology. MUC6 was positive in four cases (diffuse, n=1; focal, n=3), whereas all cases were negative for HIK1083.
Conclusion: Cervical AIS or AC concomitant with CIN or SC seems to be unrelated with adenocarcinoma with gastric phenotype.

0182

A premenopausal extramammary (vulvar) Paget's disease: case presentation

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Objective: Paget's disease of the vulva is an unusual kind of skin cancer that arises from glandular cells, and its incidence is almost exclusively encountered in postmenopausal women (65–70 years old).
Method: Vulvar skin fragments from a 46-year-old woman were submitted for definite pathological diagnosis due to a prolonged intercourse pain and itching in the genital area, thought to be linked with lichen sclerosus et atrophicus. The tissue fragments were routinely processed (buffered 4% formalin fixation, paraffin-embedded, 5-μm-thick section), stained with H&E, AB-PAS and supplementary immunohistochemical reactions with CEA, EMA, HER2/neu, pan-CK and HMB45.
Results: Intraepidermal presence of large tumor cells with vesicular, pleomorphic nuclei and well-represented, foamy cytoplasm, positive for AB-PAS, arranged in small nests with central lumen near the rete ridges or dispersed singly throughout the whole thickness of squamous epithelium raises the suspicion of Paget's disease. This opinion was confirmed by the positive immune reaction of the mucin-secreting intraepidermal tumor cells for CEA, EMA, panCK and HER2/neu. The tumor cells were negative for HMB45 and presented a high mitotic rate: 33% of tumor cell nuclei were stained with Ki67 antigen. The final diagnosis was extrammamary (vulvar) Paget's disease, Wilkinson 1a (primary intraepithelial).
Conclusion: Seldom among vulvar neoplasia, Paget's disease must be taken into count even in premenopausal women; its prompt diagnosis enables the surgeon to perform the radical cure for selected cases (those with only intraepidermal/intraepithelial spread).

0183

Characteristics of transmembranous glycoprotein CD44, PTEN protein and Ki 67 in endometrial hyperplasia and carcinoma

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Objective: The objective was to evaluate the expression of CD44 and PTEN protein in atrophy, various hyperplasias (EH), carcinoma of endometrium (EC) and its correlations with proliferation rates
Method: From 49 analyzed EC (also treated with tamoxifen) for immunohistochemical examination, we have chosen 17 and compared them with 15 hyperplasia with atypia, 17 without it and eight glandulostromal atrophy cases. Immunohistochemical evaluation was done with CD44, PTEN, Ki 67 (DAKO) antibodies. Expression of markers was stated into 3 degrees. PTEN presence and proliferation index was evaluated in percent. The results were compared with χ^2 and Fisher test ($p<0.05$).
Results: CD 44 was negative in endometrial atrophy and hyperplasia. PTEN protein expression in EH was around 100%. Expressed CD 44 marker was in atypical hyperplasia and EC. Comparison of these two groups showed that χ^2 is 4.44 ($p=0.217$), Fisher test $p=0.636$. Essential distinction was found between hyperplasia with atypia and without it: $\chi^2=9.700$, $p=0.021$, Fisher test $p=0.015$. In atypical EH, PTEN protein expression decreases in epithelium till 67.5%, but is still high in stroma. In EC, immunoreactivity of PTEN was 20.7–50.4%. Sometimes, PTEN loss goes parallel with the reduced proliferative activity in cystic atrophic glands in G0 stage of mitosis.
Conclusion: (1) CD44 expression is increasing in hyperplastic processes and EC, but there are no differences of CD44 in atypical hyperplasia and endometrial carcinoma. (2) PTEN protein expression in EH and EC was variable, but with tendency towards its diminution in cystic glands, atypical hyperplasia and EC.

0184

The association between neoplasia of the endometrium and others benign genital lesions: independent or similar mechanism?

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Objective: It is generally believed that hyperoestrogenic status is one of the most important etiopathogenic factors of premalignant and malignant lesions of endometrium. This study characterises the association between the presence of premalignant and malignant lesions and other benign clinicopathological manifestations of hyperoestrogenism in the genital area.
Method: The study comprised 83 resection specimens that were formalin-fixed and paraffin-embedded. The sections were marked with standard and special stainings (ER, PGR, Ki-67, PTEN).
Results: The study group included women with ages between 39 and 67 years. From a total of 83 hysterectomies, 25 presented both histological evidence of premalignant or malignant endometrial lesions and other associated lesions: endometrial (4), endocervical (2) polyps, leiomyoma (16), adenomyosis (4), ovarian cysts (10).
Conclusion: In conclusion, the altered expression of oestrogenic status reflects the pathogenic mechanisms of endometrial neoplasia. Assessment of these histopathological patterns may be a useful clinical and prognostic tool for

the follow-up of women with predisposition for neoplastic lesions of the endometrium.

0185

Uterine (subendometrial) schwannoma

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Objective: Schwannoma of the uterus is a benign but very rare neoplasia. We report a case of uterine schwannoma occurring in a 51-year-old woman with a recent history of abnormal uterine bleeding.
Method: The curettage material was routinely processed and stained with H&E. In addition, immunohistochemistry was performed with antibodies against S100-protein, SMA, CD 117, Caldesmon, Melan A, HMB 45, CD 10 and MIB-1.
Results: In histological investigation, very few preexisting non-neoplastic glands and a tumor with dense fibrillary matrix was seen. The nuclei were oval to bipolar and cells showed no borders. Few psammoma bodies were seen in the tumor, also incipient Verocay bodies. The tumor cells were intensely positive for S-100 protein; no reaction with all other antibodies was seen. Less than 1% of tumor cells were positive for MIB-1.
Conclusion: The histopathology and the immunohistochemistry in this case revealed an exceedingly rare example of a uterine (subendometrial) schwannoma.

0187

Expression of PTEN, ß-catenin and estrogen receptor in endometrial hyperplasia: an immunohistochemical study

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Objective: Type I endometrial carcinomas with endometrioid and mucinous morphology develop in the patients with chronic hyperestrogenism. These tumors show frequent PTEN (30-50% of cases) and β-catenin mutations (15-20% of cases).
Method: The study group consisted of 79 cases of endometrial hyperplasia (EH) and 19 cases of endometrial atypical hyperplasia (EAH). As a control group, 43 cases of anovulatory cycle endometria (ACE) were selected. The immunohistochemical analysis was performed on the endometrial curettings by means of tissue microarray. For the objective evaluation of analysed proteins, expression *H* scores (percentage of positive cells) have been used.
Results: The mean *H* score of PTEN expression was 177.16 in ACE, 111.5 in EH and 102.92 in EAH ($p<0.05$ ACE vs. EH and EAH). Mean *H* score of ER was 148.88, 173.1 and 212.12, respectively ($p<0.05$ between all groups). Mean *H* score of membrane β-catenin expression was 95.47, 89.47 and 74.92, respectively ($p<0.05$ between all groups). Percentage of cases with positive nuclear β-catenin expression was 10.8%, 12.3% and 38.5%, respectively ($p<0.05$ EAH vs. ACE and EH).
Conclusion: Expression of analysed proteins significantly distinguishes endometrial hyperplasia from disordered endometrial proliferation. The most extreme level of abnormal expression of the studied proteins was seen in atypical hyperplasia, confirming its close relationship to endometrial carcinoma. Analysis of PTEN and β-catenin expression may be a useful adjunct in small endometrial biopsies from patients with suspected precursor states for endometrial carcinoma.

0188

Immunohistochemical profile of cotyledonoid dissecting leiomyoma of the uterus

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Objective: Cotyledonoid-dissecting leiomyoma of the uterus (CDLU, also known as Sternberg tumor) is an extremely rare variant of smooth muscle neoplasm. Less than 30 cases of CDLU have been reported so far. CDLU is characterized by alarming distinctive gross appearance suggestive of a malignant neoplasm, but paradoxically universally benign clinical behavior. Although the histopathological picture of CDLU was well described, there are few if any data which may explain extrauterine overgrowth of the tumor.
Method: Four previously not described cases of CDLU were submitted to histopathologic examination and selected immunohistochemical assays (p16, Ki67, bcl-2, WT-1, p53). Results of immunohistochemical stainings were compared to those previously reported in benign and malignant uterine smooth muscle neoplasms.
Results: The age of patients with CDLU ranged from 33 to 52 years (median 50 years). Grossly, all the tumors were exophytic and composed of irregular nodular protrusions. Median diameter of tumors was 8 cm (range 3–11 cm). Microscopically, the cases were composed of smooth muscle fascicles dissecting the extratumoral myometrium and forming swirled nodules, similarly to previous CDLU reports. Nuclear p16 expression was seen in <1% of tumor cells. Ki-67 proliferative index in all

CDLU cases was below 1%. Bcl-2 was expressed focally in a single case and diffusely in three other cases. WT-1 diffuse nuclear expression was seen in all cases. All cases were p53-negative.
Conclusion: p16/Ki-67/bcl2-/WT-1/p53 immunohistochemical expression patterns in CDLU resembled those previously reported in uterine usual type leiomyomas and did not explain the distinctive gross appearance of these tumors.

0190

A panel of antibodies to distinguish the different histological subtypes of the epithelial ovarian carcinoma

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Objective: Epithelial ovarian carcinoma (EOC) is subdivided into five histological subtypes: serous, mucinous, endometrioid, clear cell, and transitional. The differential diagnosis between these subtypes is mostly unproblematic, but is getting difficult in poorly differentiated tumors. The aim of the present study was to establish an immunohistochemical marker panel in a series of unequivocal highly differentiated EOCs that simplify the differentiation in difficult EOCs.
Method: We performed an immunostaining study in a small series of highly differentiated EOCs (47 cases). After examining the cases with 23 markers, we identified 13 antibodies as useful markers (CA19-9, CD15, CA125 CD99, CD44v6, CD44H, Claudin1, EGFR, FAK, OPN, p63, p53, and PR). The percentage of the antibody-marked tumor cells per tumor was observed counted. The *F* test was used to determine differences between the means of percent expressions of the antibodies. For predicting the histological subtype from the antibodies data, a multinominal logistic regression model was performed. We additionally accomplished a leave-one-out method cross-validation experiment to mimic real-life situation of predicting an unknown histological type.
Results: In 89% of the cases, the initial multinomial regression model (using all observations) was able to predict the correct subtype. However, this number reduced to 43% in the leave-one-out experiment.
Conclusion: The match of 89% correct predictions in the full data set is in agreement with another publication (84%) using a similar panel. However, the low number of correct predictions in the leave-one-out experiment affirms that this is no valid panel to distinguish the histological types of EOCs by immunohistochemistry.

0191

Immunohistochemical aspects of differential diagnosis in ovarian and testicular malignant tumors

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Objective: 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.
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 increased size in the left testicle in last 6 months, and the second case is a 15-year-old girl hospitalized in the same department for retroperitoneal tumoral mass. CT scan shows in both cases testicular and ovarian mass with non-homogeneous structure, surrounded by liquidian areas.
Results: Histological exam revealed in both situations immature teratoma with mesenchymal, 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). Prognosis was favorable after surgery on the young man, but the young woman was given chemotherapeutical support.
Conclusion: Immature teratomas are rare germ cell tumors. The incidence in children is 2% to 3% of ovarian and testicular tumors. In both cases, the IHC confirms immature teratoma diagnosis. Immature intestinal type of glands were positive for AFP, and neuroepithelial structures were positive for chromogranin and synaptophysin that sustain immature teratoma.

0192

Giant ovarian lymphangiomas

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Objective: Vascular tumors of the ovary are rare, with only 18 lymphangiomas published in the literature so far. They

are usually asymptomatic and represent an incidental finding. Histologically, most lymphangiomas are of cavernous type and do not exceed 4–5 cm in diameter. We report two unusually large lymphangiomas measuring 7 and 9 cm in diameter, respectively, both of them presenting with ultrasonographical images of malignancy due to the large size and multicystic appearance.
Method: Patients were 50 and 51 years old, respectively, the latter with a clinical history of 20 pregnancies and associated leiomyoma and endometrial polyp. The former was operated for a large pelvic cystic mass which was on microscopic examination diagnosed as a borderline serous tumor of the left ovary.
Results: Macroscopically, they were poorly defined multicystic tumors that on cut section were dramatically reduced in size (deflated), oozing a clear, thin fluid. Microscopically, there was an anastomosing network of empty-appearing cystic spaces that were lined by flattened cuboidal cells without atypia. Immunohistochemically, their vascular nature was demonstrated as the cells lining the cystic network were positive for CD31, CD34 and D2-40 (podoplanin) and negative for epithelial markers.
Conclusion: Lymphangiomas are benign masses and their histogenesis is uncertain. They may represent a reactive process occurring after impaired or blocked regional lymphatic drainage related to conditions such as chronic follicular salpingitis, radiation therapy or various neoplastic processes. One of our cases lacked any association and the other one was associated with a surface, noninvasive tumour. We thus conclude that our cases are likely to be true neoplasms.

0194

Placental mesenchymal dysplasia (PMD) with mosaic triploidy and chorangiomas presenting as a partial hydatidiform mole (PHM)

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Objective: PHM may show radiologic, gross, and histological features overlapping with PMD. PMD with aneuplodies has been reported, but not with a mosaic triploidy.
Method: A 2,500-g male fetus without hemihypertrophy or congenital malformations was born at 34 weeks gestation. The 900-g dysmature and focally hydropic placenta showed varicose dilatation of focally thrombosed chorionic vessels, and two translucent thin-walled septal cysts grossly suggestive of PHM. The stem villi showed myxoid stromal change. There was no trophoblastic hyperplasia or pseudoinclusions. The chorionic and stem vessels were dysplastic. Two 2.5- and 2.2-cm glut-1 positive capillary chorangiomas were found.
Results: 69,XXY/46,XY karyotype was revealed by FISH. Amplification of three microsatellite loci on chromosome 11p15.5 showed that the predominant cell line was 46,XY. There was no evidence of paternal isodisomy of Beckwith–Wiedemann syndrome.
Conclusion: This unique PMD case with mosaic diandric triploidy and chorangiomas was grossly suspicious for PHM because of the presence of thin-walled translucent "hydatid" vesicles admixed with placental tissue. However, a large for gestational age and dysmature fetus without anomalies and characteristic gross and microscopic placental findings were diagnostic of PMD. The distinction between PHM and PMD is clinically valid as PHM can be complicated by persistent gestational trophoblastic disease or trophoblastic tumors. The presence of diandric triploidy is not automatically equivalent to PHM.

0197

Expression of p53 protein phosphorylated at serine 20, serine 392 and apoptosis-related proteins in ovarian carcinomas

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Objective: The biological role of phosphorylation of the p53 protein in tumour cells is still investigated. The study evaluated p53 protein overexpression, p53 phosphorylated at serine 20 (Ser20), serine 392 (Ser392), and expression of Bax, CAS proteins in ovarian neoplasms and their association with clinicopathological parameters.
Method: The expression of analyzed proteins was examined on 145 malignant and 50 benign ovarian neoplasms using immunohistochemistry.
Results: Differences between the expression of studied proteins in benign and malignant ovarian neoplasms were significant ($P<0.001$). p53-Ser20 phosphorylation was associated with advanced stage ($P=0.03$). p53 protein overexpression was associated with poor differentiated tumour ($P=0.001$), while the differences between CAS expression were observed in moderate and poor tumour grade ($P=0.03$). The correlations between Bax/p53-Ser392, CAS/p53-Ser20 and p53-Ser392 expression were found in ovarian carcinomas ($P<0.01$). p53/CAS-positive cancers were associated with poor differentiated ($P=0.005$) and advanced tumours ($P=0.001$). p53/Bax-positive cases were

found in poorly differentiated cancers (P=0.001). These immunophenotypes revealed phosphorylation of p53 at Ser20 and 392 in 60.0% of cases.
Conclusion: Our results suggest that the expression of p53 protein phosphorylated at Ser20 might depend on the morphological maturity of tumour cells. The revealed associations between Bax, CAS and p53 protein phosphorylated at serine 20 and 392 expression indicate that phosphorylation of p53 protein at Ser20 and 392 might play a role in the regulation of apoptosis-related protein expression in ovarian carcinomas.

0199
Experimental model of female hormonal sterility received by influence on a bitch rat of long constant illumination
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Objective: Female hormonal sterility is successfully treated. The experimental model of this morbid condition of ovaries is necessary for the approbation of various methods of treatment of female hormonal sterility. In this connection, we have estimated histologically the structural changes of ovaries of a rat after the long maintenance in the conditions of constant illumination.
Method: Two groups of females Vistar rats at the age of 3 months are generated: control group (Cgr), six individuals, and basic group (Bgr), six individuals. Rats of Bgr within 7 months were contained in the conditions of constant illumination and rat Cgr in other premise, in conditions of usual change of day and night. Ovaries are studied histologically.
Results: The ovary of rats Bgr has reduced dimensions. Absence of normal follicles of any degree of maturing and yellow bodies, presence of cystically variated follicles, a considerable quantity of atretic follicles, and presence of white bodies are microscopically observed. It is possible to assume that constant illumination of animals causes stimulation of the production of serotonin and inhibition of the production of a melatonin by a cone-shaped gland. In the beginning, there is a stimulation of a hypothalamus–hypophysial–ovarian axis and then exhaustion of compensatory mechanisms and development of hormonal sterility.
Conclusion: The maintenance of bitch rats in the conditions of constant illumination leads to development in ovaries of pathological changes, characteristic of hormonal sterility. Necessary level of ovarian insufficiency can be dosed by change of duration of the period of constant illumination of animals.

Thursday, 2 September 2010, Basement

PS-10 Poster Session Cytopathology

0200
Overexpression of P16INK4A can correct an underdiagnosed HGSIL?
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Objective: The expression of P16 protein is associated with a high risk of HPV infection. The purpose of this study was to evaluate if overexpression of P16INK4A protein can be a useful biomarker to detect dysplastic cells in cervical smears of women with normal colposcopic findings but HGSIL cytology.
Method: Cases were retrospectively searched in cytology data of the past 5 years. One hundred thirty-nine cervical smears from patients with HGSIL were reviewed by two cytologists. The colposcopic results concerning those cases were also obtained from the records. Forty-three of 139 (30.9%) women had no pathologic colposcopic findings. Immunocytochemistry for P16 was performed on all cases with normal colposcopy and HGSIL cytology. The immunocytochemical staining results were estimated and classified into four grades: 0, 1 (1–5% positive cells), 2 (5–25% and patchy), 3 (>25% and diffuse).
Results: The P16 protein expression was not observed in two cases (4.6%), whereas it was strong and diffuse in nine patients (20.9%). Strong and patsy P16 expression was detected in 13 patients with HGSIL cytology and normal colposcopy (30.2%). Rare positive cells (1–3% cells with nucleus stained with anti-P16 antibody) were present in 19 cervical smears (44.2%).
Conclusion: Colposcopy with guided biopsy detects approximately two thirds of CIN 3 cases (Gage et al., National Cancer Institute, USA, 2006). Increased expression of P16INK4A reflects the increasing expression of viral oncogenes in dysplastic cervical cells. P16INK4A is a specific biomarker to identify HGSILs, so it could be a useful adjunct for the reevaluation women with HGSIL cytology and normal colposcopy in order to correct the underdiagnosed HGSILs and prevent delay in therapeutic managements.

0202

A ring validation study prior to introduction of computer-assisted screening in the Dutch cervical screening programme

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Objective: Prior to the introduction of computer-assisted screening in the Netherlands, it was agreed upon to first validate this method with an a priori agreed upon uniform protocol for validation. Aim was a kappa value of 0.6 or higher.
Method: A validation sample set was made of 280 anonymized routine screen samples, enriched for abnormalities (to 15%). Two screeners from one laboratory scored these with either computer-assisted screening or conventional thin-layer cytology. Slides were scored dichotomously independently for either mode of screening as either within normal limits or outside normal limits. The procedure was supervised by an external quality pathologist. After a pilot (n=2), the 'set' was transported to other laboratories for the application of a similar protocol. All scores were computed from six different laboratories (n=3,305).
Results: This nationwide validation study required on average 4 weeks in the participating laboratories. First two kappa scores were 0.86 (95% CI 0.83–0.89) and 0.72 (95% CI 0.683–0.77) for the two techniques. After 6 months, four additional laboratories had accomplished the validation procedure. Cumulative kappa score for six laboratories was 0.77 (95% CI 0.75–0.78, n=3,305) All average scores were above the preset norm of 0.6.
Conclusion: A nationwide validation protocol could be accomplished without complications. The results showed good agreement between computer-assisted screening and conventional screening on thin-layer technique. The validation protocol allows for the implementation of computer-assisted screening in programme-based screening in the Netherlands since detection rates will not alter in a significant manner.

0204

Diagnosis of malignant mesothelioma by fine needle aspiration cytology

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Objective: Cytopathologic descriptions of mesotheliomas are limited. This study shows the results of fine needle aspiration (FNA) cytology in ten cases of malignant mesothelioma.
Method: Material was obtained by CT-guided fine needle aspiration (FNA). Smears were stained with Giemsa and Papanicolaou stain, and cell block sections, when done, were stained with hematoxylin and eosin. Immunocytochemical (ICC) studies were done in smears/cell block using adenocarcinoma markers (CEA, EMA, CD15, CK7 and TTF1) and mesothelial markers (calretinin, WT1, CK5/6).
Results: All mesotheliomas except three of peritoneal origin were from the pleura. There were nine epithelial malignant mesotheliomas (EMM) and one sarcomatous mesothelioma (SMM). All mesotheliomas except three of peritoneal origin were from the pleura. There were nine epithelial malignant mesotheliomas (EMM) and one sarcomatous mesothelioma (SMM). In FNA, all EMMs were highly cellular, with a large number of small sheets, and tridimensional clusters with smooth or lobulated contours as well as single cells. The cells were round to polygonal with abundant cytoplasm and well-defined cell borders and the nuclei were predominantly centrally located, round to oval, medium to large with fine chromatin and small but prominent nucleoli; occasional binucleation was noted. The SMM showed malignant spindle-shaped cells with scant, ill-defined cytoplasm singly and in loose clusters, in a bloody background. Immunocytochemical staining on the smears/cell block were positive for calretinin, CK5/6,CK7 and WT1 and negative for CD15,CEA and TTF1.
Conclusion: Cytologic findings coupled with immunocytochemical studies and in combination with radiologic information and clinical history are highly accurate in the diagnosis of malignant mesothelioma.

0205

Solid pseudopapillary tumor of the pancreas diagnosed on endoscopic ultrasound-guided fine needle aspiration material

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Objective: Solid pseudopapillary tumor (SPPT) of the pancreas is a rare, low-grade, epithelial neoplasm that is usually discovered incidentally in young women.
Method: We present a 42-year-old woman with 5 × 4-cm round tumor in the caput pancreatis. Endoscopic ultrasound-guided fine needle aspiration (EUS-guided FNA) was performed. Smears were stained with Giemsa and Mc Manus. Cell block was used for eosin, Mc Manus and immunohistochemical stainings.
Results: Cell-rich smears showed single cells and aggregates of uniform cells forming branching papillary clusters with delicate myxoid fibrovascular cores. The nuclei of the

cells were round or oval; nucleoli were small or inconspicuous. Cytoplasms were usually vacuolated. Intra- and extracytoplasmic myxoid globules were noticed. Tumor cells showed diffuse positivity for CD10, progesterone receptor and CD56 and focal positivity for synaptophysin and AE1/AE3. Chromogranin A, CK8/18, CK7, Tag72 were negative. Ki67 showed low (<1%) positivity. The case was diagnosed as SPPT based on morphological and immunohistochemical features, which was confirmed on the surgical material.
Conclusion: Distinguishing SPPT from other pancreatic tumors, especially pancreatic endocrine tumors, can be challenging. Clinical setting, cytomorphologic features, and immunostains of the cell block help distinguish SPPT from pancreatic endocrine tumors, acinar cell carcinoma, and papillary mucinous carcinoma.

0206
The role of breast FNAC in diagnosis: a survey of current practice in an anticancer institute

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Objective: Most countries, including Greece, have adopted the triple assessment approach to breast diagnosis, with FNAC as the first-line pathological investigation with the exception of microcalcifications. The majority of European countries use similar reporting systems for breast FNAC (C1–C5), in keeping with the European Guidelines for Quality Assurance in Breast Cancer Screening and Diagnosis.
Method: In order to explore current practice in our hospital, we reviewed 390 breast FNAs within a 1-year period, collected and interpreted by both the cytopathologists and less by the surgeons, where no clinical data were available.
Results: There were a total of 156 cancers (40%), of which 150 were diagnosed by FNA (128 as C5 and 22 as C4). Overall, 142 were diagnosed as C5 (36.4%), 40 as C4 (10.2%), 47 as C1 (12.1%) and 161 as C2 (41.3%). Of the 142 C5, 128 were confirmed histologically (mastectomy specimens), whereas in 13 patients, there was no feedback information. There was one false positive case concerning two separate fibroadenomas, one of which in the mammary tail, sent to the lab as axillary lymph node. In the C4 category, there were 22 carcinomas, two fibroadenomas, one fibrocystic disease, and 15 had no confirmatory biopsy. In the C1 category, there were six carcinomas (four ductal, one lobular, one metaplastic), two columnar cell change and one fat necrosis.
Conclusion: Our results confirm the accuracy of FNA in the diagnosis of malignancy and show the major importance of a multidisciplinary approach and knowledge of the clinical and radiologic data by the interpreters of the FNAs.

0207
Benign renal tumors mimicking malignancy in fine needle aspiration biopsy samples: report of three cases

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Objective: Fine needle aspiration biopsy (FNAB) is a well-established method to diagnose renal tumors. However, it is challenging to diagnose rare benign renal tumors with unusual cytomorphology mimicking malignant tumors. We report three such cases.
Method: All three kidney tumors were discovered by ultrasound examination, and ultrasound-guided FNAB was performed. The first case was a multicystic renal tumor in the right kidney of a 64-year-old woman. The second case was an atypical cyst in the left kidney of a 45-year-old man. The third case was a solid tumor in the left kidney of a 24-year-old woman.
Results: Case 1: Papillary-like structures and macrophages, supplemented by immunocytochemistry, rendered a diagnosis of renal carcinoma, papillary type, while disregarding stromal fragments. Histological diagnosis was a mixed epithelial and stromal tumor (MEST). Case 2: Similar morphology led to the same cytological diagnosis as in the first case with subsequent histological diagnosis of a cystic nephroma. Case 3: Small groups of severely atypical cells were suspicious of a malignant tumor, possibly a metastatic one. The histological diagnosis was juxtaglomerular tumor, confirmed immunohistochemically and by electron microscopic examination only after additional clinical data about unexplained arterial hypertension from the age of 15 were obtained.
Conclusion: Cytologic samples of cystic renal tumors should be evaluated cautiously, including the possibility of MEST/cystic nephroma group of tumours. The case of juxtaglomerular tumour shows the utmost importance of reporting the relevant clinical data to the pathologist.

0208
Cytological features of embolized meningiomas

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Objective: In some meningiomas, primarily with a large vascular supply, preoperative embolization is performed a

few days before surgery in order to soften the tumor and minimize intraoperative bleeding. Post-embolization histological changes consist mainly of necrosis, ischemic cellular changes, vascular fibrinoid necrosis and increased proliferative index. These changes may result in overgrading. We have found no description of such changes in the cytological literature.

Method: We reviewed 22 cases of meningiomas with prior embolization. In 13 of them, relevant cytological changes induced by embolization were present. Cytological material was obtained during intraoperative consultation, either by scrapping or squashing of tissue samples. On histology, all of them were grade I meningiomas and showed intravascular embolic material.

Results: Cytology revealed relevant cellular dissociation with frequent single cells. Ischemic cellular changes were a common finding and consisted of cell shrinkage, nuclear piknosis and ill-defined cytoplasmic limits. Ischemic changes were better appreciated in single cells. Eight cases showed histiocytes and neutrophils. Confluent areas of necrosis were seen in one case. None of the cases showed cellular atypia or significant mitotic activity. Embolic material was seen cytologically in four cases. Of the 22 embolized meningiomas, nine of them showed no significant cytological abnormalities.

Conclusion: Embolization of meningiomas induces cytolomorphological changes that may be relevant in some cases. Greater cellular dissociation and ischemic changes, as well as inflammation, may result in a worrisome cytological image. When faced with such changes, the pathologist should always consider the possibility of embolization, avoiding overgrading or misdiagnosis.

0210

Cytological and clinical statistic correlations in the evaluation of periodontal disease at the diabetic patients with infectious gastritis

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Objective: The cytodiagnostical marks for periodontal disease stood at the basis of the lesion framing at the diabetic patients with infectious gastritis.

Method: Cytological smears have been gathered in a period of 6 years (2004–2009) from 77 diabetic patients, *Helicobacter pylori*-positive, with a diagnostic of chronic periodontal disease. The diagnosis was made with radiological exam and clinical analysis. The samples were air-dried or alcohol-fixed before staining with cytological methods (Papanicolaou, Blue-Polichrom-Tanin-Dragan). The statistical comparison of different parameters was made using Mann–Whitney *U* test and Spearman rank order correlation, with the aim of establishing statistically relevant differences and Spearman correlation coefficients (*r*) between glucose in blood and periodontal disease, age- and sex-related, the length of the diabetes disease, the cytological periodontal stage and the length of the periodontal disease, age-related.

Results: The statistical calculation has revealed important differences of the blood glucose between the cytological periodontal stages 1 and 2 ($p<0.05$), 1 and 3 ($p<0.01$), and 1 and 4 ($p>0.00000$). In a similar way, there were also differences between stages 2 and 3 ($p<0.01$), 2 and 4 ($p>0.00000$), and 3 and 4 ($p<0.01$).

Conclusion: The neutrophils that have been drawn by the presence of bacteria or by the erosion areas of the oral and gastric mucosa can promote an increased gravity of the epithelial lesions, suggesting a particularity of the microbial infection at the diabetic patient who has hyperglucidic stress with the modification of the cellular and humoral immunity in the background.

0212

Peritoneal effusions analysis in malignant diseases: comparison of various biochemical tests with ascitic cirrotics

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Objective: Treatment of malignant peritoneal effusions is generally palliative; therefore, quality of life issues, as well as the risks and benefits of the therapeutic options, become more critical. Cytomorphologic examination alone provides only limited sensitivity for the detection of metastatic carcinoma cells in many cases of serous effusions. Early diagnosis and management of peritoneal metastases from cancer patients represent new directions of researches.

Method: The current study was aimed at differentiating peritoneal liquids encountered in 81 available cases, chosen to show both biochemical patterns (benign and malignant) and in this way to achieve a diagnostic value of the biochemical method. A panel of 17 biochemical markers (TP, ALB, LDH, TC, GL, TL, TG, AA, ALP, U, TB, DB, AST, ALT, Mg, Fe, K) were determined from the resulted supernatant after centrifugation in blood and peritoneal fluid using automatic and semi-automatic biochemistry analyzer.

Results: Thus, of all measured parameters, the highest accuracy in the differential diagnosis between malignant and the benign cases (cirrhosis) was obtained by measuring

peritoneal effusions TC (90%), peritoneal fluid LDH (85.36%) and SAAG (85%).
Conclusion: It is concluded that a suitably chosen panel, consisting of the best specific markers found, can be of great value for the initial differentiation and subsequent guidance in the diagnosis. Abbreviations: *TP* total proteins, *ALB* albumin, *LDH* lactate dehydrogenase, *TC* total cholesterol, *GL* glucose, *TL* total lipids, *TG* trglycerides, *AA* alpha amylase, *ALP* alkaline phosphatase, *U* urea, *TB* total bilirubin, *DB* direct bilirubin, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *Mg* magnesium, *Fe* iron, *K* potassium, *PE/S* effusions/serum ratio, *SAAG* serum-ascitic albumin gradient, *SAG* serum-effusion gradient.

Thursday, 2 September 2010, Basement

PS-11 Poster Session Endocrine Pathology

0213

Hyalinizing trabecular tumor of the thyroid mimicking papillary thyroid carcinoma

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Objective: Hyalinizing trabecular tumor (HTT) is an unusual type of thyroid follicular neoplasm that may represent a major diagnostic problem in routine thyroid practice.
Method: We present the case of a 31-year-old woman in which a fine needle aspiration (FNA) was performed for a 2.2-cm unique nodule, incidentally discovered in the left thyroid lobe. A hemi-thyroidectomy was performed as the aspiration specimen was reported suspicious for papillary thyroid carcinoma (PTC). Macroscopically, the nodule was light tan and well circumscribed. In microscopy, it had prominent trabecular architecture and cytological features strongly suggesting a PTC: nuclear enlargement with particularly abundant nuclear grooves and cytoplasmic invaginations. An intratrabecular abundant, homogenous and eosinophilic hyaline material was also noticed. Immunohistochemistry, including anti-thyroglobulin, anti-TTF1, anti-HBEM1, anti-CK19 and anti-MIB1 antibodies, was performed.
Results: The tumor cells were strongly positive for thyroglobulin and TTF1 and were negative for HBEM1, CK-19 and chromogranin. A characteristic membranous and peripheral cytoplasmic staining was noticed with MIB1 antibody.
Conclusion: Despite the cytological aspect, a diagnosis of HTT was made based on the well-circumscribed character of the tumor and the negativity for HBEM1 and CK-19. Moreover, the unique reactive pattern of MIB1 was an important clue to the diagnosis. Careful evaluation is needed in such cases in which cytological criteria are too obviously suggesting a PTC. The differential diagnosis is essential because unlike PTC, HTT is considered a benign tumor and has a different behavior and therapeutic approach.

0214

Fine needle aspiration is a reliable method in the preoperative evaluation of thyroid nodules

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Objective: Thyroid nodules are a common clinical problem and the fine needle aspiration (FNA) has become a standard procedure for their clinical triage. Its widespread use allows a better selection of patients in need of surgical treatment.
Method: Four thousand three hundred fifty-nine FNA were performed between 1998 and 2008, always by the same pathologist using a 27-gauge needle and a Cameco suction device. At least three aspirations were carried out, and three to five slides were obtained for each nodule. They were immediately fixed in alcohol and stained with hematoxylin–eosin or with Papanicolaou. The cytological diagnosis was classified in four categories: inadequate, benign, suspicious and malignant. Six hundred thirty-eight cases had a histological follow-up. The benefits of the cytologic diagnosis were estimated by statistical analysis (EpiInfo software version 3.4.3).
Results: The sensitivity of FNA was 79.22% and the specificity 91.54%. There were 3.46% false negative and 17.71% false positive findings. The P value was <0.0001, considered extremely significant. The main causes of diagnostic errors were sampling errors, similar cytological features for different entities, failure to recognize the follicular variant of papillary carcinoma, and coexistence of non-neoplastic and neoplastic processes in the same gland.
Conclusion: Although the sensitivity and the sensibility does not attain 100%, FNA proves to be the most reliable and cost-effective method for distinguishing benign from suspicious or malignant thyroid lesions. Limitations of the method included false negative, non-diagnostic and indeterminate or "suspicious" results. Nevertheless, the routine use of FNA reduces the rate of unnecessary surgery for thyroid nodules.

0215

Mixed medullary and papillary carcinoma of the thyroid gland. Case report

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Objective: Medullary (MTC) and papillary thyroid carcinoma (PTC) have always been considered different from each other, in their cell origin, histopathological features and incidence. We describe one rare case with simultaneous multicentric PTC, classic or oncocytic type, in one lobe and "composite thyroid carcinoma" with mixed features of MTC and PTC in the other one. These tumors were associated with Graves' disease.
Method: Surgical specimens were routinely processed, paraffin-embedded, and HE-, van Gieson- and Grimelius-stained. Immunohistochemical study included antibodies anti-cytokeratin 7 (CK7), chromograninA (CgA), thyroglobulin (TG), calcitonin (CT), and HBME1.
Results: In the right lobe, there were two, tan brown, nodular lesions measuring 1.5 and 0.7 cm, respectively, consisting of PTC oncocytic type with follicular and focal papillary pattern and a classic variant of PTC. In the lower pole of the left lobe, a grayish nodule of 0.6 cm, with trabecular, follicular and focal papillar architecture was detected, presenting small tumor cells, pleomorphic nuclei and occasional cytoplasmic pseudoinclusions. Tumor cells showed intense positive expression for TG, CK7 and moderate reactivity for CgA and CT. HBME1 was focally positive with membrane staining along lateral and abluminal surfaces.
Conclusion: Our case report emphasizes the role of detailed histopathological analysis, especially when thyroid nodules harbor various aspects, and the use of immunohistochemistry in the identification of rare types of thyroidian neoplasm. This case might be explained by the possibility of activating a common tumorigenic pathway for both follicular and parafollicular thyroid cells or a common stem cell.

0216

Thyroid metastasis of clear cell renal cell carcinoma: a series of three cases

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Objective: Thyroid metastases are extremely rare, the kidney being the most frequent primary tumor site.
Method: We report three cases of thyroid metastasis of clear cell renal cell carcinoma (CC-RCC), one of them being the primary manifestation of a renal tumor and the other two with a previous 9- and 10-year, respectively, history of renal neoplasm. The first case, a 64-year-old man, was admitted to the hospital with an initial diagnosis of bilateral goiter, while for the other two, a 72-year-old man and an 80-year-old woman, the discovery was incidental.
Results: On gross examination, white grayish ill-circumscribed solid nodules were described in the right lobe (one nodule, first case), in the left lobe (two nodules, second case) and in both lobes (multiple nodules, third case). All three cases presented similar microscopic tumoral aspects: a solid architecture, polygonal cells with abundant clear or eosinophilic cytoplasm, distinct cell borders and moderately enlarged nuclei with proeminent nucleoli. Since the microscopic aspect did not correspond to any primary tumor of the thyroid, immunohistochemistry was performed. In all cases, the tumor cells stained positive for CD10 and negative for thyroglobulin, supporting the diagnosis of a thyroid metastasis of CC-RCC.
Conclusion: Thyroid metastases of CC-RCC are uncommon and may represent the first sign of a yet undiagnosed asymptomatic renal tumor or a metastasis from a tumor surgically removed years before (up to 10 years). In setting a correct diagnosis, immunohistochemical studies together with the medical history are extremely important.

0221

Primary angiosarcoma of the thyroid gland. A case report

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Objective: Primary angiosarcoma of the thyroid is a rare and controversial entity most commonly seen in patients from the Alpine regions with a long-standing history of colloid goiter. Purpose: We describe a case of primary, poorly differentiated angiosarcoma in a 57-year-old male who presented with a rapidly enlarging thyroid mass and mild dysphagia during the preceding month.
Method: A CT scan of the neck revealed a large tumor mass in the upper pole of the left lobe of the thyroid measuring 6.5×5.5×7 cm extending into the surrounding adipose tissue and infiltrating the left internal jugular vein. Lymphadenopathy was identified in the left submandibular region and lower neck. Cytomorphologically, the aspirate was cellular, with variably cohesive clusters of atypical spindle cells and necrotic masses. Thyreoidectomy procedure was performed. Histologically, the tumor was composed of spindle, polygonal and epithelioid cells with vesicular nuclei and prominent nucleoli growing in solid sheets and occasionally forming cleft-like vascular spaces

filled with erythrocytes. Extensive necrosis was present. Mitotic activity was 28 mitoses/10 HPF. Immunohistochemical stains showed tumor positivity for CD31, vimentin, factor VIII, WT-1, M-ACT and CD99.
Conclusion: The prognosis in primary angiosarcoma of thyroid is generally poor especially if the tumor penetrates the capsule of the thyroid or if there are metastases present. The patient developed further metastases in the cervical lymph nodes within few weeks after thyroidectomy despite radio- and chemotherapy. Due to the rapid progression of the disease, the patient was scheduled for palliative radiotherapy. The patient succumbed to the disease 14 months after diagnosis.

0223

A histological and immunohistochemical analysis of 39 cases of adrenal pheochromocytoma

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Objective: Pheochromocytoma can be defined as a paraganglioma of the adrenal medulla and has been called the "10% tumor" because approximately 10% are bilateral, 10% are extra-adrenal, 10% occur in children, and 10% are malignant. Aim: In this report, we described the histopathological aspects of pheochromocytoma in correlation with immunohistochemical reaction.
Method: The study group included 39 cases of pheocromocytoma diagnosed by the chromaffin reaction, HE and van Gieson's reaction. Immunohistochemistry included monoclonal antibodies: chromogranin A, neuron-specific enolase and S100 protein.
Results: The 39 patients have encapsulated, usually soft, and, on section, yellowish-white to reddish-brown tumors. The larger tumors had areas of necrosis, hemorrhage and cyst formation. Histopathologically, the tumor cells are characteristically arranged in well-defined nests (zellballen) bound by a delicate fibrovascular stroma, which may contain amyloid. The cells vary considerably in size and have a finely granular basophilic or amphiphilic cytoplasm. The nuclei, usually round or oval with prominent nucleoli, may contain inclusion-like structures. Lipid accumulation may develop in the cytoplasm and lead to confusion with adrenal cortical tumors. Giant, hyperchromatic nuclei are common and are not an expression of malignancy. Chromogranin A and NSE were positive. Sustentacular cells form a peripheral coat around the "zellballen" and were strongly reactive for S100 protein.
Conclusion: All our cases were benign, although 10% of pheochromocytomas are malignant and have a marked tendency to metastasize to skeletal system, particularly the ribs and spine. The sustentacular cells are more numerous in pheochromocytomas associated with MEN than in sporadic cases.

0225

Primary carcinoid tumor of gallbladder

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Objective: Primary carcinoid tumor of gallbladder is a rare neoplasm. They usually present as a polypoid lesion.
Method: We report an incidentally found carcinoid tumor of gallbladder located in the cystic duct occurring in a 79-year-old male patient detected in cholecystectomy material.
Results: Histopathologically, in cystic duct, an incidental neoplasm measuring 2 mms in maximum diameter was detected, which was grossly unremarkable. The tumor constituted glandular structures. It was not invading muscular layer. Immunohistochemically tumor was positive for synaptophysin. Patient is alive and well after 8 months.
Conclusion: Definite diagnosis of carcinoid tumor of gallbladder is usually made on histopathological examination after surgery. Our case is unique in that it was found incidentally, which was grossly unremarkable. It seems so that surgery alone is a reliable definite management for these small carcinoid tumors.

Thursday, 2 September 2010, Basement

PS-12 Poster Session Molecular Pathology

0226

KRAS genotyping on formalin-fixed, paraffin-embedded (FFEP) colorectal cancer tissue in diagnostic routine: comparison of methods

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Objective: Accurate identification of KRAS mutations has great importance for target therapy. Reproducibility and cheapness of different methods are crucial to be safely applied in colorectal routine diagnostic setting.
Method: One hundred twelve FFEP specimens, previously studied by ARMS/Scorpions real-time PCR (Therascreen, Roche, Italy) designed as reference method, were in parallel analysed by other two methods: restriction fragment length polymorphism-PCR (Ampli-set-K-Ras, Bird, Italy) and PCR/reverse hybridization tests (K-Ras

StripAssay, ViennaLab, Austria), respectively, called methods A and B. The methods were compared considering the results, costs, and working times. Regarding KRAS genotype, 112 selected samples were: 40 wild type (wt), 40 mutated in codon 12, and 32 in codon 13.
Results: Six of 40 wt samples showed mutation on codon 12: Three were false positives of method A and three of method B. Two of 40 samples mutated in codon 12 resulted also positive on codon 13 by method B. Since method A is based on cleavage action of the restriction enzyme, we have increased both restriction enzyme concentration and digestion time, abolishing the false positives. On the contrary, there was no way of acting on method B.
Conclusion: Although method A does not define the codon 12 mutation type and is time-consuming, it leads to the same results of the reference method operating our slight methodological modifications. Method A resulted cheap (45% of the reference kit price), useful and reproducible for routinely KRAS testing in colorectal target therapy. Work was supported by Fondazione Cassa di Risparmio di Puglia, Italy.

0227

Mismatch repair proteins expression and BRAF V600E mutation analysis in a subset of CCR patients under 50 years

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Objective: Colorectal carcinoma (CRC) is a worldwide common malignancy; young patients represent a third of all cases, with a rise over the last years. Fifteen percent of CRC have microsatellite instability (MSI) due to alterations in mismatch repair (MMR) genes. Familiar MSI cases (5%) have germline mutations. Sporadic MSI CRC (15%) have hypermetilated MLH-1 promoter. BRAF V600E mutation exclusively occurs in sporadic MSI CRC. Immunohistochemistry detects MSI cases with high concordance with microsatellite analysis. The objective was to compare histopathology and patterns of expression of MMR proteins and BRAF V600E mutation in patients below and over 50 years old and determine if age is a risk factor for defective MMR protein expression and BRAF mutations.
Method: One hundred six patients <50 years were retrieved. Age, sex, location, histologic type, TNM, infiltration and metastatic lymph nodes data were collected. MLH1 and MSH2 immunohistochemistry and BRAF RT-PCR mutation was performed in 48 <50 years and 48 patients >50 years as control group. Student's t, χ^2 and logistic regression analysis were carried out.
Results: Medullary and mucinous types were more frequent among young patients and intestinal type in older patients ($p=0.0008$). No differences were noticed regarding clinicopathological stages between groups. MMRP expression was absent more frequently in <50 and MSH2 was negative in 13.8% of these patients. No BRAF mutations were detected in any group.
Conclusion: We found an association between young age and defective MMR expression (OR 4.28). MSH2 lack of expression is more frequently due to a germline mutation. The fact that none of our patients had BRAF mutation could be partly due to the small sample size or alternate V600E or K-ras mutations.

0228

VEGFA amplification correlates with adverse outcome in colorectal cancer

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Objective: Angiogenesis plays an important role in the progression of colorectal cancer (CRC). Evidence from preclinical and clinical studies indicates that vascular endothelial growth factor (VEGFA) is the predominant angiogenic factor in CRC. VEGFA is expressed in approximately 50% of CRCs, with minimal to no expression in normal colonic mucosa. Therefore, the aim of this study was to analyze the prognostic impact of VEGFA amplification in CRC.
Method: VEGFA gene amplification was evaluated in a large series of sporadic CRC resections ($n=1,280$) by FISH analysis using the tissue microarray technique. Spectrum Green-labeled probes originated from the FISH Clone RP1-261G23 were used together with Spectrum Orange-labeled probes for the respective centromere 6 as a reference. The VEGFA amplification status was compared to relevant clinicopathological features.
Results: VEGFA amplification was detected in 39 patients (3%) and was significantly associated with higher T stage and higher tumor grade, presence of vascular invasion, right-sided location ($p<0.001$) and B-RAF mutation ($p=0.015$). Additionally, VEGF amplification was associated with worse survival in univariate ($p<0.001$) and multivariable analysis ($p<0.001$; HR (95% CI), 2.06 (1.4–3.0)).
Conclusion: VEGFA amplification seems to highlight a small subset of CRCs with aggressive phenotype. Therefore, FISH analysis of VEGFA could represent an alternative evaluation system for identifying patients with poorer

clinical outcome who could be candidates for anti-VEGFA therapies.

0229

K-RAS and B-RAF mutation detection in routine diagnostic analysis of colorectal cancer in the Greek population

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Objective: K-Ras oncogene is frequently mutated in colorectal cancer and is currently established as a predictive biomarker for anti-EGFR targeted therapy. B-Raf is a serine/threonine kinase of the RAS/RAF/MEK /ERK signal transduction pathway which is mutated in a subset of K-Ras wild-type patients with colorectal cancer. The aim of this study was to examine the mutational status of K-Ras and B-Raf genes in correlation with tumor clinicopathological characteristics.
Method: DNA was extracted from microdissected formalin-fixed paraffin-embedded tissues. Three hundred forty-four cases were screened for K-Ras and B-Raf mutations at codons 12 and 13 and exon 15 respectively using high-resolution melting analysis, sequencing and/or pyrosequencing. Statistical analysis was performed using STATA for Windows.
Results: K-Ras mutations in codons 12 and 13 were present in 133 out of 344 cases. The most frequent types of mutation were pG12D and pG12V at codon 12 and pG13D at codon 13. B-Raf mutations in exon 15 (pV600E) were present in 4.6% of analyzed samples. B-Raf mutations were observed in a marginally higher frequency in women, whereas K-Ras mutations were positively correlated with patients' age. Moreover, there was a higher prevalence of B-Raf mutations in low-grade carcinomas ($p=0.005$).
Conclusion: The detected mutation frequency as well as the prevalence of specific mutation types is in accordance with previous studies. The observed correlation of B-Raf mutation with tumor grade implies its importance as a marker of tumor aggressiveness. The applied methods (HRM and pyrosequencing) were proven to be sensitive, fast and accurate for mutation detection in a clinical setting.

0230

Mutational analysis of PIK3CA and AKT1 genes in Greek patients with urothelial bladder carcinoma

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Objective: Aberrant activation of the phosphatidylinositol-3-kinase (PI3 kinase)-AKT pathway is frequently observed in a wide range of cancers such as breast, colorectal, ovarian and lung. Several components of this pathway such as PI3K and AKT can constitute potential therapeutic targets, and many small molecule inhibitors are in development on early clinical trials. The aim of this study was to examine the possible significance of somatic point mutations in PIK3CA and AKT1 genes as biological markers or therapeutic targets in primary urothelial bladder carcinoma.
Method: One hundred twenty-two urinary bladder cancer specimens were screened for activating mutations in exons 9, 20 of the PIK3CA gene as well as in exon 4 of AKT1 gene by PCR-SSCP and high-resolution melting analysis. Mutations were identified for PIK3CA gene with sequencing and for AKT1 gene with pyrosequencing and statistical analysis was performed.
Results: Four different mutations were detected in exon 20 of PIK3CA gene (3.3%). The mutations were identified as p.A1035T, p.A1046V, p.H1047R and p.G1049R. One out of 120 cases was mutant in exon 9 of PIK3CA gene (0.8%, p.E542K). The AKT1 p.E17K mutation was identified in 2 out of 105 bladder tumours (1.9%). Statistical analysis did not reveal any correlation with clinicopathological parameters ($p>0.10$).
Conclusion: Mutations in PIK3CA gene are quite frequently observed in bladder cancer, implying their importance as potential targets for anticancer treatment. On the other hand, mutations in AKT1 gene are not a common event in this type of cancer.

0231

Analysis of DNA integrity, morphology, antigenicity with different preservation methods in brain tissue

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Objective: Due to the potential carcinogenicity of formaldehyde, its replacement by substitute fixatives is recommended and could increase the possibility for application of molecular biology approaches. The aim of this study was to examine DNA preservation, morphology and antigenicity of brain tissues after fixation with a formalin-free fixative in comparison with formalin-fixed tissues as well as frozen ones.
Method: Calibrated specimens from six different areas (frontal, parietal, occipital lobes, hippocampus, cerebellum and basal ganglia) of 20 brains from elderly individuals (age > 70) were fixed for 24 h in two different fixatives (formalin, formalin-free fixative-RCL2) at room tempera-

ture. Tissues were stained with hematoxylin–eosin for histological examination and their antigenicity was determined by immunohistochemistry. The integrity of DNA extracted from the samples with formalin fixation or formalin-free fixation was compared with that of cryosections with gel electrophoresis and the Bioanalyzer. Furthermore, PCR-PFLPs and real-time PCR (high-resolution melting analysis) were performed in order to assess the suitability of the extracted DNA for downstream molecular pathological applications.

Results: Immunohistochemistry with antibodies against b-amyloid and tau did not reveal any significant differences in reactivity for both fixatives. On the other hand, DNA extracted from frozen samples was of comparable integrity with that from formalin-free fixation. Amplification efficiency for short DNA fragments (140–240 bp) was similar between all preservation methods; however, long fragments (854 bp) could not be amplified from formalin-fixed tissues.

Conclusion: The formalin substitute fixative used in this study provided good histopathological quality, and also the extracted DNA performed better at the subsequent molecular biological procedures.

0232

Mutational analysis of K-ras, B-raf, EGFR, PIK3CA, AKT-1 gene and pERK expression in carcinogenesis of esophagus

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Objective: The molecular mechanisms underlying the development of esophageal cancer remain elusive. The aim of this study was to examine the simultaneous presence of alterations in two main signal transduction pathways, Ras/Raf/MEK/ERK and PI3K/AKT, which are implicated in carcinogenesis. The prognostic significance of pERK expression was also investigated.

Method: Genomic DNA was extracted from 32 paraffin-embedded tissue blocks (9 squamous-cell, 2 adenosquamous cell and 21 adenocarcinomas). Additionally, in 15 cases, tumor cells were collected using laser microdissection in order to minimize contamination with stromal and normal cells. We searched for somatic mutations in K-ras, B-raf, EGFR, AKT-1 and PIK3CA genes by performing high-resolution analysis and pyrosequencing. The expression of activated ERK protein was assessed by immunohistochemistry.

Results: K-ras mutation at codon 12 was detected in one laser-microdissected adenocarcinoma out of 32 specimens (3%), whereas no mutations were found in exon 15 of B-raf gene, in exons 19 and 21 of EGFR gene, in exon 4 of AKT-1 gene and in exon 20 of PIK3CA gene. pERK nuclear expression was positively correlated with disease grade and stage and nuclear staining intensity with grade. Furthermore, pERK cytoplasmic immunopositivity was correlated with tumor grade.

Conclusion: Mutations in K-ras gene are not frequently detected in esophageal cancer, but do exist. The lack of mutations in B-raf, EGFR, AKT-1 genes implies that these may not play an important role in the pathogenesis of esophageal cancer. The correlation of pERK nuclear expression with stage and grade implicates pERK as a marker of local tumor aggressiveness.

0233

Comparison of three methods for "KRAS" mutation testing in patients with metastatic colorectal cancer

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Objective: Mutation analysis in RAS/RAF signaling pathway plays a key role in treatment decision in patients with metastatic colorectal cancer (mCRC). At this time, several methods are available for *KRAS* mutation detection; there are also commercially available kits, but validated methods and standardized testing procedures are still lacking.

Method: Genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissues after the evaluation of material by pathologists. For mutation analysis of *KRAS* gene, we used three methods: direct sequencing, allelic discrimination and high-resolution melting (HRM).

Results: We tested 21 tumor samples (primary tumors and metastasis) from 15 patients with mCRC. We identified *KRAS* mutation in 5 of 15 (33%) patients using allelic discrimination, while HRM detected mutations in 6 of 15 (40%) patients. Due to low burden of tumor cells in two cases and limited sensitivity of sequencing, only 13 patients were evaluated by this method. Out of them, four (31%) patients showed positivity. We performed sensitivity testing: allelic discrimination (5% of mutant DNA), HRM (10%) and sequencing (25%).

Conclusion: Allelic discrimination is a convenient, fast and sensitive method designed for the detection of specific mutations. Test costs are proportional to the number of tested mutations since a specific assay has to be used for each mutation. Sequencing has a limited application due to low sensitivity, while HRM represents a sensitive, rapid and cost-limited methodology, but displays higher rate of false positive results if DNA is in poor quality. This technique is suitable for routine screening; however, precise identification of mutations is not possible. Therefore, a combination of at least two methods is highly recommended.

0234

O6-methyguanine-DNA methyltransferase evaluated by immunohistochemistry: best practice for clinical and research assessment

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Objective: MGMT repairs DNA damages and act as a tumor suppressor gene in normal cells, preventing DNA mutation. Several different approaches for MGMT immunohistochemical (IHC) testing have been used, resulting in a not universally accepted standard. We evaluated the IHC expression of MGMT using five different primary antibodies in 59 invasive breast carcinomas in order to establish the most reliable marker for this protein. The results of immunostaining were compared to qRT-PCR, used as a parameter of reliability of gene expression.
Method: Cases were randomly selected to build a tissue microarray. The five primary antibodies to MGMT were provided by: MT3.1 (NeoMarkers, GeneTex and Santa Cruz), SPM287 (Santa Cruz) and MT23.2 (Zymed). Heat-induced antigen retrieval in citrate and the Advance™ (Dako) detection system were used. IHC was visually analyzed by microscope and automated analyzed by software applied to digital slides. qRT-PCR was performed in all tumors for transcript expression quantification.
Results: Antibody SPM287 (Santa Cruz) showed the highest sensitivity ($p<0.001$), and antibody MT3.1 (Santa Cruz) showed the least sensitivity ($p<0.001$). Antibody MT23.2 (Zymed) showed higher levels of cytoplasm staining, which was not observed in the other antibodies tested ($p<0.001$). qRT-PCR results showed that 94.9% of the samples showed hypoexpression of MGMT when compared to normal breast ($p<0.001$). SPM287 (Santa Cruz) was the only antibody which showed a positive and significant correlation with the results obtained by qRT-PCR ($p=0.027$).
Conclusion: This antibody seems to be reliable and effective for research and clinical practice in breast cancer. (Supported by FAPESP and CNPq).

0235

O6-methyguanine-DNA methyltransferase evaluated by immunohistochemistry: best practice for clinical and research assessment

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Objective: MGMT repairs DNA damages and act as a tumor suppressor gene in normal cells, preventing DNA mutation. Several different methods for MGMT immunohistochemical (IHC) testing have been used, resulting in no universally accepted standard. We evaluated the IHC expression of MGMT of five different primary antibodies in invasive breast carcinomas.
Method: Fifty-nine breast carcinomas were randomly selected for a TMA construction. Five different primary antibodies against MGMT were used for the IHC study: clone MT3.1 (NeoMarkers, GeneTex and Santa Cruz), SPM287 (Santa Cruz) and MT23.2 (Zymed). Heat-induced antigen retrieval in citrate and Advance™ IHC detection system were used. IHC was visually analyzed by microscope and automated analyzed by software applied to digital slides. qRT-PCR was performed in all tumors for transcript expression quantification.
Results: Antibody SPM287 (Santa Cruz) showed the highest sensitivity ($p<0.001$), and antibody MT3.1 (Santa Cruz) showed the least sensitivity ($p<0.001$). Antibody MT23.2 (Zymed) showed higher levels of cytoplasm staining, which was not observed in the other antibodies tested ($p<0.001$). Fifty-nine samples (94.9%) showed hypoexpression of MGMT when compared to normal breast evaluated by qRT-PCR ($p<0.001$). SPM287 (Santa Cruz) was the only antibody which showed a positive and significant correlation with the results obtained from qRT-PCR ($p=0.027$).
Conclusion: Antibody SPM 287 (Santa Cruz) presented to be the most sensitive and specific antibody for the IHC evaluation of MGMT. This antibody seems to be of reliable and effective use for research and clinical practice in breast cancer.

0239

MGMT as a potential prognostic marker in breast cancer

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Objective: MGMT repairs DNA damages, via alkylation, by removing a methyl group from the O6 position of guanine. It acts as tumor suppressor gene in normal cells and prevents DNA mutation. We evaluated MGMT expression in breast tumors, correlating it with other prognostic factors.
Method: Sixty-four cases of invasive breast carcinomas were randomly selected for a TMA construction. Immunohistochemistry (IHC) was performed for MGMT and also for ER, PR, HER2, Ki67, p53, p63, E-cadherin, CK5 and CK14 for luminal and basal phenotype classification. IHC was evaluated following the guidelines for each marker most recommended in the literature. Fluorescent in situ

hybridization (FISH) was performed in those cases considered 2+ in order to assess HER2 gene amplification status. qRT-PCR was performed in frozen tissue from our tumor bank for all cases in order to evaluate mRNA expression of MGMT.
Results: Fourteen cases were triple-negative (21.8%), and among those, seven cases were basal-like carcinomas (10.9%). Twenty-five cases (39%) were luminal-like type A, four cases were (6.25%) luminal-like type B, and one case (1.5%) was HER2-like type. MGMT showed significant lower expression in the basal-like tumors when compared to the luminal-like ones (p=0.007). Basal-like phenotype tumors presented higher positivity for p53 and Ki67 than the luminal types (p=0.025 and p=0.003, respectively). Positive p53 and high Ki67 tumors showed significant lower expression of MGMT (p=0.0184 and p=0.0081, respectively).
Conclusion: MGMT assessment by IHC or molecular biology techniques may represent an important prognostic factor in breast cancer.

0240

Anticol11a1 a marker of infiltration in bronchioloalveolar lung carcinoma

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Objective: The col11A1 gene codifies a protein expressed during cartilage and osteomorphogenesis which is present in the setting of several types of infiltrating malignant desmoplasia. Our team generated a monoclonal antibody against procol1A1 which was tested for infiltrating ductal pancreatic carcinoma versus chronic pancreatitis differential diagnosis and infiltrating ductal carcinoma of breast versus sclerosing adenosis differential diagnosis.
Method: To investigate its usefulness in order to assess the presence of invasion in bronchioloalveolar lung carcinomas (BAC), we carried out immunostaining with the monoclonal antibody antiCol11A1 in a series of nine BAC and six lung adenocarcinomas with bronchioloalveolar pattern.
Results: The results show lack of staining in seven of nine BAC, whereas all six adenocarcinomas with bronchioloalveolar pattern did stain (significance, p=0.0034).
Conclusion: Our conclusion is that the monoclonal antibody AntiCol11A1 could be a useful marker of invasion in BAC when invasion is doubtful.

0241

PARP-1 Val762Ala polymorphism is associated with reduced risk of non-Hodgkin lymphoma in Korean males

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Objective: Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme that plays a role in DNA repair, differentiation, proliferation, and cell death. The polymorphisms of PARP-1 have been associated with the risk of various carcinomas, including breast, lung, and prostate. We investigated whether PARP-1 polymorphisms are associated with the risk of non-Hodgkin lymphoma (NHL).
Method: Subjects from a Korean population consisting of 573 NHL patients and 721 controls were genotyped for five PARP-1 polymorphisms (Asp81Asp, Ala284Ala, Lys352Lys, IVS13+118A>G, and Val762Ala) using high-resolution melting polymerase chain reaction (PCR) and an automatic sequencer.
Results: None of the five polymorphisms were associated with overall risk for NHL. However, the Val762Ala polymorphism was associated with reduced risk for NHL in males [odds ratio (OR), 0.62; 95% confidence interval (CI), 0.41–0.93 for CC genotype and OR, 0.84; 95% CI, 0.60–1.16 for TC genotype], with a trend toward a gene dose effect (p for trend, 0.02). Asp81Asp (p for trend, 0.04) and Lys352Lys (p for trend, 0.03) polymorphisms revealed the same trend. In an association study of PARP-1 haplotypes, haplotype ACAAC was associated with decreased risk of NHL in males (OR, 0.75; 95% CI, 0.59–0.94).
Conclusion: The present data suggest that Val762Ala, Asp81Asp, and Lys352Lys polymorphisms and the haplotype-ACAAC in PARP-1 are associated with reduced risk of NHL in Korean males.

0242

H2AFX polymorphisms are associated with decreased risk of diffuse large B cell lymphoma in Korean

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Objective: H2AFX is a core histone involved in the cellular response to DNA double-strand breaks and nucleosomal organization of chromatin. Its polymorphisms have been associated with the decreased risk of non-Hodgkin

lymphoma (NHL, -417AA) and increased risk of breast cancer (−1654AG/GG and −1420GA/AA).
Method: To investigate the effect of H2AFX polymorphisms on NHL risk in Koreans, six polymorphisms were tested for association in 573 NHL patients and 721 cancer-free controls. The five polymorphisms were located in the promoter region, −1654A/G (rs643788), −1420G/A (rs8551), and −1187T/C (rs7759), −676T/G (rs2509851), and −417G/A (rs2509049). Another one, 1057C/T (rs7350), was in the 3′UTR.
Results: The −1420AA genotype was associated with decreased DLBCL risk (OR, 0.65; 95% CI, 0.43–0.97), and there was a trend for allele dose effect (p trend=0.026). The A allele showed decreased risk when compared with the G allele (OR, 0.81; 95% CI, 0.67–0.98). The −1187CC genotype demonstrated a decreased DLBCL risk with borderline significance (OR, 0.70; 95% CI, 0.48–1.02). And there was a trend for an allele dose effect with borderline significance (p trend=0.06). The T allele revealed an increased risk (OR, 0.84; 95% CI, 0.70–1.00) than the C allele. However, there were no associations between the polymorphisms and the risk of overall NHL, all B cell lymphoma, or all T cell lymphoma, respectively.
Conclusion: The results suggest that H2AFX −1420AA, and −1187CC genotype might be associated with the decreased DLBCL risk in Korean people.

0243
p14ARF–MDM2–p53 immunohistochemical surveillance in retinoblastoma

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Objective: Retinoblastoma presents between 3 and 18 months in heritable cases and 18 to 24 months in sporadic cases. The heterozygotic Rb^{+}/Rb^{-} requires a single silencing mutation of the heritable functioning Rb allele to subsequent tumor development as pRb capacity to arrest cell cycle in G1 is lost by E2F transcription factor liberty. The immunohistochemical (IHC) expression of p53 and p14ARF together with MDM2 may be a useful way to proximate the potential disruption of p53 suppressor pathway together with the known Rb function as an easy way to explore in routine diagnosis.
Method: Material: A set of 24 FFEPE tumours was collected from HUC, Ophthalmic Pathology Laboratory, Coimbra, as clinical records of age, gender, heritable pattern, Reese–Ellsworth stage and prognosis. Methods: Antibodies against p53, p14 ARF and MDM2 were applied on a representative section of each case and a uniform semiquantitative score was registered (−%, + <25%, ++ 25–70%, +++ >70% cells).
Results: There was general positivity for all antibodies: p53 (21/21), p14ARF (21/24), MDM2 (23/24). All p14ARF-positive cases also expressed p53 and MDM2.
Conclusion: The generalized IHC expression of p53, p14ARF, and MDM2, together with the accelerated malignant evolution of retinoblastoma, stresses the function of p14ARF and MDM2 as genes interfering in the p53 suppressor pathway with aggressive behaviour in the Rb pathway loss and corroborated by p53 wild-type expression as an inefficient control. This study ought to be enlarged in order to understand the real role of the aforementioned genes through their variable crossed pathways.

0244
Cytokine effects on the cell cycle and death of lung and prostate carcinoma cells

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Objective: Since cytokines are involved in cell cycle/death regulation, we investigated the effects of TNFa, IL1β, IL13, IFNγ, IGF1 and Fas/CD95 on cell cycle/death of A549 lung and LNCAP (androgen-dependent) and PC3 (androgen-independent) prostate carcinoma cell lines.
Method: Flow cytometry (PI and PI/annexin) and Western blot were used for the analysis of cell cycle/death (apoptotic and total cell death) and protein expression, respectively. Cell death was also analyzed by Hoechst and Crystal violet staining.
Results: Flow cytometry showed that (a) the TNFa or IL1β or IL13 anti-cell death effects on Fas induced A549 cell death were attenuated by inhibitors of NFkB (BAY 117082), PI3K (LY 294002), JNK (SP600125), P38 (SB203580) and ERK (UO126) pathways; (b) TNFa or IL1β did not alter Fas-induced cell death in A549 cells with suppression of the canonical (IKKβ) or the non-canonical (IKKα) NFκB pathway; (c) Fas or TNFa increased LNCAP cell death in comparison to control; and (d) TNFa increased Fas-induced LNCAP and PC3 cell death. Western blot showed (a) cleaved PARP1 protein in Fas-treated cells, (b) TRAF1 protein and decreased IkBα protein expression in TNFa treated cells and (c) no alterations of Fas, bcl2, bclxl, bax, bak and bad protein expression.

Conclusion: TNFa, IL1β and IL13 decreased Fas-induced A549 cell death, whereas TNFa increased Fas-induced LNCAP and PC3 cell death. The anti-cell death effects of TNFa, IL1β and IL13 on Fas-induced A549 cell death were mediated, at least partially, by the NFkB, PI3K and MAP kinase pathways.

0245

LGR5 protein expression in human normal and malignant tissue

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Objective: The orphan leucine-rich repeat containing G-protein-coupled receptor LGR5 (GPR49/HG38/FEX) is a novel marker for adult stem cells in small intestine, colon and hair follicles. It is suspected to have a pivotal role in cell proliferation and tumor formation and seems to be a potential therapeutic target. LGR5 expression is poorly investigated and neither its function in stem cells nor during tumor formation is known.
Method: Our aim was to identify a LGR5 antibody with a high specificity among different providers (Epitomics®, MBL®, Sigma®, Biosite®, Abgent®) for screening selected human formalin-fixed and paraffin-embedded (FFPE) tissue samples by automated immunohistochemical staining. For proving antibody specificity, in situ hybridization with a cRNA probe of LGR5 is carried out on the same tissue samples used for immunohistochemistry. As positive control, we generate a LGR5 expressing human keratinocyte cell line (HaCaT) by retroviral transduction.
Results: The LGR5 antibody from Epitomics® stains the outer root sheath of human hair follicle in anagen, whereas Sigma-Aldrich®'s anti-LGR5 antibody recognizes LGR5 in the inner root sheath. Nerve tissue is completely negative using the Epitomics® antibody, but contrarily, the anti-LGR5 antibody from MBL® shows strong staining of Purkinje cells as well as of other nerve cells in the cortex and spinal cord. The Sigma-Aldrich® antibody also shows slight nerval staining, but nuclear. Anti-LGR5 antibodies from Biosite® and Abgent® are all-around negative under the tested conditions.
Conclusion: All antibodies were optimized but the IHC results are quite incoherent; therefore, further investigations need to be done.

0246

The mutational analysis of EGFR, PIK3CA and PTEN genes in neuroblastic tumors

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Objective: Neuroblastoma is one of the most common pediatric solid tumors. Although the signal transduction pathway PIK3/AKT/mTOR is often activated in human cancer, its role and ways of activation in neuroblastoma are not well established.
Method: The aim of the study was to analyse the mutational status of EGFR (exons 18–21), PIK3CA (exons 5, 6, 10 and 21), and PTEN, as well as to assess EGFR copy number. The study was performed on paraffin-embedded tissue sections from 106 cases of neuroblastoma. For mutational analysis, polymerase chain reaction (PCR) was used and then amplified fragments were sequenced directly. Fluorescence in situ (FISH) was used for EGFR copy number evaluation.
Results: In two tumors (2.7%), c.931 A>G (p.I311V) mutation of PIK3CA (exon 5) was found. In addition to this, six polymorphisms in EGFR c.2184+19 G>A, c.2361 G>A and c.2508 C>T, in PIK3CA c.1060-17 C>A and c.1145+54 A>G and in PTEN c.285 A>T were detected. The latter one was not described previously. The most frequent polymorphisms were EGFR c.2631 G>A (73.2%), PI3KCA c.1060-17 C>A (65.6%), and PI3KCA c.1145+54 A>G (59%); however, the incidence is comparable with population frequency in Caucasians. FISH analysis showed no EGFR gene amplification, though polysomy in many cases was observed.
Conclusion: Our results show the low frequency of mutations in genes which are involved in PIK3/AKT/mTOR pathway in neuroblastoma. Study supported by grant from Polish Ministry of Science and Higher Education (N401 176 31/3867).

0248

Metabolic phenotyping of high-grade glioma biopsies

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Objective: Glioblastoma multiforme (GBM) and anaplasic astrocytoma (AA) are astrocytic neoplastic entities of the

central nervous system that appear in adults, with high biological and clinical aggressiveness. Despite the definition of new neoplasm genetic subgroups, the most relevant information in the prognostic of the patient still comes from factors as patient age, localization and size of the tumour, oedema and mass shifting. Metabolic phenotyping may provide new information for a better management of this disease.

Method: We collected molecular profiles based on HRMAS spectra for 31 high-grade glioma biopsies (25 GBM and 6 AAs). The amount of human tumor tissue analyzed for each subject ranged from 20 to 40 mg. All samples were analyzed by post-HRMAS histopathology to assess the tissue integrity and double validate histological diagnosis.

Results: Two major metabolic groups were detected, which included 16 and 11 samples, respectively. Most AAs were located in the same group. The phospholipid patterns and the glutamine/glutamate metabolic relatives seem to be the most relevant contribution to this grouping pattern.

Conclusion: HRMAS provides high-resolution glioma molecular profiles. One of the groups detected, which include most AA samples, seems to reflect a less aggressive type of tumor. Metabolic discrimination between these subgroups includes the levels of some metabolites which can be seen by MRS 'in vivo'.

0249

Mutations in the cystic fibrosis transmembrane regulator gene and relation with IVS8-poliT and Y chromosomal microdeletions

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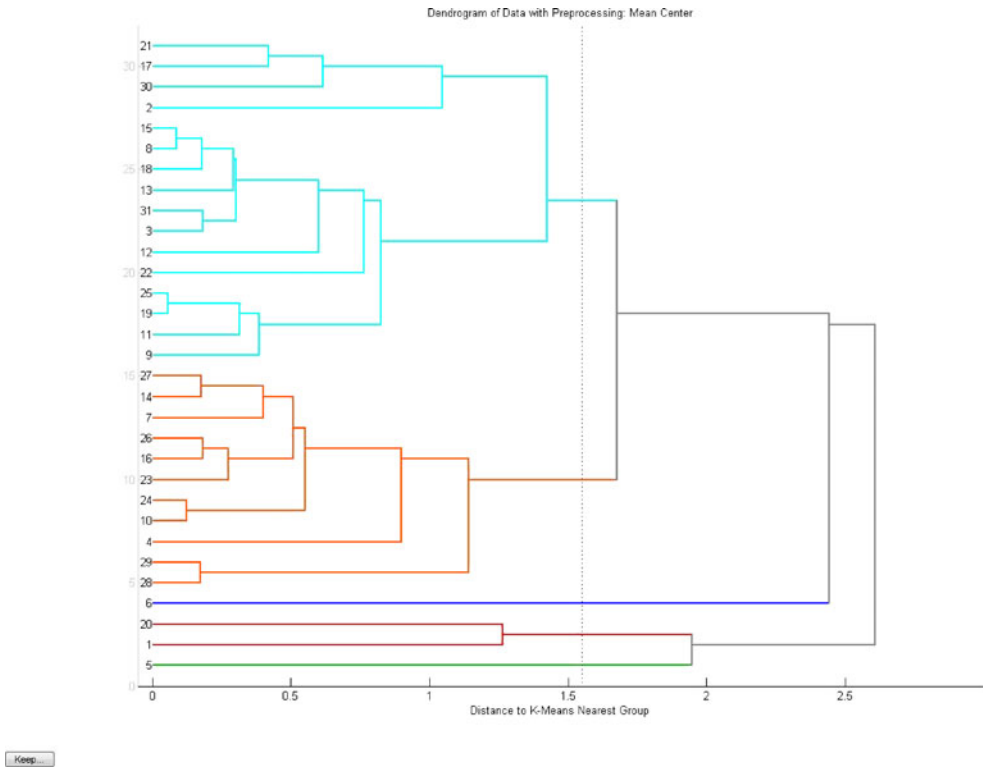
Objective: An association between cystic fibrosis transmembrane regulator (CFTR) gene mutations and infertility has been hypothesized. This study investigated the frequency of the CFTR gene mutation in a group of consecutive candidates for assisted reproductive techniques and the relation between CFTR mutation, IVS8-poliT and Y chromosomal microdeletions.

Method: Four thousand eight hundred forty-nine patients (2,817 females and 2,032 males) were screened for 53 CFTR gene mutations and IVS8-poliT polymorphism by multiplex PCR; 117/2,032 (5.8%) were azoospermic patients (APs) investigated for Y chromosomal deletion by multiplex PCR. Frequencies of mutation were separately calculated and the chi-square test was used for comparisons.



Results: CFTR mutations were detected in 4.2% of the subjects, a percentage similar to that reported in the general population. The most common mutation was ΔF508/N (1.51% of patients); only mutation N1303k/N (0.29%) showed a different gender distribution (0.54% in males and 0.11% in females, $p=0.005$). The IVS8-poly-T showed a frequency of 71.2% for 7T/7T alleles, 19.6% for 7T/9T alleles, and only 0.27% for 5T/5T alleles. One in 117 APs showed a Y chromosomal microdeletion and was negative for CFTR mutation; 4 in 117 APs had CFTR mutation. The following distribution of IVS8-poly-T polymorphism in APs was detected: 5T/5T=0.86%, 9T/9T=0.86%.
Conclusion: Our data show no evidence of associations between azoospermia, CFTR mutation, IVS8-poly-T and Y-chromosomal microdeletions.

0250
From human papillomaviruses and cervical carcinoma to HPV detection

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Objective: Human papillomaviruses (HPV) DNA was found in most of cervical carcinomas (85–99.7%) and cervical lesions. HPV are double-stranded DNA viruses, invading mucosal or cutaneous epithelium. More than 100 different types of HPV are already known. Chance for elimination of HPV infection decreases with age: HPV-positive women over 35 years are at higher risk of developing cervical carcinoma. For carcinoma development, the most important is the interaction of E6 and E7 proteins with human p53and pRb proteins, respectively.
Method: High-risk (HR) HPV test was performed by Hybrid Capture® 2 technology (Qiagen). Genotyping of HPV types was done via polymerase chain reaction and reverse hybridization with use of Inno-LiPA technology (Innogenetics). Women with positive diagnosis of gynaecological cytology—ASCUS, LSIL, HSIL—and before vaccination against HPV were tested.
Results: From March 2006 to March 2010, a total of 1,164 patients were tested. Only 291 (25%) were HR HPV DNA-positive, of whom 85 (29.2%) were over 35 years. Case report presents an interesting case of positive Hybrid Capture® 2 test with proven cross-hybridization. Result was confirmed by HPV genotyping with use of Inno-LiPA technology.
Conclusion: The use of the HPV DNA test allows the detection of HPV infection with high sensitivity and specificity. Such result can more efficiently specify unclear outcomes of other screening methods, such as cytology. HPV DNA test has been already incorporated into routine screening within several countries.

0251
HPV prevalence in chronic gastritis

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Objective: HPV is one of the best-known etiologic agents for squamous epithelia-related neoplastic lesions including the cervix, larynx and skin. In recent years, some of non-squamous epithelia-driven tumors like stomach, colon and lung adenocarcinomas were reported to be containing HPV. The prevalence of HPV in gastric adenocarcinomas, which is reasonably related to chronic gastritis and gastric intestinal metaplasia, is varied between 15% and 45% in large-scale studies. Regardless of being 'passenger' or 'driver', to show HPV prevalence in chronic gastritis with metaplasia, the well-known two predisposing lesions of gastric cancers was our aim in the present study.
Method: DNA extracted from formalin-fixed paraffin-embedded gastric biopsies of 50 cases was tested for HPV-DNA by PCR assay. Consensus primers were used. The presence of a 140-bp fragment was accepted as HPV positivity.
Results: Eight out of 50 biopsies were found HPV-DNA-positive (16%).
Conclusion: HPV may accompany gastritis and metaplasia. HPV deserves more studies for pre-neoplastic and neoplastic gastric lesions to enlighten the 'passenger–driver' dilemma.

0252
Is p. Asn680Ser polymorphism of follicle stimulating hormone receptor (FSHR) associated with regulation of FSHR gene expression?

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Objective: FSHR genetic variability in position 2039 A>G (rs6166, p.Asn680Ser) was shown clinically to correlate with response to controlled ovarian hyperstimulation with gonadotropins. The aim of the study was to correlate rs6166 with the expression of FSHR-dependent genes such as FSHR itself and LHR.
Method: Fourteen consecutive patients undergoing IVF treatment due to idiopathic or male factor-related infertility were recruited. Genotype was determined by AS-PCR. The cells were obtained by ovary puncture and cultured for 7 days to regain responsiveness to FSH in gonadotropin-free medium. Stimulation was carried out in serum-depleted

environment using 0.5 UI/ml rFSH. Total RNA was isolated after 24 h of incubation. Gene expression was accessed by real-time PCR. All analyses were performed in at least two biological replicates.
Results: Distribution of Asn680Ser genotypes was as follows: six homozygotes Asn/Asn, seven heterozygotes Asn/Ser and one homozygote Ser/Ser. Expression of FSHR in all stimulated samples was increased by a mean factor of 2.61 (CI, 0.57–12.88, $p<0.001$). Homozygotes Asn/Asn presented significantly higher ($p=0.03$) rFSH-induced expression of FSHR as compared to carriers of Ser allele with mean fold change of 3.56 (CI, 0.84–16.2) vs 2.22 (CI, 0.67–5.9), respectively. Induced expression of LHR, although significantly upregulated, showed no difference with respect to FSHR genotype.
Conclusion: This is a preliminary report of correlation of FSHR genotypes of rs6166 polymorphism with FSHR mRNA expression. Altered FSH-induced FSHR transcript level is likely to explain different clinical behavior of patients with FSHR gene variants.

0254

The role of adamantinoma of long bones and osteofibrous dysplasia stromal cells in tumour osteolysis

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Objective: Adamantinoma of long bones (ALB) and osteofibrous dysplasia (OFD) are rare, osteolytic primary bone tumours of uncertain origin. To investigate the nature of the proliferating fibroblastic stromal cells in ALB and OFD and to determine cellular mechanisms of osteolysis in these tumours, we carried out cell culture and molecular studies on two cases of ALB and two cases of OFD.
Method: ALB and OFD cells were cultured on coverslips and dentine in the presence and absence of macrophage colony-stimulating factor (MCSF)-primed $CD14^+$ monocytes + MCSF + receptor activator of nuclear factor kappa B ligand (RANKL). Cultures were examined for the osteoclast formation markers tartrate-resistant acid phosphatase (TRAP), CD51 and lacunar resorption. ALB and OFD cells were examined for osteoblast markers (mineralisation nodule formation, alkaline phosphatase).
Results: Few cultured ALB and OFD stromal cells expressed epithelial markers (epithelial membrane antigen [EMA] and cytokeratin). OFD cells expressed osteoblast markers. Cultured ALB and OFD stromal cells showed no ultrastructural evidence of epithelial differentiation. ALB and OFD cells were not capable of lacunar resorption, but co-cultures of ALB or OFD stromal cells and monocytes resulted in the formation of multinucleated $TRAP^+$ and $CD51^+$ cells capable of lacunar resorption.
Conclusion: Our findings indicate that OFD and ALB stromal cells express epithelial and osteoblast markers. These cells support osteoclast formation from mononuclear phagocytes via a RANKL-dependent mechanism, which may contribute towards the aggressive bone destruction associated with this tumour.

Thursday, 2 September 2010, Basement

PS-13 Poster Session Neuropathology

0255

Radiotherapy induced sPNET in a 40-year-old female 11 years after subtotal excision and radiotherapy of oligoastrocytoma: report of a case

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Objective: Supratentorial primitive neuroectodermal tumor (sPNET) is an embryonal tumor usually developing supratentorially in children or young adults and composed of undifferentiated or poorly differentiated neuroepithelial cells. It is a relatively rare tumor in middle-aged people and extremely rarely develops after radiotherapy. We report an extraordinary case of sPNET in a 40-year-old female that developed 11 years after radiotherapy of oligoastrocytoma.
Results: A 40-year-old female was regularly followed after treatment of oligoastrocytoma of the left frontal lobe. The tumor was subtotally resected and treated with radiotherapy 11 years ago. The patient has persistent mild aphasia and mild right hemiparesis. CT performed due to increased incidence of headaches revealed a cystic lesion of the left frontal lobe, 4 cm in diameter, located below the previous tumor. The recurrence of oligoastrocytoma was suspected; however, the diagnosis of sPNET was rendered.
Conclusion: Long survival in patients successfully treated for primary brain tumors may yield in independent treatment-induced primary malignancies of the central nervous system.

0256

Relation between MMP-2, MMP-9 and Ki-67, CD44v6 in human malignant brain tumors. Preliminary study

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Objective: The interaction between different proteins plays an important role in the metastatic ability of brain tumor cells. The purpose of this study was to evaluate the expression of MMP-2, MMP-9, CD44v6, and Ki-67 in gliomas of different grades of malignancy and correlate the expression between the studied proteins in tumors.
Method: Expression of MMP-2, MMP-9, CD44v6, Ki-67 was evaluated on 48 formalin-fixed paraffin-embedded tissue blocks divided into: glioblastomas multiforme (n=28), anaplastic astrocytomas (n=11), anaplastic oligodendrogliomas (n=2) and normal brain tissue (n=7) using immunohistochemistry.
Results: MMP-2, MMP-9, CD44v6 and Ki-67 expression was found in 40.6%, 46.8%, 59.3%, 34.3% of gliomas, respectively.MMP-2 immunopositivity was significantly higher in glioblastoma multiforme than anaplastic astrocytomas (P=0.03). No immunoreaction for all proteins was found in normal brain tissues. MMP-2, MMP-9, CD44v6, Ki-67 immunoreactivity was significantly higher in glioblastomas when compared with anaplastic gliomas (P=0.05). In gliomas, the percentage of positive cells and the intensity of the immunostaining were proportional to the degree of malignancy. Positive correlations between Ki-67 and MMP-9 (P=0.002), MMP-2 and MMP-9 (P=0.02), MMP-9 and CD44v6 (P=0.01) were found in malignant brain tumors.
Conclusion: These results suggest that relationship between MMP-2, MMP-9, and MMP-9 and Ki-67 and CD44v6 indicate that expression of both matalloproteinases may facilitate the migration of tumor cells and increase their proliferative activity.

0257

Methylation status of MGMT (O6-methylguanine DNA methyltransferase) promoter in high-grade gliomas

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Objective: Gliomas are most common among primary brain tumors, and glioblastomas (GBL) are most fatal among gliomas. Surgical operation concomitant with chemoradiotherapy is adopted to treat gliomas. Temozolomide (TMZ) is a new oral cytotoxic agent with less myelosuppression. Epigenetic silencing of O6-methylguanine-DNA-methyltransferase (MGMT) by promoter methylation is associated with improved survival in GBL treated with TMZ. In this study, we investigated MGMT promoter methylation in gliomas treated with concomitant chemoradiotherapy (CCRT) following operation.
Method: In 40 cases of gliomas including 31 GBL, six anaplastic astrocytomas (AA) and three anaplastic oligodendrogliomas (AO), formalin-fixed, paraffin-embedded archival samples were used to evaluate the methylation status of MGMT promoter via methylation-specific PCR.
Results: MGMT promoter methylation was detected in 15 cases (37.5%) of gliomas. MGMT promoter methylation was detected in 41.5% of GBL, in 16.7% of AA, and in 33.3% of AO. GBL with methylation had significantly more prolonged overall survival (mean 32.46 months) compared with unmethylated GBL (mean 12.95 months, p=0.012). Methylated GBL also had more prolonged progression-free survival (mean 21.74 months) compared with unmethylated GBL (mean 7.41 months, p=0.068).
Conclusion: The frequency of MGMT promoter methylation in Korean glioma patients was similar to those in Western countries. These data indicate that MGMT promoter methylation is prognostically significant in GBL given CCRT. MGMT promoter methylation status is considered to be a valuable predictive factor in the routine clinic for GBL.

258

Spinal cord glioneuronal tumor with rosetted neurophil islands: case report

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Objective: Three new entities have been recently added to the group of glioneuronal tumors: papillary glioneuronal tumor (PGT), rosetted glioneuronal tumor with neurophil-like islands, and rosette-forming glioneuronal tumor (RGNT) of fourth ventricle. In particular, rosetted glioneuronal tumor with neurophil like islands needs to be distinguished from RGNT and PGT. The lesion currently is considered a variant of astrocytoma, WHO grade II or III.
Method: We present a 14-year-old female patient having been under observation because of scoliosis and urinary incontinence. Radiologically, on MRI, a mass lesion was detected located intramedullary at C5 and T5 and an expansion in spinal canal. Solid component of the mass extends between C7-T3 vertebrae and measures 51×18×22 mm. This solid component of the tumor was heterogeneously hypointense on T1 and heterogeneously hyperintense on T2 images. Surgically, C5, 6, 7 and T1 hemilaminotomy and intradural intramedullary tumor excision were performed. Histopathological examination showed a mixed glioneuronal tumor composed of oligodendrocyte-like cells constituting neurophil-like islands, astrocytic cells and neurons scattered in glial tissue. Immunohistochemically, glial tissue was GFAP-positive and neurophil-like areas and big neurons were synaptophysin-positive. In astrocytic component Ki-67, the proliferation index was below 1%. P53 was negative.

Results: Consequently, the final diagnosis was spinal cord glioneuronal tumor with rosetted neurophil-like islands—WHO grade 2. These cases can be confused with oligodendrogliomas, astrocytomas and ependymomas. Oligodendrogliomas are distinct with their molecular genetic features.
Conclusion: Our case is unique in that it is the youngest reported patient having this tumor located in the spinal cord.

0260

Proliferative activity in all subtypes of the glioblastoma

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Objective: Glioblastoma is the most malignant and the most frequent primary brain tumour in adults. It accounts for 60–75% of astrocytic tumours. The tumour has two variants: giant cell glioblastoma and gliosarcoma. The purpose of the study was to show the differences between values of the MIB-1 proliferative indexes of glioblastoma, giant cell glioblastoma and gliosarcoma.
Method: Surgical specimens from 60 patients were formalin-fixed, paraffin-embedded immunostained using MIB-1 antibody. Next, proliferative index (PI) was calculated. Analysis of variance (ANOVA) was used to test the hypothesis that mean values of MIB-1 PI were equal for each diagnosis group.
Results: The mean values of MIB-1 PI were as follows: glioblastoma, 25.7% (14.7–43,7%); giant cell glioblastoma, 21.7% (4.9–39.7%) and gliosarcoma, 27.2% (12.5–46.9%), but there were no statistically significant difference in the MIB-1 PI mean value between diagnostic groups.
Conclusion: We observed that proliferative activity shows regional variation and was the most prominent in glioblastoma with small cells. In giant cell glioblastoma, it was much lower. Because of the absence of significant differences of MIB-1 PIs in our study, in the future, the study will be repeated on 40 cases in each group of that tumour.

0264

The extracellular matrix and diffusion barriers in the focal cortical dysplasias of brain—an immunohistochemical study

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Objective: Changes in the geometry and composition of the extracellular space (ECS) may influence the epileptogenesis in focal cortical dysplasias (FCD) of the brain.
Method: Tissue ECS volume and geometry (the geometrical factor "tortuosity", reflecting various ECS diffusion barriers) were studied in cortical samples of 21 patients surgically treated for epilepsy, including nine patients with FCD type I and of six patients with FCD type II, by real-time iontophoretic method. Consequently, the samples were subjected to immunohistochemical analysis of the composition of the extracellular matrix (ECM) and morphology of the $GFAP^+$ glial cell processes.
Results: In both FCD type I and FCD type II, the tortuosity of the ECS was significantly increased: 1.61±0.01 and 1.69±0.02, respectively; control = 1.46±0.01 (mean ± SEM). The ECS volume fraction was not significantly changed. When compared to controls, no significant changes in ECM composition were noted in FCD type I. However, we observed an increase in $GFAP^+$ glial processes in both types of FCD and pathological accumulation and distribution of some ECM molecules (tenascins C and R, hyaluronate, chondroitin sulphate, reelin) in the ECS of FCD type II.
Conclusion: The ECS of FCD has increased tortuosity, reflecting the increase of diffusion barriers in the ECS due to gliosis and pathologic accumulation of some ECM molecules. We propose that disturbed extrasynaptic transmission, mediated by the diffusion of neuroactive substances through the ECS of such cortex, represents another factor of epileptogenicity in FCD. Support: IGA MZCR NS9915-4 and MZOFNM2005.

0265

Hemangiopericytoma of the pineal body: case report

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Objective: Hemangiopericytomas are rare mesenchymal neoplasms that presumably derived from pericytes. Although HPC mas generally attached to the cranial and spinal dura, intraparenchymal and intraventricular rare tumors have been reported.
Method: A 27-year-old male patient presented with headache, nausea and blurred vision 2 months ago. Cranial MRI revealed contrast-enhanced lesion 3 × 3 cm in diameter in pineal gland location. Given the findings of the preoperative imaging studies, the lesion was consistent with parenchymal tumors of the pineal gland. A right occipital craniotomy and resection of the lesion was performed. Histologically, the tumor was composed of clusters of monotonous, closely packed, small neoplastic cells. Nuclei are oval to round with granular chromatin and inconspicuous nucleoli. Mitosis was rare and necrosis was not found. Stroma contained dilated, slit-like vascular channels lined by flattened endothelial cells. Reticulin stain

showed increased reticulin network of investing group of neoplastic cells. Neoplastic cells were diffusely immunoreactive with BCL-2, but were negative with epithelial membrane antigen, chromogranin, synaptophysin, PGP 9.5, S-100 protein, and CD 34. A MIB-1 labeling index was 5%.
Results: A diagnosis of hemangiopericytoma was made based on light microscopy and conventional histochemical and immunohistochemical studies. Differential diagnosis included pineal parenchymal tumors and meningiomas.
Conclusion: The present case provides one unique example of a rare entity to the diverse spectrum of the pineal region neoplasms encountered in neuropathology.

0267

Cancer metastasis to intracranial meningioma

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Objective: Tumor-to-tumor metastases are very rare. The most common intracranial tumor recipient is meningioma.
Method: Case study.
Results: (1) A 70-year-old man was admitted in 2009 due to a rapid onset of the right hemiparesis. His history revealed: 2003—nephrectomy due to renal clear cell carcinoma (RCC) T1N0M0, 2003—detection of parieto-temporal meningioma 2.5/2.2 cm; he denied the operation; 2005—diagnosis and hormonotherapy of prostatic cancer; 2003–2008 radiological follow-up of meningioma. In CT at admission, the tumor was 6.2/3.2 cm and contained intensively contrast-enhanced 1.5-cm area with surrounding edema. The tumor was removed but complicated with a hematoma. Histologically, it consisted of whorled clusters of spindle cells with focal necrosis and mitotic index of 5/10 HPF. The separating, well-vascularized area was composed of clear cells. Meningioma immunophenotypes were S-100^{+}, vimentine^{+}, progesterone receptor^{+}, and CD10^{-}, while focus were S-100^{-}, vimentine^{-}, progesterone receptor^{-}, and CD10^{+}. The diagnosis was atypical meningioma (G2) with RCC metastasis. (2) A 57-year-old man with a 2 year's history of a colorectal cancer (T3N1M1) was admitted in 2009 due to severe headaches. CT revealed an enhancing temporal lobe tumor 2.5/3.2 cm surrounded by edema. The neurosurgeon resected brain metastasis and the radiologically undescribed meningeal tumor 0.9/0.4 cm. Histologically, meningeal tumor was a meningothelial meningioma (G1) with a 0.3-cm focus of metastatic adenocarcinoma (CK^{+}, CDX2^{+}).
Conclusion: Cancer metastasis to meningioma can be the first manifestation of the metastatic stage of disease. Metastasis to meningioma should be included into differential histopathological diagnosis in patients with systemic malignancies.

0268

A solitary laryngeal plexiform neurofibroma in a pediatric patient

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Objective: Neurofibroma is the most frequent neurogenic tumor in the first year of life. It is a benign proliferation of Schwann cells, perineurial cells, and fibroblasts, which may occur in association with neurofibromatosis type 1 or sporadically. The most common sites of laryngeal involvement are the aryepiglottic folds and the arytenoids, areas of the larynx rich in terminal nerve plexuses. Clinical findings: A 1-year-old girl presented with airway obstruction and stridor with a progressive nature since birth. Direct laryngoscopy: laryngeal tumoral mass, located in the supraglotic area. Complete surgical excision was performed.
Method: All anatomical pieces were fixed in 10% formaldehyde solution, paraffin-embedded, cut and stained (hematoxylin–eosin and Van-Gieson).
Results: Macroscopically: 1-cm whitish multinodular mass, firm consistency, myxoid cut surface Microscopically: The submucosal nerve fibers were expanded in a plexiform pattern, infiltrating between the submucosal glands due to a proliferation of small, delicate, wavy, spindle cells with markedly elongated, wavy nuclei with pointed ends and coarse collagen fibres, embedded in a myxoid matrix. Immunohistochemistry: positivity of the nervous fibers for CD34 and S100 protein. ACT was negative in the nervous fibers and positive in the blood vessels. Ki67 was negative. Histopathology diagnosis: plexiform neurofibroma of the larynx.
Conclusion: Neurofibroma of the larynx is a rare condition that should be considered in the differential diagnosis of children presenting with a submucosal laryngeal mass even without the other clinical signs of NF1 or NF2. It has a high rate of recurrence or residual disease due to the possibility of malignant transformation.

0271

Magnetic resonance microscopy of brain biopsies for detecting microstructural patterns

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Objective: Brain lesions are highly heterogeneous both clinically and morphologically. The morphological study of these lesions is based in their cellular characteristics, different tissue organization patterns, vascular network, collagen distribution, presence of calcium, etc. MR imaging of excised tissue samples may provide valuable information about lesion structure and microarchitecture, increase the diagnosis specificity and help for better tissue characterization.
Method: Fifteen brain lesions biopsies from the Hospital Clinico Universitario de Valencia were fixed with formalin and embedded in a gel matrix for minimizing motion artifacts. MR microscopy images and optical microscopy images were obtained for all of them. MR microscopy images included T1, T2, T2* and DWI. Images were subsequently analyzed by hierarchical clustering.
Results: MR microscopy of brain biopsies shows high resolution and quality. Major histological findings (for example, healthy, vascular, proliferative or necrotic tissue) exhibit differential MR parameters values (T1, T2 and diffusion coefficient among others). Hierarchical cluster analysis of these parameters revealed different MR patterns correlating with relevant histopathological and immunohistochemical features.
Conclusion: MR microscopy provides functional, microstructural and biophysical information complementary to that obtained by conventional histopathology. Our approach and findings may help in multidisciplinary groups for better integration of histopathology and MRI in brain lesion diagnosis.

0272
Interaction of vesicular monoamine transporter 2 (VMAT2) and neuromelanin pigment among the midbrain dopaminergic neurons in man

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Objective: Neuromelanin (NM) pigment accumulate with age catecholaminergic neurons in man, and ventral substantia nigra dopaminergic neurons that are most vulnerable to degeneration in Parkinson's disease (PD) contain the greatest amount of this pigment. In vitro data indicate that NM pigment is formed from the excess cytosolic catecholamine that is not accumulated into synaptic vesicles via the vesicular monoamine transporter2 (VMAT2).
Method: Using semiquantitative immunohistochemical methods in human postmortem brain, we sought to examine the relationship between the contents of VMAT2 and NM pigment. The immunostaining intensity (ISI) was measured for VMAT2 in two regions of the midbrain dopaminergic cell complex. The ISI of the cells was related to the density of NM pigment within the cells. We also measured the ISI for tyrosine hydoxylase (TH) and examined the noradrenergic neurons in the locus coeruleus (LC) in brain 22–-65 years of age.
Results: (1) Ventral substantia nigra neurons had the lowest VMAT2 ISI of all neurons in the midbrain cell complex, whereas over twofold higher levels are found in most ventral tegment area neurons. (2) There was an inverse relationship between VMAT2 ISI and neuromelanin pigment in midbrain dopaminergic neurons. (3) Neurons with the highest VMAT2 ISI resided in the LC. (4) Neurons with high VMAT2 ISI also had high TH ISI.
Conclusion: These data support the hypothesis that among the midbrain dopaminergic neurons, the ventral substantia nigra dopamine neurons accumulate the highest levels of NM pigment because they have the lowest levels of VMAT2, which thereby renders them especially vulnerable to degeneration in PD.

0273
Histopathological aspects and protein aggregation in mitochondrial myopathies

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Objective: Human mitochondrial diseases have a relatively high incidence, multiple genetic causes and quite diverse clinical, biochemical and morphological phenotypes requiring a multidisciplinary approach for diagnosis. The study of protein aggregation in muscle fibres in these disorders may offer new insights on their complex pathogeny.
Method: We studied morphological aspects of the "ragged red" fibres—targeting our interest on the types and location of different protein aggregates. We selected diagnostic muscle biopsies from seven unrelated patients and used histological, histochemical, enzyme histochemical, ultrastructural and immunohistochemical techniques.
Results: The expression of cytoskeletal, transsarcolemmal, sarcomeric, chaperone type and nuclear proteins revealed that "ragged red" fibres and "ragged red" regions of the fibres accumulate diverse proteins, of which desmin, alpha B crystallin, as well as N-CAM, dysferlin and heat shock protein appear to be regular ones. None of our cases had any accretion of plectin, actinin, beta and gamma dystroglycan. Other proteins showed variable expression.
Conclusion: As the list of members in the "protein aggregate myopathies" family is constantly enlarging, the study of protein accumulation in mitochondrial myopathies is of clear interest for the future. Note: Zurac

and Bastian should be regarded as first authors with equal contribution.

Friday, 3 September 2010, Basement

PS-14 Poster Session Uropathology

0275

Audit of prostate core biopsies 2005–2009

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Objective: Prostate biopsy is performed to detect carcinoma in those at high risk on the basis of elevated PSA and abnormal digital rectal examination. Re-biopsy is often performed following an initial atypical diagnosis or rising PSA.
Method: All histopathological reports of prostate biopsies at Beaumont Hospital, Dublin, during the time period 1 January 2005 to 1 January 2009 were analysed. A total of 1,483 reports were retrieved and the data were recorded and analysed.
Results: All prostate biopsies were grouped into initial biopsy (no previous biopsy recorded) and repeat biopsy (at least one previous biopsy recorded). Multiple report parameters were recorded for each biopsy including diagnosis, number of cores taken, number of positive cores and Gleason grade, and these were compared for the two groups. In the original biopsy group, all biopsies reported as atypical (but not meeting criteria for carcinoma) were further analysed for changes on follow-up biopsy. In the repeat biopsy group, repeat biopsies performed for increased PSA and for previous atypical diagnosis were analysed separately and compared.
Conclusion: Repeat prostate biopsy is often performed for persistently elevated or rising PSA and previous diagnosis of atypical changes. Our study assessed the likelihood of finding carcinoma in a follow-up biopsy performed for these two reasons. Our study also compared the biopsy adequacy and extent and grade of carcinoma found in both initial and repeat biopsies. The findings are significant in determining the likely clinical course and appropriate management of patients with atypical diagnoses and elevated PSA.

0276

Cytological features, loss of basal cells and AMACR expression in some hyperplasic and precancerous lesion of the prostate

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Objective: Since atypical adenomatous hyperplasia (AAH), post-atrophic hyperplasia (PAH) and proliferative inflammatory atrophy (PIA) of the prostate demonstrate both overlapping histological features and generality of biological properties with prostatic intraepithelial neoplasia (PIN) and atypical small acinar proliferation (ASAP), these processes were proposed in probable precancerous lesions of the prostate.
Method: We studied the cytological features with the use of morphometry. The expression of AMACR and cocktail HWC+p63 was estimated with the use of immunostaining. For the image analysis, the software WSIF ImageJ and ImageScope was used.
Results: BC layer was fragmented in most of cases PAH, PIA, AAH. The BC loss in PAH–PIA was 6% and 20% accordingly and was below that in PIN—42%, AAH—83%, and ASAP—84%. Analysis of cytological features and nucleolus frequency showed that PAH–PIA was similar to PIN–ASAP and above that in AAH. Prominent nucleolus was identified in atrophy, precancerous lesions and PCa. Frequency of prominent nucleolus in PAH (63% cases, 4% in cases) and PIA (44% and 13% accordingly) was below that in PIN (58% and 11%), ASAP (59% and 27%) and PCa (78% and 55%). AMACR expression was moderately strongly positive in precancerous lesions and PCa and weakly positive in other groups. Area of AMACR expression in PIN and ASAP was above that in AAH, PAH, PIA and below that in cases PCa.
Conclusion: We identified that PAH and PIA showed more aggressive cytological properties than AAH and was indistinguishable from PIN-ASAP according to cytological features. Meanwhile, PAH and PIA take intermediate position between BPH and PIN-ASAP according to BC loss, frequency of prominent nucleolus and AMACR expression.

0277

Rare tumors of the prostate diagnosed by a core needle biopsy

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Objective: Presentation of 2 rare lesions of the prostate.
Method: A 69-year-old man, with PSA 16,04 underwent a needle biopsy of the prostate. A histological examination of 11 biopsique carrots showed an infiltrating tiny lesion within adipose periprostatic tissue at the level of one biopsy of the left lobe nodule. The lesion consisted of little irregular glands and solid nests of atypical cells PSA^{-} and $CK7^{+}$, and $CK20^{+}$. We proposed a diagnostic of a metastasis/infiltration of unknown primary origin, likely from the urinary tract. This was confirmed by a cystoscopy revealing a tumor which had a histologic appearance of a choriocarci-

noma, finally classed as a poorly differentiated urothelial carcinoma with a choriocarcinoma differentiation.
Results: The second case concerns a 35-year-old patient with decompensated cirrhosis admitted to the hospital for a gastroenterology bilan. Rectal examination revealed an enlarged prostate. The hemostase was necessary after a core needle biopsy of the prostate. The histological examination demonstrated a highly vascular tumor composed of chief and sustentacular cells arranged in an alveolar pattern. Immunohistochemical study confirmed a paraganglioma. There were no signs of malignancy; however, we did not observe any prostatic parenchyma at the tumoral level.
Conclusion: Many significant non-epithelial tumors may arise in the prostate gland; although they are rare, their recognition by the pathologist is essential as their treatment and prognosis are quite variable. The core needle biopsy allows for a diagnostic of a variety of tumors, and one should not ignore, in spite of the absence of clinical symptoms and complementary information, the risk of metastatic lesion in the prostate.

0278
Latent prostate cancer in association with benign prostatic hyperplasia after the Chernobyl accident in Ukraine

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Objective: During the 24-year period subsequent to the Chernobyl accident, the morbidity of prostate cancer in Ukraine has increased from 12.0 to 28.6 per 100,000 male population. The prevalence and immunohistochemical (IHC) study of latent, incidentally found prostate cancer (LPC) in patients who underwent surgery for benign prostatic hyperplasia (BPH) were studied.
Method: BPH samples were obtained by prostatectomy from 120 Ukrainian patients operated between 2007 and 2009 consisting of 30 patients from the so-called clean (without radio-contamination) areas (control group 1) and of 90 patients living in Cesium 137 (^{137}Cs)-contaminated areas of Ukraine (group 2). Ki-67, p53, p27Kip-1, p63 and Bcl-2 proteins were IHC-investigated in BPH from all patients.
Results: The incidences of LPC (Gleason score 4), chronic prostatitis, proliferative inflammatory atrophy (PIA) and prostatic intraepithelial neoplasia (PIN) were 16.67%, 53.34%, 20%, and 26.67% in group 1 and 12.23%, 64.45%, 43.45%, and 36.67% in group 2, respectively. Greatly elevated levels of p53, Ki-67, and Bcl-2 associated with decreased levels of p27Kip-1 and p63 in areas of PIA and less LPC and PIN in group 2 compared with group 1 patients were obtained with statistically significant differences.
Conclusion: Our study suggests that chronic long-term low-dose radiation exposure might result in the increase of chronic inflammation, and it is now found to be associated with increased incidences of PIA and PIN in BPH accompanied by p53, p27KIP-1, and Bcl-2 alteration, which in turn could lead to prostate carcinogenesis.

0280
Mixed peripheral ductal prostatic adenocarcinoma in radical prostatectomies: a five-year experience in a small Spanish Community Hospital

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Objective: Ductal prostate cancer (DPC) is a rare morphologic variant of prostatic cancer. They can arise centrally or peripherally. They have characteristic growth patterns and morphology. They usually appear combined with common acinar adenocarcinoma.
Method: We reviewed 71 consecutive radical prostatectomies from 2005 until the end of 2009. Immunohistochemical analysis were performed with P504S, p63, 34BE12, p63, PSA, Bcl2, chromogranin A, synaptophysin, p53, Ki-67, CK7, CK20, CEA and CD56 using the L-SAB method.
Results: Four mixed DPCs were found (5.6% of the total) with no previous treatment. Two cases had a minor DPC component: case 3: age 66, RP with 5% of cancer (bilateral), 25% of DAP, GS 4-3, focal EPE and seminal vesicle (SV) invasion and positive margin; case 4: age 53, 20% of cancer(bilateral), 20% of DPC, GS 4-3, no EPE or SV invasion, lymph node (LN) negative. Two cases had a major ductal component: case 1: age 63, 25% of adenocarcinoma (bilateral), 40% of DPC, GS 3-4, multifocal EPE, SV and LN negative; case 2: age 60, 40% of carcinoma, 70% DPC; occasional EPE, no SV invasion, LN negative. It showed prominent sclerotic stroma. Focally, it had a peculiar pattern: central solid bland spindle/basaloid areas (HMWK, neuroendocrine markers, Bcl-2, PSA and CEA^{+}).
Conclusion: That low incidence correlates with that described in the literature. They all shared typical ductal features and conventional acinar carcinoma at least focally. Case 2: predominantly ductal, had an additional morphology: basaloid–neuroendocrine differentiation.

0281
Preoperative data predictive for final tumor volume in radical prostatectomy specimens

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Objective: Tumor volume is a powerful predictor of patient outcome in prostatic adenocarcinoma. Preoperative assessment of prostate cancer tumor volume is still a big challenge. The present study attempted to identify the most predictive biopsy variables for final tumor volume in radical prostatectomy specimens.
Method: We reviewed 128 prostate needle biopsies in patients who subsequently underwent radical prostatectomy. The preoperative data collected were: PSA, preoperative ultrasound, total prostate volume, number of positive biopsies, total percentage of cancer in the biopsy and Gleason score. Prostatectomy specimens were entirely embedded and mounted whole. Tumor volume was measured using the grid method. Preoperative data were compared with final tumor volume in radical prostatectomy specimens by univariate and multivariate analysis.
Results: The number of positive biopsies ($p<0.001$), total percentage of cancer in the biopsy ($p<0.001$) and Gleason score ($p<0.001$) were significant predictors of tumor volume on linear regression analysis. PSA value ($p=0.102$) and preoperative ultrasound total prostate volume ($p=0.07$) were not significant predictors of tumor volume. On multivariate logistic regression analysis, we designed a model to predict final tumor volume. This model contains all the predictors mentioned above and significantly correlates with final tumor volume ($p<0.001$).
Conclusion: Preoperative data (Gleason score, number of positive biopsies and total percentage of cancer in the biopsy) can be used to predict the volume of prostate cancer with acceptable accuracy. These variables must be mentioned to the clinician in order to help in the therapeutic decision.

0282

"Negative" histopathology (stage pT0) in a radical prostatectomy specimen after a preoperative diagnosis of prostatic adenocarcinoma

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Objective: In rare cases, histologic assessment of the entire radical prostatectomy specimen (RPS) after a positive biopsy does not reveal residual tumour. We analyze the incidence of "vanishing prostatic carcinoma" in our institution and present algorithm of pathologic workup of "negative" RPSs.
Method: Among 589 RPSs from patients treated for prostatic adenocarcinoma (January 1997–December 2009), ten cases (1.70%) showed no residual carcinoma despite complete sampling. A five-step protocol of additional pathologic workup included: (1) preoperative biopsy review; (2) "second look" review of the RPS; (3) immunohistochemistry (34betaE12, p63) performed on suspicious foci; (4) deeper levels of each block with PIN high grade and/or of blocks with areas where cancer was seen in the biopsy; and (5) block-flipping of regions specified above with deeper levels.
Results: Cancer diagnosis had been made in transurethral resection (TURP) specimen (two patients) and core biopsy (eight patients). The TURP cases were excluded on the assumption of complete pre-prostatectomy carcinoma resection. Biopsy review (protocol step 1) confirmed prostatic carcinoma in seven core biopsy specimens (1.18%). In one case, diagnosis of adenocarcinoma made in another institution was not confirmed. Steps 2–5 revealed residual carcinoma in a further four RPs. Despite full workup, no residual tumour was seen in three RP specimens (0.51%). All three "negative" cases had undergone MAB prior to RP and showed no clear evidence of residual carcinoma.
Conclusion: "Vanishing prostate carcinoma" is a rare phenomenon, with higher incidence after neoadjuvant hormone therapy. Extensive histologic workup of RP specimen reduced the rate of pT0 RP after a positive core biopsy from 1.18% to 0.51%.

0284

CD10 expression in prostate cancer—preliminary results

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Objective: CD10 is a transmembrane neutral endopeptidase strongly expressed by normal prostatic epithelium. Loss of CD10 expression is an early event during prostatic carcinogenesis. The aim of this study was to evaluate CD10 expression in different stages of prostate tumors and its correlation with classical prognostic factors.
Method: Twenty cases of prostatic cancer with different pathological stages (four cases—organ-confined disease, four cases—locally advanced disease, six cases—metastatic disease and six cases—hormone-resistant disease) were selected and further CD10 immunostained. The malignant foci were evaluated for the presence, pattern and intensity of reaction. We also assessed the classical prognostic factors like tumor grade, pathological stage and resection margin status.
Results: Our study showed a marked heterogeneity of CD10 expression. We noticed a membranous and/or cytoplasmic pattern of reaction. CD10 stained positively predominant at the apical membrane of the malignant glands ($n=14$). Gleason pattern 2 carcinomas showed a focal membranous apical reaction, while some of Gleason pattern 3 tumors expressed CD10 in both membranous and

cytoplasmic compartments. Cytoplasmic CD10 expression was almost exclusively noticed in Gleason pattern 4 and 5 tumors and in metastatic and hormone-resistant cases (n=6).
Conclusion: Our study suggests some prognostic value for membranous and cytoplasmic pattern of CD10 expression in prostate tumors. To eliminate discrepancies on this issue, further standardization of both immunostaining procedure and interpretation is mandatory. Larger prospective studies are also required to confirm predictive CD10 significance in prostate cancer progression.

0285

High CD10 expression in lymph node metastases from surgically treated prostate cancer independently predicts early death

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Objective: Patients with nodal positive prostate cancers are an important cohort with poorly defined risk factors. CD10 is a cell surface metallopeptidase that plays a role in the progression of various cancers and has been shown to be associated with early biochemical failure in surgically treated prostate cancer.
Method: CD10 expression was evaluated in 119 nodal positive, hormone treatment-naïve prostate cancer patients using tissue microarrays constructed from primary tumors and lymph node metastases. All patients underwent radical prostatectomy and standardized extended lymphadenectomy.
Results: In the primary tumor, high CD10 expression was significantly associated with earlier death of disease (5-year survival: 73.7% vs. 91.8%, p=0.043) when compared with low CD10 expression. In the metastases, high CD10 expression was significantly associated with larger total size of metastases (p=0.015), earlier death of disease (5-year survival: 87.3% vs. 71.5%, p=0.037) and death of any cause (5-year survival: 87.2% vs. 70.0%, p=0.009) when compared with low CD10 expression.
Conclusion: CD10 expression in metastases added independent prognostic information for overall survival (p= 0.018) that might help schedule adjuvant therapies.

0286

Cytoplasmic cyclin D1 in lymph node metastases independently predicts survival in surgically treated nodal positive prostate cancer patients

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Objective: Prognostic factors in nodal positive prostate cancer are poorly defined. Cyclin D1 is expressed in prostate cancer cells and might be prognostically relevant.
Method: Nuclear and cytoplasmic cyclin D1 expression was evaluated in 119 nodal-positive prostate cancer patients undergoing radical prostatectomy and extended lymphadenectomy. Cyclin D1 was correlated with various tumor features and biochemical recurrence-free (bRFS), disease-specific (DSS) and overall (OS) survival.
Results: In primary tumors, high nuclear cyclin D1 expression was significantly correlated with poor tumor differentiation and large nodal tumor burden. In metastases, high cytoplasmic cyclin D1 expression was significantly correlated with low nodal tumor burden and predicted independently unfavourable outcome compared to patients with low expression (5-year bRFS: 26.4% vs. 12.5%, p= 0.008; 5-year DSS: 80.7% vs. 56.3%, p=0.023; 5-year OS: 78.7% vs. 56.3%, p=0.011). These patients had a 2.5-fold elevated risk of dying from cancer compared to patients with low cytoplasmic cyclin D1 expression (p=0.018).
Conclusion: The subcellular location of cyclin D1 expression in prostate cancer might impact on important biological properties. Survival stratification according to biomarker expression in metastases indicates an important role for tumor sampling from these tissues. Cyclin D1 in metastasizing prostate cancer might serve for targeted therapies.

0287

Immunohistochemical assessment of lymph vessel architecture and lymphangiogenetic marker vascular endothelial growth factor-C (VEGF-C) in prostate carcinoma. Correlation with tumor grade and lymph node status

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Objective: Lymphangiogenesis is considered an essential procedure that facilitates lymphatic invasion in many tumors. It is not clear, however, whether lymph node metastasis depends on preexisting or newly formatted lymph vessels. We immunohistochemically evaluated the lymph vessel architecture and the expression of VEGF-C in prostate carcinoma cases and examined the correlations with tumor grade and lymph node status.
Method: Mean lymph vessel density (LVD) was assessed by lymph vessel markers D2-40 and LYVE-1 in 87 cases (64 with positive L+ and 23 with negative L− node status) of prostate carcinoma. Correlations of lymph vessel density with VEGF-C expression, tumor grade and lymph node status were evaluated.
Results: Lymph node status was marginally correlated with Gleason grade (chi-square, p=0.052). Mean LVD assessed

by D2-40 and LYVE-1 showed no differences between L+ and L− cases (*t* test, $p>0.05$ in both cases). When LVD was correlated to tumor grade, no statistically significant differences were present. On the other hand, VEGF-C expression was increased in L+ cases and correlated well with lymph node status (chi-square, $p=0.041$). Interestingly, VEGF-C correlated well with D2-40-assessed LVD (but not LYVE-1) in both intratumoral and peritumoral lymph vessels (ANOVA, $p<0.0001$).

Conclusion: Our results indicate that VEGF-C is essential for tumor lymphangiogenesis in prostate carcinoma. Both D2-40 and LYVE-1 may reveal lymph vasculature, the latter, however, showing lower expression, probably limited to a specific lymph vessel subpopulation. It is possible that lymph node metastasis is independent of newly formed lymph vessels.

0288

Bmi1 is repressed in primary prostate cancer but re-expressed in bone metastatic disease

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Objective: Polycomb group genes (PcGs) actively control epigenetic regulation of gene expression in human progenitor cells. Expression of the PcG Bmi-1 has been associated with cancer stem cell activation. High Bmi-1 mRNA expression in primary prostate cancer (CaP) has been shown to be a sign of poor prognosis, and activation of the PcG pathway has been associated with metastatic CaP cells in vitro and in vivo. Therefore, Bmi1 expression in the primary tumor may be a predictor of potentially lethal disease in CaP.

Method: A tissue microarray (TMA) consisting of control samples and primary tumor tissue samples of 284 CaP patients and tissue samples of 71 patients with CaP bone metastases was generated, stained and evaluated for Bmi1 expression. Median follow-up of the patients was 7.7 years. Bmi1 expression was correlated with clinical parameters.

Results: Non-malignant prostate tissue generally showed high Bmi-1 expression. In contrast, only 12% of the localized prostatic carcinomas demonstrated Bmi1 expression. Along with a significant enrichment of Bmi1-positive cancer cells in bone metastases, a significantly higher fraction of Bmi1-positive samples was found in bone metastases as compared to primary tumors (41% vs 12%, $p<0.01$). No correlation was found for Bmi1 expression and clinical parameters in CaP.

Conclusion: Bmi1 expression in the primary CaP is not a predictor of worse outcome or bone metastatic disease, although bone metastases are associated with Bmi1 re-expression. This finding suggests a potential role of PcG Bmi1 in prostate cancer progression and metastasis.

0289

Large cell neuroendocrine carcinoma in urinary bladder

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Objective: Large cell neuroendocrine carcinoma (LCNEC) of the urinary bladder is a rare tumor with only a few cases reported. The diagnosis is based on the criteria established in lung tumors. We report the clinical and histological data of six patients with LCNEC in urinary bladder.

Method: The clinical charts and histological slides of six patients with the LCNEC diagnosis in urinary bladder from a single institute were reviewed. The diagnosis was confirmed by the use of immunohistochemistry with at least two positive neuroendocrine markers (synaptophysin, chromogranin and/or CD56).

Results: All patients were male. Their age ranged from 38 to 74. The most frequent clinical presentation was gross hematuria. Their histology had overlapping features with urothelial carcinoma and was difficult to differentiate without immunohistochemistry. The most useful features were extreme hypercellularity (6/6), lack of exophytic papillary component (5/6), nuclear molding (4/6), finely stipled chromatin (4/6) and exuberant mitotic rate (most >10/hpf) with innumerable apoptotic figures. In three cases, there were foci of admixed invasive or in situ urothelial carcinoma, in one case admixed adenocarcinoma. All tumors were infiltrative to muscularis propria at the time of diagnosis, except the most recent one which did not have muscle sampled in resection. Distant metastases were found in liver, spleen and bones of one patient. Three patients underwent radical cystoprostatectomy; all patients received adjuvant or neoadjuvant chemotherapy, and one was given additional radiotherapy.

Conclusion: Distinction between LCNEC and urothelial cancer is important because of likely different therapeutic options. LCNECs are highly aggressive tumors with at least pT2 disease at diagnosis.

0290

Myofibroblastic stromal reaction in pTa and pT1 papillary urothelial carcinoma of the urinary bladder

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Objective: The most important element in pathologic evaluation of urothelial cancer is recognition of the

presence and extent of invasion. Our research objective was to analyze myofibroblastic stromal reaction in papillary urothelial carcinoma of the urinary bladder with lamina propria invasion and compare it with lamina propria of noninvasive carcinoma.
Method: Immunohistochemical analyses of vimentin, smooth muscle actin (SMA) and desmin reactivity were performed in 50 pTa and 50 pT1 papillary urothelial carcinomas of the urinary bladder obtained by transurethral resection. The reaction was graded as 0—no reaction, 1—positive reaction in <33% of lamina propria cells, 2—positive reaction in 33–66% of lamina propria cells and 3—positive reaction in >66% of lamina propria cells. Distribution patterns of vimentin- and SMA-positive cells were graded as fascicular (organized) and reticular (disorganized), respectively.
Results: Reactive stroma in pT1 carcinoma showed increased vimentin and SMA expression ($p<0.001$, chi-square test) compared to lamina propria of pTa carcinoma. There was no significant difference in desmin expression. The distribution pattern of both vimentin- and SMA-positive cells was reticular in invasive and fascicular in noninvasive carcinoma ($p<0.001$, chi-square test).
Conclusion: Our study indicates that an increased number of cells with myofibroblast immunophenotype as well as an altered, disorganized pattern of myofibroblast distribution can be demonstrated in invasive (pT1) papillary urothelial carcinoma of the urinary bladder when compared to pTa carcinoma. These findings, especially the latter, may be used as a helpful additional diagnostic tool in cases suspicious of lamina propria invasion.

0291
Gemcitabine vs sirolimus in chemically induced bladder tumours: preliminary results

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Objective: Bladder cancer's incidence is increasing worldwide. To improve the results of current bladder cancer treatments, new drugs are being tested in single use or in associations. Gemcitabine is metabolized to the active diphosphate and triphosphate, which is integrated into the DNA and causes cessation of DNA polymerization. Sirolimus inhibits mTOR protein kinase. Our aim was to study and compare the effect of gemcitabine and sirolimus in bladder cancer chemically induced in mice.
Method: *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine was administered to ICR male mice in drinking water for 12 weeks. One group was treated with gemcitabine (0.05 mg/kg, i.p.) and the other with sirolimus (1.5 mg/kg, i.p.). All animals were killed at the end of the experiment.
Results: Animals not treated showed a higher incidence of neoplastic and pre-neoplastic lesions than animals treated (77% simple hyperplasia, 69% nodular hyperplasia, dysplasia 100%, CIS 15% and invasive carcinoma 54%). Gemcitabine group showed simple hyperplasia 66.7%, nodular hyperplasia 46.67%, dysplasia 100%, squamous carcinoma 40%, low level papillary carcinoma 6.67%, high-grade papillary carcinoma 6.67%, and invasive carcinoma 20%. Sirolimus showed 71.4% simple hyperplasia, nodular hyperplasia 28.6%, dysplasia 85.7%, squamous carcinoma 35.7%, and invasive carcinoma 7.1%. Cis was not observed in either sirolimus or gemcitabine groups.
Conclusion: Histopathological studies showed that both drugs reduce tumour incidence. Despite that the differences were not statistically significant, sirolimus was more effective than gemcitabine. Given their different mechanisms of action and the synergistic effect observed with these two drugs in vitro in other types of tumours, further trials with their combination should be considered.

0293
The most appropriate immunohistochemical panel for discriminating poorly differentiated invasive tumors in transurethral resection specimens

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Objective: The accurate diagnosis of poorly differentiated invasive carcinomas in transurethral resection (TUR) specimens using only H&E-stained sections continues to be a challenge for the pathologist. The aim of this study was to evaluate and select an optimal immunohistochemical (IHC) panel of antibodies to establish the urothelial or prostatic origin of poorly differentiated tumors in TUR specimens.
Method: IHC was performed to detect cytokeratin (CK) 7, CK20, high-molecular-weight CK (HMWCK), p63, prostate-specific antigen (PSA) and P504S/AMACR in 24 cases of poorly differentiated invasive tumors: 12 poorly differentiated prostatic adenocarcinomas (Gleason score ≥ 8) and 12 high-grade urothelial carcinomas. The youngest patient was 56 while the oldest was 84 years of age.
Results: The sensitivities for labeling prostate cancers were: PSA (92%), AMACR (92%), CK20 (17%), CK7 (8%), p63 (0%), and HMWCK (0%), while urothelial high-grade carcinomas demonstrated the following rates of positivity: HMWCK (92%), CK7 (92%), p63 (83%), CK20 (67%), AMACR (33%), and PSA (0%). Co-

expression of CK7/20 was noticed in 67% of the urothelial carcinoma cases, but it lacked in prostate carcinomas.
Conclusion: Our results indicate that a panel of four markers, PSA, HMWCK, CK7 and p63, can determine, in the majority of the cases, the definite primary site of origin of a poorly differentiated invasive tumor diagnosed in TUR specimens. In spite of the high rate of positivity in prostate carcinomas (92%), AMACR is less useful for this purpose since 33% of high-grade urothelial invasive carcinomas expressed this marker too.

0294
Histological and ultrastructural analysis of seminiferous tubules wall layers in ageing testis

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Objective: The authors evaluated possible morphological changes of basement membrane (BM) and lamina propria (LP) of seminiferous tubule wall (ST) related to ageing.
Method: Surgical samples of testicular tissue from 28 cases with orchiectomy for prostate adenocarcinoma were processed for light microscopy and transmission electron microscopy (TEM) examination. Seven age groups (AgGr) between 50 and 80 years were designed. Tissue samples were immunomarked for collagen IV and smooth muscle actin. Images were acquired and measured with a specialized software. Thirty STs were randomly selected, with ×40 objective, for each case. Five random determinations for each ST and each parameter were performed. Mean values/tubule, case and AgGr were calculated for each parameter. Regression line (RL), slope and significance test for slope were determined for each parameter correlation with ageing.
Results: BM mean value was around 0.5 μm, with narrow limits of ranging in AgGr but more extended individual limits. RL showed discrete decreasing trend with ageing, but without an obvious statistical correlation. LP mean value was around 6 μm, also with narrow limits of ranging in AgGr and more extended individual limits. RL decreased discretely with ageing, but without an obvious statistical correlation. TEM showed more prominent BM material and more collagen fibers and less fibroblasts in LP of older AgGr and higher fibroblasts density in LP of younger AgGr.
Conclusion: Our results showed that BM thickness is apparently decreasing with ageing, whereas LP presents extremely variable degenerative changes, with a "mosaic", focal distribution and no tendency to advance with ageing.
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0295
Age-related changes of testicular intralobular interstitial tissue

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Objective: The authors evaluated the possible influences of ageing on the morphology of testicular intralobular interstitial tissue (TIIT).
Method: The studied material consisted of surgical samples of testicular tissue from 192 cases with orchiectomy for prostate adenocarcinoma. Tissue samples were fixed in neutral buffered formalin embedded in paraffin, stained with hematoxylin–eosin and Goldner trichrome and immunomarked for CD34. Images were acquired and measurements were performed with a specialized image analysis software after previous calibration. The assessed parameters were TIIT amount (P-TIIT) and intralobular vascular network (IVN). Ten fields were randomly selected for each case, with ×20 objective. Mean P-TIIT (mP-TIIT)/field, mP-TIIT/case and mP-TIIT/age group were determined. Regression line (RL), slope (m) and significance test for slope (p) were determined in order to assess the correlation of TIIT amount changes with ageing.
Results: Intralobular septae showed variable amount of collagen fibers, from simple linear fibrillary assemblies to marked thickening with obvious septal enlargement. The latter were seen mostly in the outer region of the lobules, but without any significant spread. TIIT amount had a mean value of 25% of intralobular parenchyma. RL showed a discrete decreasing trend with ageing ($m=-0.01$) confirmed by "p" value ($p=0.018$). Arteriolar wall presented degenerative changes with limited extension and variable intensity. Peritubular and intramural capillaries were present in all age groups. Endothelial cells revealed no significant changes.
Conclusion: Our results showed that intralobular fibrosis had no obvious increasing trend with ageing and IVN revealed only focal changes, not related with ageing.
Acknowledgement: Work supported from the Research Project 41-015/2007 financed by Romanian Ministry of Education and Research through CNMP.

0297
Bilateral malignant Leydig cell tumor with unilateral undescended testis and multiple metastases

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Objective: Leydig cell tumors (LCTs) are usually benign tumors, but approximately 10% of them are malignant. It has been reported that approximately 3% of the LCTs are bilateral. Data regarding a relationship between sex cord stromal tumors and cryptorchidism are scarce.
Method: We present an unusual case of bilateral testicular malignant LCT with left-sided undescended testis, metastatic to subcutaneous tissue, lymph nodes, bones and brain.
Results: Clinical course was aggressive, resulting in the death of the patient in 30 days after the diagnosis was given.
Conclusion: We still need for further studies about optimum management of malignant LCTs.

0298

Primary testicular carcinoid

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Objective: Primary carcinoid of the testis is a rare neoplasm, making up for <1% of testicular neoplasms. Only a few studies were published on this subject presenting the clinicopathological characteristics, immunohistochemical features and the controversial histogenesis of primary testicular carcinoid tumor and its differential diagnosis.
Method: We present the case of a 52-year-old man who sought care for a painful testicular tumefaction which had been discovered 3 months prior and developed rapidly. The patient underwent radical right orchiectomy. Standard and immunohistochemical stains were performed.
Results: Gross examination revealed a 2-cm intraparenchymatous, well-circumscribed, pale yellow, solid and homogeneous tumor. Microscopic examination showed a nested or trabecular architecture associated with tubular and papillary structures within a scarce stroma without necrosis. Tumor cells had an abundant cytoplasm, with regular, round or oval nuclei, moderate anisokaryosis and rare mitoses. Surrounding parenchyma was only slightly modified. Immunohistochemistry showed positivity for cytokeratin (AE1\\AE3), chromogranin, synaptophysin and serotonin and negativity for inhibin, calretinin, PLAP and alpha-fetoprotein. Proliferation index (MIB1) was under 2% and p53 staining was negative.
Conclusion: Primary testicular carcinoid is an extremely rare neoplasm whose histogenesis remains disputed. For a correct diagnosis, a metastatic carcinoid tumor from other site should be excluded in the first place.

0300

Evaluation of the prognostic value of peritumoral inflammatory response in patients with penile carcinoma

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Objective: In spite of the low incidence in industrialized countries, penile carcinoma (PC) represents 2.1% of all malignancies among Brazilian men. Peritumoral immune response has been described to have influence in prognosis in some tumor types, but its role in PC has not yet been addressed. The purpose of the present study was to evaluate inflammatory infiltrate cell components and to evaluate its relationship with clinicopathological features in order to establish the prognostic value of immune response in patients with PC.
Method: One hundred fifty-five patients diagnosed at our hospital, with a minimum 5-year follow-up were included in the study. Representative paraffin-embedded tissue was submitted to immunohistochemical evaluation using antibodies to CD1a, CD3, CD4, CD8, CD20, CD56, CD68, CD138, granzyme B, S100 protein, HLA-DR and FOX-P3.
Results: Elevated plasma cell infiltrate detected by conventional morphology and CD138 correlated with more favorable event-free survival ($p=0.04$), indicating a protective mechanism of elevated plasma cell counts. However, when the presence of lymph node neoplastic infiltration was evaluated in a multivariate analysis, only this parameter remained as having prognostic value.
Conclusion: It is concluded that the role of inflammatory response on prognosis is relevant, especially for CD138, but this importance does not prevail over the value of lymph node status. (Supported by FAPESP and CNPq).

0301

Unusual morphologies in adult epithelial kidney tumors

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Objective: Light microscopic diagnosis of renal tumors using the World Health Organization (WHO) is often

difficult due to variable histology. Six separate epithelial renal tumors with distinct morphologies are described in the present study.
Method: Formalin-fixed paraffin-embedded tissue specimens from patients diagnosed with renal tumors described on computed tomography and surgically treated in Fundeni Clinical Institute in the last 2 years were sectioned at 4 μm. The slides were stained with the usual hematoxylin&eosin staining for histological diagnosis. We selected cases with unusual morphologies for conventional epithelial parenchymal renal tumors on light microscopy; clear cell renal cell carcinomas with variable nuclear grading and pure non-epithelial kidney tumors were excluded. Immunohistochemical studies were performed in the Department of Pathology and Immunohistochemistry at "Victor Babes" National Institute. This work was supported by the Grant PNII no. 62089/2008.
Results: The selected cases were classified as renal oncocytosis, collision renal tumor, metastatic undifferentiated carcinoma in clear cell renal carcinoma, clear cell carcinoma with partly sarcomatoid morphology and two unclassified renal cell carcinoma.
Conclusion: The importance of recognizing renal tumors with unusual morphologic features lies in the potential diagnostic, prognostic or therapeutic implications that accompany these particular types of renal tumors. Clinical and imaging data, tumor sampling and ancillary studies can be helpful in rendering the correct diagnosis.

0302
Mixed epithelial and stromal tumor of the kidney

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Objective: Mixed epithelial and stromal tumor of the kidney (MESTK) is a recently described rare neoplasm. Malignant transformation, recurrence and metastasis are rare; therefore, histopathological distinction from other renal neoplasms, especially from renal cell carcinoma, is important. Histologically, the tumor is composed of biphasic components, including cysts and tubules embedded in the spindle cell stroma.
Method: We report a case of a MESTK in a 60-year-old woman who presented with an incidental renal mass but without any urinary complaints.
Results: Clinical course is uneventful after 5 months.
Conclusion: This is a unique case resulting in differential diagnosis which should be distinguished from malignant tumors of kidney.

0303
Primitive neuroectodermal tumor—a very unusual and highly aggressive tumor of the kidney

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Objective: Primitive neuroectodermal tumors (PNET) occur predominantly in childhood in the central nervous system and soft tissue. PNET of the kidney are rare and highly aggressive tumors that can be mistaken for a variety of other small blue cells tumors. We present a rare case of kidney PNET in an adult with rapidly fatal outcome.
Method: This case was of a 55-year-old patient who was hospitalized for a left renal tumor discovered during an ultrasound examination. On CT scan, a solid non-homogeneous tumor with 15-cm maximum diameter was detected in the left kidney; there were also visualized enlarged hilar and paracaval lymph node masses which included the left renal vein. A left perifascial nephrectomy with adrenalectomy was performed.
Results: On gross examination, the tumor presented as a large, lobulated, friable, grey-white mass with hemorrhagic foci which replaced most of the kidney. The tumor exhibited primitive undifferentiated round cell morphology on H&E-stained slides. Immunohistochemical study revealed positive reaction for vimentin, NSE and CD99, while CK (MNF116), EMA, LCA, CD20, CgA, CD34, desmin, actin were negative. Soon after surgery, bilateral pleurisy with secondary lung involvement images precipitates the fatal outcome of the patient.
Conclusion: The occurrence of PNET in the kidney of an adult is very unusual, this case being the first diagnosed in our department in a period of 10 years. It confirms the advanced stage at presentation and the aggressive behavior of the renal PNET. For an accurate diagnosis, immunohistochemical reactions play an extremely important role.

0304
Synchronous appearance of carcinoma, adenoma and leiomyoma in kidney: a case report

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Objective: Papillary carcinomas comprise 10% of the renal cell carcinomas. The patients are usually middle-aged men. Papillary adenomas are often found in patients undergoing haemodialysis. Leiomyomas are benign

mesenchymal neoplasms that are considered to arise from the renal capsule.
Method: Six months ago the patient, a 71-year-old woman, suffered from pneumonia and was hospitalised. After a few months, in a follow-up CT scan, a tumour was found on her right kidney and a nephrectomy was scheduled.
Results: On cut sections, we found a well-circumscribed yellowish tumour, 4.5 cm in maximal diameter, a capsular, whitish, elastic nodule sized 0.5 cm, as well as a small, cystic-like formation. Microscopically, they corresponded with a papillary carcinoma type 1 with numerous foamy macrophages and calcifications, a papillary adenoma and a benign mesenchymal neoplasm, immunohistochemically identified as a leiomyoma.
Conclusion: Papillary carcinomas can be bilateral or multifocal, usually in hereditary cases. Predictive factors are identical to the ones of clear cell carcinomas. Type 1 tumours with extensive necrosis, abundant macrophages and absence of a sarcomatoid dedifferentiation are associated with a better prognosis. The presence of adenomas or leiomyomas does not to seem to have an affect on the outcome. A year after the nephrectomy, the patient is alive and well.

0305

Renal angiomyolipoma associated with endometriosis

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Objective: Angiomyolipoma is a rare neoplasm composed of varying admixtures of blood vessels, smooth muscle cells and adipose tissue. There is a strong association with tuberous sclerosis. We describe the first case report of multifocal renal angiomyolipoma developed on tuberous sclerosis and associated with endometriosis.
Method: Imaging studies in an asymptomatic 37-year-old woman, affected by tuberous sclerosis with TSC1 gene mutation, revealed a partially solid and cystic mass measuring 7 cm involving the left kidney and a solid mass measuring 0.6 cm in the right kidney.
Results: The surgical specimen consisted of two left nephron-sparing procedures and one at the right side with firm, pale nodules measuring 6.5, 1.5 and 0.6 cm. The tumors were composed of spindle and epithelioid cells with an abundant pale eosinophilic or clear cytoplasm with round nuclei, mild atypia and low mitotic activity. Rare islands of adipose tissue were observed. In the two left tumors, the predominant smooth muscle cells were associated with some foci of endometriosis. The spindle and epithelioid cells were immunoreactive with HMB45 and smooth muscle actin. The estrogen and progesterone receptors were positive in stromal and epithelial cells of endometriosis. The spindle cells of angiomyolipoma were positive with progesterone receptors.
Conclusion: Multifocal PEComa of the female genital tract associated with diffuse endometriosis was described by Froio et al in 2008, but to our knowledge, this is the first case report of renal angiomyolipoma associated with endometriosis. This association represents a supplementary proof of potential role for hormones in the pathogenesis of angiomyolipoma.

0306

Translocation-associated renal cell carcinoma (RCC) in young woman: case report

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Objective: A renal masse was found incidentally on imaging in a 30-year-old woman. It was classed as a Bosniak type III cyst.
Method: Gross findings showed a 3.5 partially cystic, well-circumscribed and tan-yellow renal tumor. Histologically, it contained cells having voluminous cytoplasm (ranging from eosinophilic granular to clear) arranged in sheets, nests, trabeculae, pseudoalveolar structures and papillae. There were no psammoma bodies. Diagnostic hypothesis: variant of multilocular RCC, papillary or chromophobe carcinoma?
Results: Immunohistochemistry: $CK7^{-}$, VIM^{-}, $p504S^{+}$, HMB45/ MelanA $focally^{+}$, CD10 $focally^{+}$. Complementary study: TFB^{+}. Our final diagnostic was: RCC with translocation, known as tumor associated with MiTF/ TFE family translocations, found mainly in young patients and children. In contrast to TFE3 (Xpll.2) translocation tumors, TFB-RCCs are extremely rare (12 cases published). They are characterized by a translocation between the α gene at 11q12 and the first intron of the TFEB gene at 6p21. Translocation-associated RCCs are likely to be present at higher TNM stage. Our patient (pT1N0) presented no evidence of disease 1 year after resection.
Conclusion: Literature analysis confirmed an increase in advanced stage presentation and a worse outcome in pediatric translocation ($TFE3^{+}$) RCC compared with classic ($TFE3^{-}$) RCC. Little is known about TFB RCC's behaviour. Taking into consideration a morphologic heterogeneity of these tumors and their supposed worse prognosis, a screening for translocations with consistent use of antibodies against TFE3 and TFB in all unusual cases of RCCs, regardless of patient age, however, especially in young patients, seems necessary to determine the true incidence of these tumors.

Friday, 3 September 2010, Basement

PS-15 Poster Session Digestive Disease Pathology

0307

Acute onset alendronate-induced esophageal ulcers: a case report

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Objective: Alendronate sodium is used primarily in the treatment of osteoporosis. Severe esophageal injury has been reported as a rare adverse effect of alendronate.
Method: We present a case of widespread longitudinal esophageal ulcers between 26 and 34 cms appearing within 8 h of oral alendronate treatment. A 46-year-old woman who had hypothyroidism began alendronate treatment for osteoporosis prevention. She presented with severe dysphagia and abdominal pain 8 h after taking the first pill. Upper endoscopy has been performed and biopsy samples from the esophageal ulcers have been taken. Histologically, epithelial infiltration of neutrophils and eosinophils was seen and within leukocytic exudate fragments neutrophils and eosinophils were detected, suggesting a hypersensitivity reaction. No sign of organisms or a neoplasm was present.
Results: Alendronate treatment was discontinued. After 2 weeks, repeat endoscopy showed marked improvement of esophageal ulcers.
Conclusion: This is a unique case reporting acute onset alendronate-induced esophageal ulcers.

0309

CagA, CagA EPIYA motifs and histopathological features in gastric *Helicobacter pylori* infection

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Objective: The aim of the study was to identify carboxyl terminal regions of the CagA (EPIYA motifs) in Turkish *Helicobacter pylori* strains and compare cagA positivity and EPIYA motifs with demographic characteristics and histopathological findings in patients with dyspepsia.
Method: Antrum and corpus biopsies from 124 patients with dyspepsia were evaluated by histopathology, rapid urease test (RUT) and culture. *H. pylori* cagA polymerase chain reaction (PCR) products ranging from 370 to 570 bp were amplified and products purified by QIAquick PCR kit (Qiagen) and sent to Macrogen for sequencing.
Results: Eighty-nine (71.8%) patients were *H. pylori* positive by at least two of the tests, including RUT, histopathology and culture. Thirty (48.4%) patients were colonized with cagA-positive strains from the 62 *H. pylori* culture-positive patients. We found significantly higher scores of inflammation of pits in antrum and corpus and atrophy in corpus in CagA-positive patients; there were no differences in relation with intestinal metaplasia and with inflammation of the lamina propria. Of the 30 CagA *H. pylori* strains, 21 (70%) had three EPIYA motifs (EPIYA-ABC) and seven (23.3%) had more than three EPIYA motifs (EPIYA-ABCC). However, no association was found between inflammation scores, intestinal metaplasia or atrophy with differences in the EPIYA motifs. We found two patients (6.7%) colonized with more than one strain (EPIYA ABC and ABCC) based on phylogenetic analysis and variation in cagA genotype.
Conclusion: CagA positivity strongly correlated with gastric histopathological inflammation patterns, but there was no association with the number or type of EPIYA motifs.

0310

Do gastric lymphoid infiltrates remain a challenge for pathologists?

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Objective: Gastric MALT (mucosa-associated lymphoid tissue) lymphoma is closely related with *Helicobacter pylori* (Hp) colonization and represents a multistage process from gastritis to lymphoma. For the differential diagnosis of MALT lymphoma and reactive inflammation, Wotherspoon proposed a histomorphological scoring with two intermediate categories, grades 3 and 4. Genetic studies (Ig clonality and/or translocations and chromosomal aberrations) have not brought so far definite arguments to confirm or discard lymphoma in these problematic scores.
Method: We reviewed 14,071 HE biopsies containing "gastric lymphoid infiltrates", retrieved from our files from January 2004 to December 2008 in order to evaluate the relevance of ambiguous Wotherspoon score on the follow-up.
Results: From these biopsies, 124 samples (0.9%) from 77 patients were ambiguous or lymphomatous. Thirty-four patients (44%) suffered from lymphoma with a predominance of MALT lymphoma (50%), as well as diffuse large B cell lymphoma (29%), mantle cell lymphoma (15%), Burkitt-like lymphoma (3%) and follicular lymphoma (3%). The remaining 43 patients were affected by Wotherspoon grade 2 to 4 gastritis, with a complete follow-up available for 17 (40%). Lymphoma was present in further biopsies in

six cases (35%), initially coming from either grade 4 (5/6) or grade 3 (1/6). Hp was only observed in two of six lymphoma cases in the first biopsy.
Conclusion: Wotherspoon grades 3 and 4 remain a challenge for pathologists. Clinical follow-up combined with histology and immunohistochemistry should be still considered as the gold standard for the diagnosis. Moreover, pathologists must keep in mind that aggressive lymphomas can also involve the stomach.

0311
Correa's model—comprehensive or partial explanation for carcinogenic process triggered by the *Helicobacter pylori* infection?

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Objective: As a group 1 carcinogen for gastric cancer, *Helicobacter pylori* (HP) was involved in many studies and researches focused on physiopathology and morphopathologic changes induced by this bacterium. We proposed to study the precancerous epithelial alteration in patients with HP chronic gastritis, their regression or progression after anti-infectious treatment.
Method: The study included 3,069 gastric endoscopy biopsies performed between 2005 and 2009 in our hospital. Histopathologic diagnosis of these biopsies was made using Sydney criteria. The patients were divided into two groups based on the presence or absence of HP: Group A included 1,414 HP-positive patients and group B included 1,653 HP-negative patients. We evaluated several histopathological parameters, correlating the degree of inflammation, atrophy, metaplasia, regenerative hyperplasia and dysplasia with the presence of HP.
Results: Our study identifies an overall tendency towards regression of premalignant lesions of gastric epithelium after HP eradication, as well as an increasing number of patients diagnosed with early gastric cancer, thus consolidating the results of studies who foretell the significant decrease of gastric cancer mortality. These lesions are present years before becoming clinically manifest and consequently treatable.
Conclusion: In respect to carcinogenic mechanisms, some of our results confirm the carcinogenic cascade triggered by the HP infection, as was proposed by Correa et al. in 1975. However, we obtained data leading to the idea that the "precursor lesions" could appear (and histopathologically evaluated) independent from one to the other, which seemed to be through other possible steps. Note: Zurac and Micu should be regarded as first authors with equal contribution.

0312
Intestinal metaplasia and cytokeratin 7/20 pattern of cardiac mucosa in patients with *Helicobacter pylori* infection

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Objective: Specialized Barrett-type intestinal metaplasia (SIM) seems to be a stronger risk factor for malignancy than non-Barrett cardiac intestinal metaplasia (CIM). The pathogenesis of SIM and that of CIM are thought to be associated with gastro-oesophageal reflux disease and *H. pylori* infection, respectively, but are still controversial. Recent studies regarding the expression of cytokeratin CK7 and CK20 in intestinal metaplasia (IM) found at the gastroesophageal junction are conflicting, especially in regard to associated *H. pylori* infection.
Method: We studied the expression of CK7 and CK20 on 45 biopsy specimens of gastric cardia from patients with *H. pylori* infection and histological evidence of IM (group 1) and 36 biopsy specimens from patients with *H. pylori* infection but without evidence of IM (group 2). The distinction between SIM (18/45 cases) and CIM (27/45 cases) was defined morphologically rather than by biopsy site.
Results: On the intestinalized cardiac mucosa, most often we observed the so-called Barrett-type CK7/20 staining pattern consisting of band-like CK20 staining of the surface epithelium and superficial glands and moderate to strong CK7 staining of both superficial and deep glands, i.e. it was found in 12 of 18 cases of SIM, in 19 of 27 cases of CIM and in 23 of 36 cases of non-intestinalized cardiac mucosa or in 14.8%, 23.5% and 28.4% of patients, respectively.
Conclusion: We have found no statistically significant differences in Barrett-type CK7/20 staining pattern between SIM and CIM as well as between intestinalized and non-intestinalized cardiac mucosa in patients with *H. pylori* infection.

0314
Expression of Her2/neu in gastric adenocarcinomas

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Objective: In gastric adenocarcinoma, overexpression of HER2 has been correlated to tumour location and histologic

type and varies considerably in the few published studies, probably due, at least in part, to the racial heterogeneity of patients included. The aim of the present study was to investigate the immunohistochemical expression of HER2 protein in gastric adenocarcinomas of Greek patients who live in the area of Epirus.

Method: A total of 90 primary gastric adenocarcinomas were retrieved from the archives of the Department of Pathology (63 men and 27 women). They were all located in the stomach, away from the oesophageal–gastric junction. Forty-eight were intestinal type, 37 diffuse type and 5 mixed (Lauren classification). Immunohistochemical analysis of samples was performed using the polyclonal rabbit anti-human c-erb-2 Oncoprotein (DAKO) antibody on paraffin sections. HER2 scoring was performed according to published consensus panel recommendations.

Results: Overexpression of HER2 ($IHC3^+$ and 2+) was detected in eight adenocarcinomas (8.9%). Specifically, score 3+ was assigned to five cases (5.6%, four intestinal type, one diffuse type) and 2+ to three cases (3.3%, two intestinal type, one diffuse type). Score 1+ was detected in ten cases (11.1%, eight intestinal type, one diffuse type and one mixed), and 0 in 72 cases (80%). In two cases with score 3+, adjacent dysplastic epithelium was noted, which was also 3+.

Conclusion: The results of the current study are comparable with those of the literature, indicating that assessment of immunohistochemical expression of HER2 in gastric adenocarcinomas could lead to the stratification of patients, the goal being the optimal personalized treatment.

0316

The prognostic value of Her-2/neu expression in gastric cancer patients from lower Silesia in Poland

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Objective: Overexpression of HER-2/neu was most widely explored in breast cancer. HER-2 positivity is reported as 6–35% in gastric cancer (GC). The aim of the study was to assess the prognostic significance of HER-2 in GC patients from Lower Silesia, the southwestern region of Poland.

Method: Immunohistochemistry (IHC) for HER-2 monoclonal antibody was performed on 82 paraffin-embedded specimens. IHC expression of HER-2 was evaluated using the Remmele scale (IRS) and standardised HercepTest modified for GC.

Results: The status of HER-2 protein expression was observed as the following: (1) IHC 0, IRS 0—37 cases (45.1%); (2) IHC 1+ (22, 26.8%), IRS 1/2/3 (21, 25.6%); (3) IHC 2+ (17, 20.7%), IRS 4/6/8 (20, 24.4%); and (4) IHC 3+ (6, 7.3%), IRS 9/12 (4, 4.9%). HER-2 overexpression was detected in 23 (28%) tumors in HercepTest (IHC 2+/3+) and in 24 (29.3%) in IRS scale (IRS 4-12). No correlations were found between HER-2-postive GC and tumor size, local and distant metastases. Tumor stage at diagnosis and age were the only covariate with negative prognostic significance in patient survival ($p=0.028$ and $p=0.0048$). There was no significant difference in survival between HER-2-positive and -negative tumors ($p=0.454$).

Conclusion: HER-2 positivity rate observed in GC in patients from Lower Silesia is as high as in breast cancer. Immunohistochemical detection of HER-2 expression is neither a predictor of survival of patients with GC nor does it identify subgroups of patients at higher risk for recurrence of disease.

0317

MMP-2, MMP-9/TIMP-1, TIMP-2 immunophenotype identifies clinical outcome in gastric cancer patients

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Objective: Breakage or degradation of extracellular matrix (ECM) and basement membrane (BM) is a critical step in tumor progression. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) take important roles in these processes acting in a coordinated manner to form an integrated system. The aim of the study was the evaluation of MMP-2, MMP-9, TIMP-1 and TIMP-2 expression and the correlation with clinicopathological parameters and survival in GC.

Method: Formalin-fixed paraffin-embedded tissue sections from 82 gastric cancers were studied for the expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 protein by immunohistochemistry. Immunoreactivity of neoplastic/epithelial tissue and tumor stroma was scored according to Remmele IRS. Results were correlated with tumor grade, Lauren classification, stage, lymph nodes status, distant metastases and overall cancer specific survival (OS).

Results: MMP-2 and MMP-9 expression was positive in 78 of 82 cases (95.1%). Seventy-two of 82 (87.8%) and 40 of 82 (48.8%) GC expressed TIMP-1 and TIMP-2, respectively. High expression of MMP-2 ($p=0.047$) and TIMP-2 ($p=0.011$) was associated with Lauren intestinal tumor type. High epithelial TIMP-2 expression was associated with distant metastases ($p=0.036$). High epithelial TIMP-2 expression was associated with shorter OS ($p=0.025$) and high epithelial MMP-2 with better survival ($p=0.017$).

Conclusion: High epithelial TIMP-2 immunoreactivity is significantly associated with shorter survival and distant metastases. Epithelial MMP-2 overexpression identifies GC

patients with better clinical outcome. Increased TIMP-2 expression reflects its crucial role in predicting the aggressive GC behavior.

0318

Expression of chemokine SDF-1a and its receptor CXCR4 is a marker for an unfavorable prognosis in gastric cancer

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Objective: To investigate the effects of the expression of the chemokine stromal cell-derived factor (SDF)-1α and its receptor CXCR4 on the prognosis of human gastric cancer, the clinicopathological features and the clinical outcomes were analyzed.
Method: The expression of SDF-1α and CXCR4 proteins were studied by immunohistochemistry in 221 specimens of primary gastric cancer.
Results: The patients were categorized into three groups according to the expression of CXCR4 and SDF-1α; high CXCR4/high SDF-1α, low CXCR4/low SDF-1α, and high CXCR4/low SDF-1α–low CXCR4/high SDF-1α. There were no significant differences in age, gender, histology, tumor location, lymphovascular invasion, or proportion of tumor size >5 cm between three groups. However, high CXCR4/high SDF-1α tumors were significantly associated with invasion depth of the tumor (T status, P=0.001), lymph node involvement (N status, P=0.029), and higher stage III/IV (P=0.001) compared with low CXCR4/low SDF-1α tumors and high CXCR4/low SDF-1α–low CXCR4/high SDF-1α tumors. Furthermore, the patients with high CXCR4/high SDF-1α expressions had worst prognosis (5-year survival rate, 26.7%; median, 2.2 years; range, 0.8–3.6 years), whereas patients who had low CXCR4/low SDF-1α expressions showed the most favorable prognosis (5-year survival rate, 57%; median, not reached; log-rank test, P = 0.01).
Conclusion: Thus, CXCR4 and SDF-1α are considered to be useful prognostic factors in gastric cancer, and the combination of high CXCR4 protein expression with high SDF-1α expression indicates a dismal prognosis.

0321

HER2 amplification and overexpression in gastric cancer: analysis of subtype specificity and optimal detection methods

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Objective: The recent ToGA trial indicates that Herceptin is a new, effective, and well-tolerated treatment for HER2-positive gastric cancer (GC). Current diagnostic methods to detect HER2 amplification and overexpression should be carefully evaluated. Furthermore, potential subpopulations of GC (intestinal vs diffuse, cardia vs distal, early-onset GC) should be evaluated for their frequency of amplification and overexpression.
Method: Tissue microarray (TMA) blocks of 108 early-onset GCs and 91 conventional GCs were stained with immunohistochemistry (IHC, Hercep test, DAKO) and chromogenic in situ hybridization (CISH, SPoT-Light, Invitrogen).
Results: Overall, there was 3% HER2 overexpression (5/199) and 5% (9/199) amplification. Furthermore, 1.5% of the patients showed a 2+ IHC score and 3.5% showed a low level CISH amplification. Early-onset GC showed a lower frequency of amplification/overexpression than conventional GC: no overexpression and 2% amplification versus 6% overexpression and 8% amplification. IHC and CISH showed 94% concordance. In 5 of 199 cases (2.5%), IHC showed clinically relevant heterogeneity not seen by CISH. Cardia tumors had more amplification and overexpression than distal tumors: 9% versus 3% and 7% versus 2%, respectively (NS). HER2 CISH showed more amplification in the intestinal type (7%) compared to the mixed (5%) and diffuse type (3%) GCs (p=0.03). A similar trend was seen for HER2 IHC and histology type (p=0.067).
Conclusion: This study reports lower amplification and overexpression frequencies than previously described but confirms previous studies showing different frequencies depending on the type and localisation of the GC. There was a lower amplification and overexpression frequency in early-onset GC and a lower heterogeneity rate by CISH compared to IHC.

0322

Alfa-metyl-acyl-CoA racemase (AMACR) expression in gastric cancer—prognostic factor or a dead end?

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Objective: AMACR plays role in beta oxidation of branched fatty acids. It is expressed in several neoplasms like prostatic, hepatic and thyroid cancers. It is also believed to be expressed in gastric carcinoma (GC), particularly in better differentiated and less advanced lesions. The aim of the study was to assess AMACR expression in GC and its correlation with clinical and histological prognostic factors.

Method: Eighty-two consecutive GC cases (M/F 52:30, mean age 64.3 (30–81) were analyzed after a detailed histological and clinical reevaluation. Immunohistochemical staining was performed and the expression of AMACR was assessed semiquantitatively (0—no expression, 3—intensive, diffused expression). For statistical analysis, Fisher's exact test was used.
Results: There were 33:39:10 cases with glandular, diffused and mixed histology, respectively, according to Lauren classification. Thirty-three localized in antral part and 49 in corpus or fundus. AMACR expression was present in 49 GC (59%). No statistically significant correlation between AMACR expression and age, sex (p=0.63), localization (p=0.81), tumor histology (p=0.375) and differentiation, Lauren groups (p=0.27) or tumor staging (p=0.48) was revealed.
Conclusion: According to our results, the correlation of AMACR expression with the aforementioned parameters cannot be confirmed. Negative results might have been caused by low statistical power due to the limited number of patients. Therefore, its utility as prognostic factor needs further validation.

0326

Concordance of HER2 dual-color, dual-hapten in situ hybridization (DDISH) with fluorescence in situ hybridization on gastric carcinoma

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Objective: It is well established that the HER2 gene, located on chromosome 17, is amplified in ~30% of breast cancer patients. Determination of HER2 gene amplification and/or protein overexpression dictates the eligibility of patients for Herceptin® (trastuzumab) therapy, a monoclonal antibody that targets HER2 and can significantly improve prognosis. Recent studies indicate that HER2 is overexpressed in a percentage of gastric cancers, and Herceptin also is a promising therapy for these patients. Thus, accurate diagnosis of HER2 status is required for breast and gastric patients. Here, a new fully automated, HER2/CHR17 dual-color, dual-hapten brightfield in situ hybridization method (DDISH) was compared with Dako HER2 FISH PharmDx Kit on gastric samples.
Method: Two hundred five invasive gastric carcinoma cases were evaluated with DDISH and FISH. A HER2 repeat-depleted, dinitrophenyl (DNP)-labeled probe targeted the gene and was detected with silver in situ hybridization. A digoxigenin (DIG)-labeled chromosome 17 probe was detected by an alkaline phosphatase-driven Red ISH detection. All assay steps, from deparaffinization through counterstaining, were fully automated and required ~12 h. HER2 SISH and CHR17 Red signals were enumerated using conventional light microscopy, the HER2/CHR17 ratio calculated, and the gene status compared to FISH.
Results: Overall concordance between the HER2/chromosome 17 DDISH assay and FISH was 94.5%.
Conclusion: The automated HER2 (DDISH) assay yielded excellent correlation with FISH. These data indicate that DDISH is a valuable diagnostic test for the determination of HER2 gene status in gastric carcinoma.

0328

A rare case of pyloric obstruction due to Brunner's gland hyperplasia

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Objective: Brunner's gland hyperplasia is a rare lesion, with fewer than 240 cases having been reported in the literature. It is considered to be a hamartoma, developing from the submucosal glands, usually in the first or second part of the duodenum. Most of the lesions described were polypoid and <2 cm in greatest diameter. Often, there is an association with peptic ulcer disease.
Method: We report the case of a 31-year-old man who presented with abdominal pain, vomiting and upper gastrointestinal bleeding. The anamnestic data showed chronic ulcer disease. He also was anaemic, with a slight raise in blood eosinophilia.
Results: The radiological examination of the upper gastrointestinal tract revealed pyloric stenosis due to a polypoid mass measuring 1 cm. Preoperative diagnosis of the mass could not be established. The mass was excised and at the same time a vagotomy with pyloroplasty (Finney) was performed. Brushing cytology of the surgical specimen revealed a great number of aggregated Brunner's glands without any atypia, as well as many lymphocytes. A frozen section was negative for malignancy. The histologic diagnosis was that of diffuse nodular Brunner's gland hyperplasia (hamartoma) accompanied by lymphocytic infiltration. The rate of cellular proliferation was low. During the postoperative period, there were no complications. A subsequent gastroscopy showed no pathologic findings, and the patient is well at present.
Conclusion: Our case is reported because of its rarity and the difficulties in preoperative diagnosis. The literature is also reviewed.

0329

East European picture of coeliac disease

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Objective: Although the autoimmune process targets mainly the intestinal mucosa, coeliac disease (CD) present with a variety signs and symptoms affecting any organ or tissue. Aim: The aim was to assess the clinical behavior of coeliac disease in a secondary care setting in Iasi Romania.
Method: From April 2002 to December 2009, 175 adults were screened for CD. Patients were referred because of malabsorption and classical gastrointestinal symptoms (chronic diarrhoea, abdominal pain, weight loss and iron deficiency anemia). The diagnosis was based on clinical presentation, serology including IgA EMA or IgA-tTGA and duodenal biopsy. Results of intestinal biopsy were classified according to the revised Marsh criteria 1999.
Results: Of the 175 subjects suspected cases, 91 underwent small bowel biopsy and 55 of 91 serology. The most common clinical presentation was weight loss in 60 of 91 and chronic diarrhoea and anemia in 78 of 91. Histology revealed microscopic enteritis (Marsh 0–II) in 43 of 91 and macroscopic lesions (Marsh IIIa–IIIc) in 48 of 91, respectively.
Conclusion: There are not many studies on the epidemiology, investigation or clinical behavior of CD in East European countries. Most of the cases in this study were diagnosed from 2005 onward, indicating increasing awareness on CD. Classical presentations were predominant and more prevalent compared to atypical forms of disease. This still may indicate a high threshold for performing serology and small bowel biopsies in cases with atypical presentations.

0330

Synchronous small intestinal inflammatory fibroid polyp and GIST

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Objective: In the differential diagnosis of GIST, associated mesenchymal lesion represents an additional challenge.
Method: A 51-year-old woman with intestinal occlusion at surgery showed an ileal invagination on polypoid mass of 4 cm and an ileal suberosal nodule of 1 cm.
Results: The polypoid lesion, ulcerating the mucosa and growing in the muscular wall with subserosal hourglass-like expansion, showed a monotonous hypocellular mesenchymal proliferation ($CD34^+$, $CD117^-$) with prominent vascular network in myxoid inflammatory stroma. Considering the peculiar degenerative, inflammatory pattern of the polyp due to ischemia and the existence of a subset of GIST c-Kit negative, differential diagnosis was GIST ($c\text{-}Kit^-$) or inflammatory fibroid polyp ($CD34^+$). After a careful review of the clinical and immunohistochemical and pathological findings, final diagnosis was inflammatory fibroid polyp (IFP). The minor nodule showing a spindle cell proliferation ($CD117^+$) was diagnosed as GIST. To our knowledge, no other studies report IFP and GIST occurring synchronous in the ileum.
Conclusion: The correct definition of these lesions is crucial: Defining both tumors as GISTs, prognostic and therapeutic assessment becomes poor (multiple familial or advanced metastatic GIST). The recent discovery of PDGFR mutation (the mutually exclusive mutation of wild-type cKIT GISTs) in the IFP questions his reactive nature and raises the possibility of a neoplastic process. Then, the questions arising are whether the two tumors represent a fortuitous coexistence or involve the same carcinogenic agents on the same gene (located in the chromosome 4q) and if the proliferation can be inhibited by STI571.

0332

Colonic mantle cell lymphoma in situ: a report of a unique case

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Objective: Colonic mantle cell lymphoma (MCL) represents 1–4% of malignant tumors of the GI tract. They are extremely difficult to diagnose due to the small sampling size, immune hyperplastic responses which may mimic lymphoma, and very early phase of neoplasia.
Method: H&E-stained slides from 2007 were reevaluated. Immunohistochemical (IHC) stain for BCL1 (cyclin D1) was performed. H&E-stained slides and IHC stain for CD20, BCL1, CD5, kappa, lambda, CD19, CD20, CD5, CD10, and CD23 were evaluated. Flow cytometry was done on bone marrow and lymph node biopsy. FISH study for t(11;14) (q13,q32) was done.
Results: A 45-year-old man was admitted to the hospital in 2007 for a bright red blood per rectum (BRBPR). Colonoscopy showed rectal erythema thought to be prep-related. Random biopsies taken were signed out as colonic mucosal lymphoid aggregates and revised to MCL in situ upon reevaluation. In 2009, he was readmitted for BRBPR

and a 10-lb weight loss, lower abdominal discomfort, fatigue and soaking night sweats. Colonoscopy displayed mucosal edema in cobblestone pattern with erythema, ulcerations and a mass-like appearance of folds which were biopsied and signed out as MCL.
Conclusion: In situ MCL lymphoma is not a so well-recognized entity, much less in the GI tract where it has never been described before. We emphasize the need to be aware of GI lymphomas in general, specifically a rare entity such as in situ MCL, knowing that this may represent a disease in progress.

0333
Undifferentiated carcinoma versus acromic melanoma of rectum—case report

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Objective: Gastrointestinal (GI) involvement by malignant melanoma is predominantly metastatic. The concept of primary malignant melanoma of the GI tract has been validated for lesions involving the esophagus, stomach, small bowel, and anorectum, (the most common site). The existence of primary melanoma of the large bowel is a controversial topic. Six cases of apparent primary colonic melanoma have been reported to date, two of which in the rectum.
Method: A 65-year-old Caucasian man presented in emergency following a massive rectal bleeding. Rectal examination revealed an anterior fleshy mass situated 4 cm from the anal verge. The patient said he had been bleeding intermittently for 4 months but without any pain or change in bowel habit. Rigid sigmoidoscopy demonstrated a polypoid lesion at the anorectal angle. The surgical specimen was analyzed macroscopically and microscopically using routine and immunohistochemical staining. Dermatological and ophthalmological examinations revealed no evidence of a cutaneous or an ocular primary lesion.
Results: Histopathologic examination on routine staining demonstrated undifferentiated malignant cells with pleomorphic nuclei and atypical mitosis, suggesting the possibility of an acromic melanoma or undifferentiated carcinoma. Immunostains for pancytokeratin, CEA and EMA were positive and showed negative reaction for HMB 45, S-100 and Melan-A, establishing the diagnosis of undifferentiated carcinoma.
Conclusion: Malignant melanoma of the anorectum is an uncommon condition. An expeditious diagnosis and care within a multidisciplinary team can have an important bearing on prognosis. Morphologically, the differential diagnosis of melanoma includes undifferentiated carcinoma, GIST, clear cell sarcoma, and epithelioid malignant peripheral nerve sheath tumor.

0334
Primary anorectal melanoma—immunohistochemical and genetic analysis

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Objective: Melanoma is a rare tumor of the anorectal area and accounts 0.1% to 1.7% of all anal tumours and approximately 1% of all primary melanomas. Besides the positivity with conventional melanocytary immunohistochemical markers S100, HMB45, Melan A are primary anorectal melanomas positive also with marker CD117 and negative with monoclonal antibody CEA. The primary anorectal melanoma is a typical BRAF mutation (exon 15+11) and the allelic loss NF1 associated with neurofibromatosis.
Method: The melanocytary lesion of a 79-year-old woman in the anorectal canal was examined with immunohistochemical antibodies: S100, HMB45, CK AE1/AE3, CD117 and monoclonal CEA (DAKO), and FISH method with the use of combined sound Vysis PREB1/LSI MYB/LSI CCND1/CEP 6 (Abbot, USA).
Results: Our examination stated positivity with S100, HMB45, Melan A and sporadic positivity with CD117 and negativity with monoclonal antibody CEA. Further, amplification of the gene RREB1 (locus 6p25) and CCND1 (locus 11q13) was found.
Conclusion: The results of our analysis confirm genetic abnormalities associated with melanoma, and our immunohistochemical analysis prefers primary melanoma before metastatic melanoma of the anorectal canal.

0336
Human crypt stem cell dynamics in familial adenomatous polyposis

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Objective: Stem cells in the intestinal crypt are believed to be the target cells for the initiation of tumourigenesis and adenoma development. Macroscopic and histological normal-appearing colorectal mucosa of patients with symptomatic familial adenomatous polyposis (FAP) harbour cell kinetic and stem cell abnormalities, suggesting the exis-

tence of pre-tumour progression. Pre-tumour progression can be made visible by studying methylation patterns, or tags, in stem cells of the crypt where heterogeneity among these tags is a representation of a genetic drift and stem cell survival. Increased stem cell survival may be a predictive marker of an increased risk of neoplastic outgrowth. The objective of this study was to investigate potential biomarkers predictive of adenoma development; the focus lies on alterations in stem cell survival.

Method: DNA was isolated from laser-microdissected crypts of normal colonic FAP tissue and age-matched controls. DNA was bisulphite converted and methylation tags were created by amplifying the CpG islands in the CSX gene. Sequence analysis of this pool of methylation tags allows characterisation of the heterogeneity among the different tags recovered from multiple stem cell pools.

Results: The bisulphite treatment, with subsequent sequencing, showed a significantly ($p=0.030$) greater number of unique methylation tags for FAP patients (2.8 unique methylation tags) compared to control patients (2.1 unique methylation tags), which indicates enhanced stem cell survival.

Conclusion: This increased stem cell survival supports the idea of pre-tumour progression since longer lived stem cells have the potential to gain more mutations with higher risk to develop into a carcinoma.

0337

Colonic carcinogenesis induced by 1NC and PCNA and p53 expression

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Objective: 1-Naphtyl *N*-methylcarbamate (1NC) is considered of low toxicity when applied according with the good practice rules. The evaluation of morphology and proliferation rates of experimentally induced adenocarcinomas by 1NC and DMH was tempted.

Method: Thirty Wistar 8-week male rats were distributed into three groups (ten rats each) submitted to dimethylhydrazine (DMH) 15 mg/week during 8 weeks (group II), 1NC 60 mg/l in drinking water for 8 weeks (group III), and without manipulation (group I). Killed at the end of the 28ª week, lesions were collected and FFPE, PCNA and p53 antibodies were applied to sections of all tumours by the streptavidin–biotin/HRP method.

Results: In groups II and III, lesions were exophytic (2–4 cm), well-differentiated adenocarcinomas, except for one case 1NC-induced a moderately differentiated adenocarcinoma. Proliferation rate—PCNA was higher in group III (1NC-induced): six negative cases in group II against three negative cases in group III; the cutoff: + (<25% positive nuclei), ++ (25–75%) and +++ (>75%), demonstrating five cases ++ and +++ in group III and one case +++ in group II (as the other three cases were +). For p53 nuclear immunostaining, six were positive in 1NC-induced cases against four by DMH.

Conclusion: Despite the small sample of this preliminary study, 1NC revealed to induce more aggressive adenocarcinomas with a higher proliferation rate when compared with the DMH, probably by apoptosis blockage. In general, macroscopic tumors were pediculated and developed from deep crypts where the functional stem cells pool is supposed to be.

0341

Tumor budding added to K-RAS analysis improves the individualized prediction of response to anti-EGFR therapies for metastatic colorectal cancer patients

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Objective: Tumor budding or dedifferentiated single cells/small clusters at the invasive front of colorectal cancer is a histological feature highly predictive of lymph node and distant metastasis and an independent prognostic factor. We hypothesized that the evaluation of tumor budding could complement K-RAS analysis to improve the individualized prediction of response to anti-EGFR-based therapies in metastatic colorectal cancer (mCRC) patients.

Method: Forty-three patients with mCRC treated with cetuximab or panitumumab were entered into this study. According to the RECIST criteria, 30 patients had stable or progressive disease (non-responsive, NR), while 13 patients had a partial response (PR). Tumor buds were evaluated from whole tissue sections stained for pan-cytokeratin, evaluated in the densest region using a ×40 objective, and "high-grade" tumor budding was defined as 15 buds/high-power field.

Results: Tumor buds and K-RAS mutation both correctly classified 68% of patients. All patients with K-RAS mutation ($n=7$) or high-grade tumor budding ($n=11$) were NR, of which four patients had both features. All 13 PR were K-RAS wild type with low-grade tumor budding. Combined, the predictive value of K-RAS and tumor budding was 80%. Additionally, high-grade tumor budding was significantly related to worse

progression-free survival (HR (95%CI), 2.8 (1.3–6.0, p=0.008)).
Conclusion: The combined analysis of tumor budding and K-RAS mutational status accurately predicted response in 80% of patients. If confirmed on larger cohorts, the addition of tumor budding to K-RAS analysis may represent an effective approach for individualized patient management in the metastatic setting.

0342
Consensual proposal for centralized KRAS testing of patients with colorectal carcinoma in Slovakia
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Objective: Identification of gene KRAS somatic mutations (in codons 12, 13) represents accepted biomarker predicting resistance to EGFR inhibitors therapy in patients with colorectal carcinoma (CRC). It is expected that approx. 40% of patients with CRC show one of KRAS mutations. The objective was to develop a centralized programme of KRAS testing in bioptically examined both frozen and/or formalin-fixed paraffin-embedded CRC tissues.
Method: Inter-institutional and interdisciplinary cooperation (of pathologists, geneticians, molecular biologists and oncologists of all three centers) during the last 15 months based on mutual exchange of ideas, materials and laboratory protocols and common meetings and seminars. In the centres, the following three methodical options were tested—sequencing, RT-PCR (TheraScreen®) and SNaPshot multiplex analysis—and the results of the examinations were retested using another method to verify specificity and sensitivity of the testing.
Results: Numbers of tested cases/percentage of KRAS unmutated cases were the following: Center 1: n=290/59.3% unmutated cases, center 2: n=679/61% and center 3: n=841/65.4% of unmutated cases. Altogether, from 1,810 analyzed patients, 62.9% showed CRC with unmutated and 37.1% with mutated KRAS gene. The advantages and disadvantages of all methods tested were evaluated.
Conclusion: On the basis of the described programme, we have developed national guidelines for centralized KRAS testing of CRC in Slovakia, accepted by the authorities of the Ministry of Health and insurance companies. The main outcome of the programme is represented by a possibility offered to every patient with CRC to have the tumor tissue tested for KRAS mutations.

0343
Possible correlations of the K-ras gene mutational status in colorectal carcinoma
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Objective: Gain-of-function K-ras point mutations, present in 20–40% of the colorectal carcinomas, maintain the active form of the ras p21 protein and lead to epidermal growth factor receptor (EGFR)-independent activation of intracellular signaling pathways, making the anti-EGFR tumor therapy ineffective. The aim of the present study was to identify possible correlations between the mutational status of the K-ras gene and the histopathological findings in patients with colorectal adenocarcinoma.
Method: We studied 34 patients with colorectal adenocarcinoma (30) or liver metastases of colorectal adenocarcinoma (4; male/female, 19:15; age range, 34–87; average age 61). The formalin-fixed paraffin-embedded tissue samples were analyzed using an indirect bistadial immunohistochemical (IHC) technique, performed with a Dako EnVision+ Dual Link System-HRP, with antibodies for the EGFR and the ras protein. Mutations in exon 2 and codon 12 of the K-ras gene were detected by PCR–restriction fragment length polymorphism analysis, with *Mva*I restriction enzyme.
Results: K-ras mutations were present in 23.52% of the cases, seven adenocarcinomas and one liver metastasis. EGFR was positive in ten cases in tumor cells and in seven cases in the vessels. Immunohistochemical overexpression of ras protein was detected in 15 samples. The relationship between the positivity of the K-ras mutation and the positive immunohistochemical reaction for EGFR and the ras protein did not reach statistical significance.
Conclusion: There were no significant correlations between the mutational status of the K-ras gene and the IHC reaction for EGFR and ras p21 protein, proving once more the major role of molecular analyses in colorectal carcinoma anti-EGFR therapy.

0344
Abnormal expression of MUC2 associated with p53 overexpression in colorectal carcinomas
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Objective: We reported a case of the colonic choriocarcinoma, of which the accompanying intramucosal adenocarcinomatous area consisted of two components: One revealed normal supranuclear expression of MUC2, and the other revealed abnormal cytoplasmic expression of MUC2 and overexpression of p53 due to a point mutation in exon 5 of TP53. It is thus possible that overexpression of p53 is related to abnormal expression of MUC2 during progression of colonic choriocarcinoma. To investigate whether this relation is seen generally, we examined expressions of p53 and MUC2 in colorectal adenocarcinomas.
Method: This study was based on the analysis of 31 surgically and endoscopically resected colorectal carcinomas. H&E and immunohistochemical stainings with the monoclonal antibodies p53, MUC2 and CDX2 were carried out.
Results: All areas of 12 lesions and a part of two lesions revealed overexpression of p53 (45.2%). For CDX2, the nuclei of cancer cells were positive diffusely in 29 lesions and partially in one lesion. The remaining one lesion showed an abnormal, dot-like cytoplasmic staining pattern. For MUC2, cancer cells were negative or weakly positive in 28 lesions and abnormally positive in five lesions (16.1%). Three out of 31 lesions revealed not only overexpression of p53 but also abnormal expression of MUC2 (9.7%).
Conclusion: In this study, colorectal adenocarcinomas with overexpression of p53 and abnormal expression of MUC2 were relatively rare. Therefore, such a pattern of p53 and MUC2 expressions may be specific to colonic choriocarcinoma, possibly related to the occurrence of a choriocarcinomatous component from the background adenocarcinoma.

0345

Mismatch repair proteins expression and BRAF V600E mutation analysis in a subset of CCR patients under 50 years

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Colorectal carcinoma (CRC) is a worldwide common malignancy, with a rise over the last years. Fifteen percent of all CRC have microsatellite instability (MSI) due to alterations in mismatch repair (MMR) genes. Germline mutations occur in 5%. Sporadic MSI CRC (15%) have hypermethylated MLH-1 promoter. BRAF V600E mutation exclusively occurs in sporadic MSI CRC. Immunohistochemistry detects MSI cases with high concordance with microsatellite analysis.

Objectives: The objective was to compare histopathology and patterns of expression of MMR proteins and BRAF V600E mutation in patients below and over 50 years old and determine if age is a risk factor for defective MMR protein expression and BRAF mutations.
Methods: One hundred six patients <50 years were retrieved. Clinocopathological data were collected. MLH1 and MSH2 immunohistochemistry and BRAF RT-PCR mutation were performed in 48 <50 years and 48 patients >50 years as control group. Student's t test, χ^2 and logistic regression analysis were carried out
Results: Medullary and mucinous types were more frequent among young patients, intestinal type in older patients. No differences were noticed regarding clinicopathological stages between groups. MMRP expression was absent more frequently in <50 and MSH2 was negative in 13.8% of these patients. No BRAF mutations were detected on any group.
Discussion: We found an association between young age and defective MMR expression (OR 4.28). MSH2 lack of expression is more frequently due to a germline mutation. The fact that none of our patients had BRAF mutation could be partly due to the small sample size or alternate V600E or K-ras mutations.

0347

Expression of E-cadherin and beta-catenin in primary and metastatic foci of colorectal carcinoma. Correlation with selected clinical and morphological parameters and the assessment of its prognostic value

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Objective: Colorectal carcinoma is the second most often diagnosed malignant neoplasm in Poland, being the second most common malignancy causing death among men and the third among women. Staging is one of the most important prognostic factors. Patients with tumor invading the whole thickness of bowel wall without lymph node metastases have 5-year survival rates between 70% and 80%. With additional lymph node metastases, the 5-year survival rate is much shorter, between 25% and 50%. Currently, the new prognostic factors such as adhesion molecule expression are extensively studied, which potentially may influence further treatment. Aims: (1) Comparative assessment of E-cadherin and beta-catenin expression in primary and metastatic foci of colorectal carcinoma and (2) correlation with selected clinical and morphological parameters, study of survival time and the assessment of its prognostic value.

Method: One hundred twenty-nine patients after surgery with stage III (106) and IV (23) colorectal carcinoma were qualified to this investigation. The expression of the E-cadherin and beta-catenin was studied by immunohistochemistry in primary and metastatic foci.
Results: E-cadherin was expressed in 60.5% while membranous staining of beta-catenin was reduced in 38.7% of primary tumors. In lymph nodes, 72.8% and 70.5% of metastatic tumors were positive for E-cadherin and membranous beta-catenin staining, respectively.
Conclusion: E-cadherin expression was correlated with membranous beta-catenin expression in the primary tumor and in the lymph node metastases. Expression of E-cadherin correlates with the larger amount of neoplastic stroma. No correlation was found between survival time and the expression of E-cadherin and beta-catenin in primary and metastatic foci of colorectal carcinoma.

0348

Comparison of transforming growth factor Beta 1 with GLUT-1 and bak in human colorectal cancers

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Objective: Transforming growth factor beta1 (TGF-beta1) is a crucial agent in colorectal carcinogenesis which interferes in growth signaling pathways of intestinal cells and adjacent immune cells. We aimed to evaluate relations among TGF-beta1, a cytoprotective and hypoxia-induced protein–glucose transporter 1 (GLUT-1) and proapoptotic protein Bak in human colorectal cancer.
Method: Expressions of TGF-beta1, GLUT-1 and Bak were detected by immunohistochemistry in 108 primary tumors of colorectal cancers.
Results: Immunoexpression of TGF-beta1 was observed both in the cytoplasm of cancer cells and adjacent inflammatory cells. GLUT-1 was visualized in membranous fashion and Bak was detected in the cytoplasm of malignant cells. Cancer immunoreactivities to TGF-beta1 correlated with GLUT-1 ($p>0.001$, $r=0.355$) and Bak ($p=0.001$, $r=0.320$) in all patients and subgroups of different clinical and pathological traits. TGF-beta1 of immune cells failed to associate significantly with GLUT-1 and Bak expressions in cancer cells.
Conclusion: The accumulation of TGF-beta1 cancer expression is associated with augmentation of apoptosis highlighted by Bak immunostaining and with increase of glucose uptake marked by higher index of GLUT-1-positive cancer cells. Diversity of Bak and GLUT-1 association with TGF-beta1 depends on the origin of TGF-beta1 expression and probably reflects different impacts of TGF-beta1 on malignant cells and immune cells.

0349

Expression of E2F1 and prognosis of patients with Astler–Coller stage B2 and C colorectal cancer treated with 5-FU-based adjuvant chemotherapy

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Objective: E2F1 affects the genes coding thymidylate synthase, which is the main molecular target for 5-fluorouracil. The aim of the study was to assess the association of E2F1 expression in Astler–Coller stage B2 and C colorectal cancer (CRC) cells with survival.
Method: Nuclear E2F1 was detected by immunohistochemistry on tissue microarrays from 259 CRCs. One hundred ninety-two patients received adjuvant chemotherapy.
Results: High expression of E2F1 was found in 37.5% of CRCs. It was more commonly found in B2 group as compared with group C ($p=0.01$). High expression of E2F1 was associated with worse overall survival (OS, $p=0.028$) in stage B2+C group of patients. Low expression of E2F1 was associated with better OS in the whole B2 group ($p=0.039$) and in B2 subgroup not treated with chemotherapy ($p=0.035$).
Conclusion: The expression of E2F1 may have prognostic significance in stage B2 group of patients, but not in the group of patients treated with chemotherapy. This work was supported by grant KBN 2P05B 174 28.

0350

Liver regeneration and its clinicopathological impact

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Objective: To our knowledge, this study is the first concerning human liver regeneration (HLR) evaluated by the hepatic extraction fraction (HEF) calculation using radioisotopic methods. We studied the HEF as an indicator of HLR of patients who underwent hepatectomy (HR).
Method: Ninety-five patients with colorectal metastases ($n=69$), hepatocellular carcinoma ($n=16$) and other tumors ($n=10$) were included. Thirty-five underwent major hepatectomy (MAHR) and 63% minor hepatectomy (miHR). HLR was assessed after intravenous bolus injection of ^{99m}Tc-Mebrofenin. In this study, we evaluated the preoperative HEF (T0) and in the fifth day (T5) and

1 month after HR (T30). HEF values of 98.8±0.4% were normal.
Results: In the overall patient population, the mean HEF values in our series were 97.3±9.6% for T0, 97.5±8.3% for T5 and 98.7±4.1% for T30 (ns). Related to the subgroup of 35 patients treated by MAHR, the HEF values were 97.2±5.3% (T0), 95.6±12.6% (T5) and 98.9±1.8% (T30) (ns). For the 60 patients who underwent miHR, the HEF values were 97.4±11% (T0), 98.8±2.4% (T5) and 98.6±4.7% (T30) (ns).
Conclusion: Our results strongly support the concept that the HLR is early enough to normalize the HEF at day 5 after HR. We have demonstrated that HLR is early, fast, non-anatomical and functionally complete 5 days after liver resection. This fast functional liver recovery has high clinical importance because considering this quick functional recovery, adjuvant chemotherapy can begin much earlier after surgical resection than the 3 week's dogma.

0351
Clinical and histopathological study of nonalcoholic steatohepatitis

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Objective: Non-alcoholic steatohepatitis (NASH) is a liver injury characterized by a spectrum of histological alterations which closely resemble alcoholic hepatitis steatosis, but occur with no significant alcohol consumption. The aim of the study was to examine the relationships between the degree of hepatic steatosis and various histological features of the accompanying hepatitis.
Method: In our study, we included 75 liver biopsies of patients aged between 38 and 64 years. Because clinicopathological correlation is essential in the diagnosis of NASH, we included in our study only patients with a complete report: clinical data (gender, age, BMI and associated diseases) and results of laboratory tests.
Results: Out of 463 consecutive liver biopsies, 75 showed steatosis, hepatocyte injury (ballooning degeneration, necrosis), lobular inflammation, with or without pericellular fibrosis and Mallory bodies, all consisting with the diagnosis of NASH. Mild, moderate and severe steatosis was present in 21, 46 and 8 cases, respectively. Cirrhosis was present in five cases, and 11 cases showed severe necro-inflammatory activity. The cases with moderate and severe steatosis were more often associated with higher degrees of necroinflammatory activity and fibrosis. Lesions were zone 3 predominant (centrilobular region). A Perls' Prussian blue stain for iron was positive in six cases. We performed multifactorial analysis of the cases establishing correlations between histological (activity, fibrosis and steatosis) and clinical data.
Conclusion: The increasing prevalence of NASH and the lack of standardized diagnostic criteria confirm the need of more studies to define a strategy of approach of this important liver disease.

0353
GFAP, SMA and CK7 expression in liver biopsies with chronic B and C viral hepatitis

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Objective: Hyperplasia of quiescent or activated stellate cells and also oval cells reflects the reactive changes leading to fibrosis in liver tissue.
Method: A retrospective comparative study between chronic B and C viral hepatitis using GFAP, SMA and CK7 antibodies was performed. We have analyzed portal and/or lobular localization and quantified the intensity of those cells which were marked by these antibodies.
Results: Our study included 100 cases, 50 with chronic B and 50 with chronic C viral hepatitis. In chronic B viral hepatitis, we observed a high lobular and portal reactivity which was marked only by SMA. In chronic C viral hepatitis, high expression of CK7, GFAP and SMA was revealed by portal areas.
Conclusion: The reactive changes observed in biopsy specimens provided by patients selected for biopsy examination seem to have greater importance in C than B viral chronic hepatitis regarding a successful antifibrotic treatment.

0355
Impact of hepatic artery selective clamping in hepatocellular function

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Objective: Hepatic pedicle triad occlusion is often used to prevent bleeding during hepatectomy despite the ischemia and reperfusion liver injury iatrogenically induced. This study aimed to estimate the impact of selective clamping of the hepatic artery (SCHA) in hepatocellular function.
Method: (1) Forty Wistar rats were divided into four groups: three groups were subjected to a SCHA ischemia period for 60 min: (A (n=13) submitted to a continuous SCHA; B (n=13) underwent an intermittent SCHA for

30 min with 5 min of reperfusion; C (n=6) underwent an intermittent SCHA for 15 min with 5 min of reperfusion)) and group D (n=8) without SCHA. (2) Determination of liver blood markers and hepatic extraction function (HEF) using ^{99m}Tc-mebrofenin 3 days before and after surgery. (3) Isolation of hepatocytes from the biopsy performed after surgery to evaluate oxidative stress (DCFH2-DA), characterization of cell death (annexin-V/propidium Iodide) and assessment of mitochondrial membrane potential (JC-1 probe) by flow cytometry.
Results: (1) There was significant increase of blood markers before and after SCHA, but without differences between groups (ns). (2) HEF maintained normal values without differences between groups (ns). (3) There were no significant differences in viability and in the type of cell death, as well as in the production of reactive oxygen species between groups (ns).
Conclusion: The SCHA compared to previous studies performed by us where total hepatic pedicle triad was clamped shows an increase of cell viability with a decrease of hepatocyte necrosis and/or apoptosis. SCHA is a potential alternative to decrease preoperative bleeding, maintaining hepatocellular function.

0356

Impact of selective portal vein clamping in hepatocellular function in the murine model

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Objective: The selective portal vein clamping (SCPV) is a potential alternative to decrease preoperative bleeding, maintaining hepatocellular function. This study aimed to evaluate the effect of SCPV in hepatocellular function.
Method: (1) Fifty-two Wistar rats were divided into four groups: Three groups of animals were submitted to a SCPV ischemia period for 60 min (A (n=21) was submitted to a continuous SCPV, B (n=12) underwent an intermittent SCPV for 30 min with 5 min of reperfusion, C (n=10) underwent SCPV for 15 min with 5 min of reperfusion)) and group D without SCPV (n=9). (2) Determination of liver blood markers and hepatic extraction function (HEF) using ^{99m}Tc-mebrofenin 3 days before and after surgery. (3) Isolation of hepatocytes from the biopsy performed after surgery to evaluate oxidative stress (DCFH2-DA), characterization of cell death (annexin-V/propidium Iodide) and assessment of mitochondrial membrane potential (JC-1 probe) by flow cytometry.
Results: (1) Mortality: A—62%, B—17%, C—30% and D—0% (p<0.03). (2) There was a statistically significant increase of the AST values (p<0.025) and LDH (p<0.002), but without differences between groups (ns). (3) HEF significantly decreased (p<0.0001), but without differences between groups (ns). (4) There were no significant differences in viability and in the type of cell death or in the production of reactive oxygen species between groups (ns).
Conclusion: The SCPV compared to previous studies performed by us where total hepatic pedicle triad was clamped shows an increase of cell viability with a decrease of hepatocyte necrosis and/or apoptosis. However, SCPV above 30' should be avoided given the high mortality observed.

0357

Oxidative stress and liver morphology of rats received doxorubicin and tirapazamine

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Objective: Tirapazamine (TPZ) selectively kills hypoxic tumor cells. Doxorubicin (DOX) is one of the most effective drugs against a wide variety of cancers. It is widely accepted that oxidative stress is involved in DOX organ toxicity. TPZ, similarly to DOX, is activated to TPZ free radical by the action of several NADPH-dependent reductases and generate reactive oxygen species. NADPH is also an essential cofactor for glutathione-dependent enzymes that constitute major cellular defenses against oxidative damage. Aim: The aim of the study was to evaluate oxidative stress and liver morphology changes in rat that received TPZ in addition to DOX administration.
Method: Male Wistar rats were treated i.p. with DOX (1.8 mg/kg b.w.) and TPZ (5 or 10 mg/kg b.w.) weekly for 6 weeks. Seven days after the last treatment, animals were killed and liver samples were analyzed for NADPH, MDA, GSH levels and histopathological changes. Blood samples were analyzed for ALT, AST activities and bilirubin concentration.
Results: ANOVA test revealed significant higher levels of NADPH and MDA in liver in group receiving a higher dose of TPZ with DOX vs. DOX group. There were no differences between group TPZ+DOX vs. DOX of liver GSH concentration and plasma ALT activity, but levels of GSH and ALT in all these groups were significantly higher compared to control. Microscopically, hydropic degeneration, necrosis, inflammatory cell infiltration and vascular lesion were observed in TPZ+DOX group.
Conclusion: Tirapazamine causes oxidative stress in liver of rats receiving DOX. This study suggests enhanced toxicity by TPZ in rat livers which received DOX.

0358

The influence of tetraiodothyronine-supplemented diet on selected redox equilibrium markers and liver morphology in rats treated with doxorubicin

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Objective: The toxicity of doxorubicin results from reactive oxygen species generation. ROS production depends on tissue-specific enzymatic performance. Liver is an organ that is characterized by very intensive activity of these enzymes. As a result, redox equilibrium disorders may appear. Moreover, changes in thyroid hormone concentrations are accompanied by ROS production. Thus, disorders in iodothyronine hormones status may result in the intensification of doxorubicin-induced oxidative stress. Aim: The aim of this study was to evaluate the influence of DOX and T4-associated treatment on selected redox equilibrium markers and histopathological evaluation of the liver.
Method: Rats were treated with DOX (.5 mg/kg, i.p.) once a week for 10 weeks. Apart from DOX, thyroxin was simultaneously given in drinking water 0.2 and 2.0 mg/l, respectively. The concentration of lipid peroxidation products—malondialdehyde (MDA) and total glutathione—were measured in liver homogenates. Liver morphology was evaluated in H+E and PAS diastase staining.
Results: Higher levels of MDA in liver of all tested groups and at the same time in rats treated with DOX plus T4 lower concentrations of total glutathione compared to control were observed. Morphological evaluation of liver did not show any symptoms of necrosis and steatosis, but a decrease in glycogen content in DOX+T4 group compared to DOX treatment was noticed.
Conclusion: Thyroxin supplementation causes redox equilibrium disorders and oxidative stress in liver of rats receiving DOX. The study revealed the normalizing influence of thyroxin on glycogen deposits that were observed after doxorubicin treatment.

0359

Dysadherin expression in human hepatocellular carcinoma: preliminary results

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Objective: Dysadherin is a cancer-associated cell membrane glycoprotein that inhibits cell–cell adhesion. In many types of cancer, dysadherin is related to decreased E-cadherin expression, promotes metastasis and is an independent predictor of poor patient survival. Dysadherin expression in human hepatocellular carcinoma (HCC) has not been studied to date.
Method: We studied by immunohistochemistry 70 HCC (grade I, 11; II, 28; III, 24; and IV, 7) from 70 patients (M/F 3.5, mean age 65.7 range 45–85 years) with monoclonal antibodies specific for dysadherin (clone NCC-M53) and E-cadherin (clone 36/E-cadherin). Immunostaining of inflammatory and endothelial cells was used as internal positive control for dysadherin (DYS) expression.
Results: Non-neoplastic hepatocytes and cholangiocytes were DYS-negative. In neoplastic cells (NC), DYS-specific immunostaining was mainly membranous, while some cases showed additional NC cytoplasmic positivity. The majority of HCC (43/70, 71.4%) had 0–9.9% DYS (+) NC, 18.6% (13/70) showed 10–50% (+) NC, while 10% (7/70) had >50% (+) NC. DYS expression was negatively correlated with E-cadherin expression ($p<0.05$), while a positive statistically significant correlation was observed between strong DYS immunostaining (>50%+NC) and tumour grade ($p<0.05$). There was no correlation with tumour size, vascular invasion or other clinicopathological parameters examined.
Conclusion: Normal hepatocytes and cholangiocytes do not express dysadherin. In HCC, increased dysadherin expression is observed in approximately one third of the cases; it is more extensive in high-grade tumours and is related to decreased E-cadherin expression.

0363

Klippel–Trenaunay syndrome with clinical presentation as metastatic liver disease: a case report

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Objective: Klippel–Trenaunay Weber syndrome (KTWS) is a rare congenital malformation characterized by hemangiomata, varicose veins and bone and soft tissue hemihypertrophy. KTWS can include additional vascular and lymphatic system abnormalities in various organs, but involvement of the liver is rare. We report a case of KTWS simulating metastastic liver disease.
Method: A 39-year-old male with KTWS presented with a tumor mass in the right abdomen. Ultrasonography revealed a 15-cm cystic abdominal tumor and additional hypodense lesions in liver segments I, II, and VIII. A CT scan additionally showed multiple hypodense lesions in the spleen, peritoneum, lung, right scrotum, soft tissue and enlarged abdominal lymph nodes, highly suspicious for metastatic disease.

Results: Laboratory data values were: gammaGT 90 U/l (normal 10–71), LDH 765 U/l (normal 240–480), albumin 29 g/l (normal 34–48), and alpha feto-protein 121.6 μg/l (normal <7). Histopathology of the cystic abdominal tumor mass showed a complex vascular malformation of lymphangioma–hemangioma type. Evaluation from two different tumorous liver lesions revealed nodular hepatic tissue separated by fibrous septa with reactive ductules and atypical vessels, consistent with multiple focal nodular hyperplasia (FNH) in the setting of KTWS.
Conclusion: The spectrum of KTWS can include arterial and lymphatic system abnormalities beyond the classical manifestation. Our findings support the concept that multiple FNH characteristically occurs in a syndromic form and is induced by an irregular blood supply in the liver, with localized hyperperfusion leading to reactive nodular proliferation of liver tissue.

0364

Microscopic evaluation of metaplastic and dysplastic changes in the mucous of the gallbladder

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Objective: In the intra- and extrahepatic biliary ducts, precancerous changes such as hyperplasia, metaplasia and dysplasia are diagnosed. In the gallbladder, cholecystitis chronica is diagnosed often with a disregard of the features of hyperplasia and metaplasia. Generally accepted markers of precancerous changes of the gallbladder and biliary ducts are still to be found. Aim: The aim was to define the accumulation of TP53 protein and MIB1 activity in hyperplasia, metaplasia and dysplasia of the mucous membrane of the gallbladder.
Method: The material comprises 325 gallbladders operated by laparoscopy and fixed in formalin. Specimens were embedded in paraffin. In the microscopic sections with hyperplasia, metaplasia and dysplasia immunohistochemical stainings for TP53 and MIB1 were done along with mucycarmin and PAS with Alcian blue stainings (mucous presence).
Results: The histopathologic diagnosis revealed 295 cases (91%) of chronic calculous cholecystitis, 23 cases of acute inflammation and secen cases of cancer. Hyperplasia, metaplasia or dysplasia were found in 44 cases (13.5%) with chronic inflammation and accounted for hyperplasia without metaplasia—26 cases, pseudopyloric or intestinal metaplasia—16 cases, dysplasia—2 cases. Mucous stainings confirmed the presence of pseudopyloric and intestinal metaplasia, which helped distinguish them from adenomyosis focuses. IHC confirmed the accumulation of TP 53 protein and proliferative activity in metaplasia and dysplasia of the mucous membrane.
Conclusion: Mucous stainings and positive TP53 and MIB1 reactions suggest that metaplasia and dysplasia of the mucous membrane of the gallbladder can be classified as intraepithelial neoplasia changes. More research should be done.

0365

Correlation of clinical and histopathologic features of hepatocellular carcinomas diagnosed in hepatectomy specimens

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Objective: We aimed to investigate the relationship between the findings in total or partial hepatectomy specimens.
Method: One hundred six cases were collected who had total transplantation or partial hepatectomies because of liver cirrhosis (LC), HCC or other hepatic disorders from the archives. Then, clinical, histopathologic 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 comparison tests.
Results: For the last 2-year period, data from 106 patients who underwent transplantation or hepatectomy were collected. Ninety-five had total, ten had partial hepatectomies, and one had metastasectomy. After the gross and microscopic examination, 86 cases were diagnosed as LC, 32 had HCC, three had parasitic cystic disease and ten had other unusual diseases. Among LC cases, 34 had hepatitis B virus (HBV), seven had hepatitis C virus (HCV), three had mixed virus etiology, six were alcoholic and 14 were due to other causes. Among LC patients, 32 had HCC. Fourteen cases were found to have one, seven had two and 11 had more than two tumors. Twenty-two of the HCCs were located in the right, while nine had tumors in the left and right; one case had tumors in the right, left and caudate lobe.
Conclusion: The results demonstrated that the most common hepatic disorder was cirrhosis due to HBV in the hepatectomy specimens and HCC seen in one third of them. Studies with follow-up and response to therapy will form the basis of our future projects.

0366

Metastatic solid pseudopapillary tumor of the pancreas. Experience at Hospital Clinic, Barcelona, Spain

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Objective: Solid pseudopapillary tumor of the pancreas (SPTP) represents 1–2% of pancreatic tumors, it affects young women, and surgery is the treatment of choice. Histologic features include loosely cohesive, relatively uniform polygonal cells surrounding delicate capillary-sized blood vessels. Most tumors are positive for beta-catenin, CD10, progesterone receptors and neuroendocrine markers. Mutations in the beta-catenin gene are frequent. Although considered benign, they are currently classified as low-grade malignant epithelial neoplasms.
Method: A 31-year-old woman presented with abdominal pain. A tumor in the pancreatic head with liver metastasis is diagnosed by TC. Fine needle aspiration cytology is diagnostic of SPTP. We reviewed four more cases of SPTP.
Results: All five tumors occurred in women <50 years old that presented with abdominal pain or asymptomatic. Tumors ranged from 1.4 to 18 cm. Four were located in the tail and one in the pancreatic head. All patients underwent surgery with negative margins. One patient had hepatic and nodal metastases as well as lymphatic and perineural invasion. All tumors were positive for CD56 and CD10, three for progesterone receptors. None of the patients presented tumor recurrence (2 months to 14 years).
Conclusion: Solid pseudopapillary tumor of the pancreas should be included in the differential diagnosis of pancreatic masses. Surgery is usually curative and should also be attempted in rare cases with an aggressive behaviour. Despite the characterization of the morphologic and molecular features of this enigmatic neoplasm, more work is needed to uncover its cell of origin and true histogenesis.

0368

Serous cystic neoplasms of the pancreas. Histopathologic study of 31 cases

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Objective: Serous cystic neoplasms (SCN) are rare pancreatic tumors. The aim of the study was to describe histopathological characteristics of 31 SCN diagnosed in a single center during a 20-year-long period.
Method: Pathological reports including macro- and microscopic descriptions of SCN cases as well as routine slides were retrieved from institutional databases (1989–2010) and retrospectively reevaluated. Only cases with available original slides were included in the study.
Results: Several clinicopathologic SCN subtypes were distinguished: 25 serous microcystic adenomas (80.6%), five serous oligocystic and ill-demarcated adenomas (16.1%) and a single case of microcystic adenoma coexisting with multiple serous cysts in von Hippel–Lindau patient (3.2%). There were 25 females (80.6%) and six males (19.4%). Median patients' age was 63 years (range 31–93 years). Fifteen cases (48.4%) were localized in the head of the pancreas, 11 (35.5%) in the pancreatic body, and five (16.1%) in the tail. Median tumor diameter was 4.0 cm (range 1–12 cm, the diameter of three tumors was not known). Eleven cases (35.5%) were diagnosed in pancreaticoduodenectomy specimens, six (19.4%) in middle segment pancreatectomy specimens, and four (12.9%) in distal pancreatectomy specimens. Six cases (19.4%) were enucleated, two cases (6.4%) were diagnosed in open biopsy and two (6.4%) cases were found during autopsy.
Conclusion: SCN is found in middle-aged or older patients, mainly women. SCN may be localized in each pancreatic segment and grow to large masses. The reasons for preferential existence of SCN in females remain unknown.

0369

P53, Ki-67, CD 117 expressions in gastrointestinal and pancreatic neuroendocrine tumors and the evaluation of these tumors with clinicopathological and prognostic parameters

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Objective: Gastrointestinal and pancreatic neuroendocrine tumors (GEPNETs) originate from the cells of the diffuse endocrine system. Their molecular genetic mechanism of development and progression is complex and remains largely unknown. The purpose of this study was to review the gastrointestinal and pancreatic neuroendocrine tumors and to investigate these tumours with an emphasis on their clinicopathological characteristics.

Method: Twenty-one patients were reviewed and classified as well-differentiated neuroendocrine tumor, well-differentiated endocrine carcinoma and poorly differentiated endocrine tumor. We performed immunohistochemistry to characterise the expression of the immunoreactivity for synaptophysin, chromogranin, p53, Ki67 and CD 117.
Results: Mean age of the 21 patients was 43.86 ± 3.78. Thirteen (61.9%) of the patients were males and eight (38.1%) of the patients were females. The method of statistics we used was SPSS 11.0. According to Spearman's correlation test, there was a good correlation between Ki-67 expression and tumor type ($p<0.005$, $r = 0.597$). Also, there was a good correlation between p53 expression and tumor type ($p<0.05$, $r = 0.521$). But there was no correlation between CD 117 expression and tumor type ($p>0.05$, $r = 0.471$). Of the tumors, 38.1% were located in stomach, 38.1% located in appendix, 4.8% located in duodenum, 4.8% located in ileum, 9.5% located in colon and 4.8% located in pancreas, respectively.
Conclusion: The depth of invasion, tumor size, the presence of solid pattern, large tumor cells and local distant metastases correlated with the degree of malignancy ($r = 0.699, 0.833, 0.671, 0.705, 0.882$, respectively, $p<0.001$).

0370
CK19 and Ki-67 immunohistochemical staining in gastroenteropancreatic neuroendocrine tumours (GEP-NETs)

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Objective: Ki-67 is used in GEP-NETs as a marker of biologic behavior. CK19 is a marker of poor prognosis in pancreatic neuroendocrine tumours, but its importance in gastrointestinal neuroendocrine tumours is less clear.
Method: Blocks from GEP-NETS were stained with Ki-67 and CK19 antibodies. Ki-67 was used to group tumours according to the ENETs grading system. CK19 was evaluated for intensity and percentage of cell staining.
Results: Sixteen of 18 (89%) pancreatic tumours and 26 of 29 (90%) GI tumours expressed CK19. With respect to Ki-67 in pancreatic tumours, 9 of 18 (50%) were G1, 3 of 18 (17%) were G2, and 6 of 18 (33%) were G3. In GI tumours, 18 of 29 (62%) were G1, 10 of 29 (34%) were G2, and 1 of 29 (3%) G3. Four pancreatic tumours and six GI tumours had distant metastases. Of the four pancreatic tumours, two were G1, one was G2, and one was G3; two stained diffusely with CK19, while the remaining two showed weaker staining. Three metastatic GI tumours were G1 and three were G2; Allred score for CK19 was 4–5 in five of six (83%). There was no significant difference between tumours with and without metastases with respect to Ki-67, but in GI tumours, CK19 had a higher mean Allred score in tumours with metastases (4.3 versus 3.3) which approached statistical significance ($p=0.07$).
Conclusion: CK19 staining is common in GEP-NETs. This near-ubiquitous positivity may limit its potential as a prognostic marker. Further study is needed regarding the prognostic value of Ki-67 since half of the tumours with metastases had low proliferation rates.

0372
Adenocarcinomas of the pancreas in patients in the age of 45 or younger. Clinicopathologic study of 33 patients

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Objective: Pancreatic adenocarcinoma (PA) is a very rare lesion in young patients. Patients in the age of 45 or younger reported previously constituted <5% of all patients with PA. The clinical and histopathological data concerning these patients are therefore scanty. The aim of the study was to describe characteristics of 33 young patients with PA treated and diagnosed in a single center during a 20-year-long period (1989–2010).
Method: The original gross descriptions, routine hematoxylin–eosin slides as well as available clinical data of 33 PA cases were reevaluated. Only cases with available original slides were included in the study.
Results: There were 30 (90.9%) cases of pancreatic ductal adenocarcinomas (not otherwise specified), two (6.1%) cases of adenocarcinoma associated with intraductal papillary mucinous neoplasm and a single (3%) case of adenosquamous carcinoma. Twenty (60.6%) patients were males and 13 (39.4%) were females. Median age of patients was 42.0 years (range 23–45). Twenty-one (63.6%) patients were treated with pancreaticoduodenectomy, three (9.1%) with distal pancreatectomy, and two (6.1%) with total pancreatectomy. In seven (21.2%) patients, PA was diagnosed in open surgical biopsy specimen (the majority of these patients were treated with palliative surgery). Follow-up data were available for 23 patients—among these patients, 21 died of the disease. A single patient died in

postoperative period. Another patient survived for 11 years and died of unrelated cause.
Conclusion: PA occurs infrequently in young patients. Although some cases are diagnosed early and may be treated with potentially curable surgery, long-term survivals are rather exceptional.

0373
Granulomatous pancreatitis in a patient with LADA type diabetes mellitus

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Objective: The aim of this presentation was to demonstrate an unusual case of granulomatous pancreatitis discovered in a patient suffering from the LADA type diabetes mellitus.
Method: Samples of pancreatic tissue obtained during the postmortem examination were processed by a routine histological technique. The slides were stained with H&E, trichrome and van Gieson method. Immunohistological methods were used to detect markers of endocrine cells of islets of Langerhans and of macrophages.
Results: A 71-year-old female patient suffering from diabetes mellitus LADA type, generalized atherosclerosis and hypertension died due to pulmonary embolism. Lipomatosis of pancreatic tissue was observed during the postmortem examination. Histologic examination of pancreatic tissue discovered multiple small non-caseating epithelioid cell and giant cell granulomas replacing the islets of Langerhans.
Conclusion: To our knowledge, our case represents the first description of non-infectious granulomatous pancreatitis associated with diabetes mellitus of LADA type.

0374
Assessment of Crohn's disease inflammatory and fibrostenotic lesions. Histologic correlation with magnetic resonance

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Objective: Evaluation of Crohn's disease (CD) activity is crucial to optimize therapeutic strategies. Differentiation between inflammatory and fibrostenotic lesions is decisive since the former are treated with drugs, while the latter require surgery. CD activity is based on a combination of clinical, biochemical, endoscopic and radiologic findings, which cannot reliably distinguish between both entities. In patients with refractory symptoms, therapy decisions rely heavily on histologic diagnosis.
Method: Preoperative MR imaging was performed in 27 CD patients undergoing elective bowel resection. MR evaluated wall thickness, pre- and post-contrast wall signal intensity, relative contrast enhancement, presence of edema, and luminal stenosis. Matched histological sections of the MR images were stained with hematoxylin and eosin (HE). Evaluation of wall and submucosa thickness, percentage of inflammatory and fibrous components in the mucosa and submucosa (Masson's trichromic and reticulin stains), submucosal edema, and vascularity (CD31 stains).
Results: Cases assessed as inflammatory by HE had higher early post-contrast signal intensity by RM than fibrostenotic cases ($p=0.009$). Submucosal thickness correlated with early fibrosis (reticulin, $p=0.007$), but only a trend with high vascularity (CD31). Fibrostenotic cases had submucosal thickness inversely correlated with early signal capture and with relative contrast enhancement. Mucosal inflammation was inversely correlated with submucosal fibrosis and thickness ($p=0.007$). Established fibrosis (trichromic) had no delayed signal capture ($p=0.038$).
Conclusion: There is a good correlation between histologic and MR findings. MR may reliably differentiate between inflammatory and fibrostenotic lesions, and it may have an important role as a new pre-surgical biomarker in the management of CD.

0376
The role of standard endoscopic biopsy in diagnosis of gastric gastrointestinal stromal tumors managed in The Holycross Cancer Centre, Kielce, Poland, in years 2000–2010

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Objective: Intramural character of growth makes gastrointestinal stromal tumor (GIST) difficult or impossible to access by endoscopic biopsy forceps, and therefore, the diagnostic yield of the biopsy does not exceed 40%, even with the use of special techniques. The aim of the study was to evaluate the role of standard endoscopic biopsy in preoperative assessment of gastric GISTs.

Method: From among 24 cases of all gastric GISTs managed in our institution in the years 2000–2010, we selected 17 cases where standard forceps biopsy from subepithelial mass was obtained. We excluded five cases with no subepithelial mass visible endoscopically and two cases of small incidental GISTs in patients operated for other neoplasms. Biopsies diagnosed as negative for GIST were analysed retrospectively with serial sectioning and immunostains including, where appropriate, CD-117, CD-34, SMA, S-100 and calponin in search for neoplastic tissue which could have not been apparent in original HE-stained slides.
Results: None of the biopsy samples analysed retrospectively contained neoplastic tissue. Material which enabled histopathological and immunohistochemical diagnosis of GIST was obtained in 5 out of 17 cases. Mucosal ulceration was present in all cases of diagnostic biopsies and in 4 out of 12 non-diagnostic biopsies.
Conclusion: No GIST tissue was originally missed in biopsy material, so the diagnostic yield of standard endoscopic biopsy in diagnosis of gastric GISTs approached one third of the cases.

0377

Immunohistochemical markers utilized in differential diagnosis between primary ovarian carcinoma from metastatic colorectal carcinoma

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Objective: Colorectal adenocarcinoma is the most common tumor that metastasizes to the ovary and is often difficult to distinguish from primary ovarian mucinous carcinoma. An immunohistochemical marker CDX-2 was found to have a diagnostic value in establishing the gastrointestinal origin of metastatic tumors. Obtaining the correct diagnosis is difficult but crucial to treatment and prognosis.
Method: In our study realized in Clinical County Emergency Hospital Constanta, we evaluated the immunohistochemical expression of CDX2 and also the expression of cytokeratin 7, cytokeratin 20, CEA, MUC2 in 86 cases representing 45 ovarian adenocarcinoma and 41 metastatic colorectal adenocarcinomas involving ovaries.
Results: Searching the tumor registry and pathology database of our hospital, we found that the median age of the study group was 50 years (range, 24–78). Metastatic colorectal adenocarcinoma were almost always negative for MUC5 (98.6%), often negative for CK7 (89.9%), focal or diffuse positive for CDX2 (98.2%), diffuse positive for CK20 (56.9%), focal or diffuse positive for MUC2 (51.2%), and diffuse positive for CEA (43.4%). Almost all of the primary ovarian carcinomas lacked immunoreactivity for CDX-2. In contrast, metastases to the ovary from colorectal primaries showed CDX-2 immunoexpression.
Conclusion: CDX-2 is a useful marker for differentiating primary ovarian carcinoma from carcinomas metastatic to the ovary. CK7, CK20, CDX2 and MUC2 IHC staining is a useful adjunctive diagnostic tool to differentiate metastatic colonic tumours from primary ovarian tumours, in addition to clinical history and gross and microscopic findings.

0378

Multicystic mesothelioma—a case report

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Objective: Multicystic mesothelioma or multilocular peritoneal inclusion cyst is a rare tumor with a predilection for pelvic surfaces of the peritoneum. Most frequently, it occurs in women of reproductive age who have a history of previous pelvic surgery or infection.
Results: A 20-year-old man with no significant past medical history presented with abdominal pain lasting 5 days in the ileocecal region. Despite normal white cell count, acute appendicitis was suspected and the patient underwent surgery, during which a multicystic mass measuring 6.5 cm in the longest diameter was found. Cysts were thin-walled, translucent, with a smooth outer surface, and mostly filled with clear and some with hemorrhagic fluid. Histologically, they were lined by a single layer of cuboidal polygonal and focally hobnail-shaped cells with small uniform nuclei without mitotic activity. Cells stained positive for cytokeratin, calretinin and WT-1, but were negative for endothelial markers, CEA and estrogen and progesterone receptors. The loose connective tissue between cysts was infiltrated by lymphocytes and granulocytes. The diagnosis of multicystic mesothelioma was made. Three months after surgery, the patient is doing well.
Conclusion: The majority of investigators considers this entity to be an unusual type of mesothelial neoplasm that has a tendency to recur locally and may rarely transform into a conventional mesothelioma. Some, however, consider the lesion to be a non-neoplastic reactive mesothelial proliferation. Multicystic mesothelioma has an indolent course, but approximately one half of cases recur. The differential diagnosis includes malignant mesothelioma, cystic lymphangioma, pseudomyxoma peritonei and mesenteric and omental cysts.

0380

Significance of histopathological tumor regression after neoadjuvant chemotherapy in adenocarcinomas of the upper gastrointestinal tract: what we can learn from 589 cases

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Objective: We analysed a series of 589 upper gastrointestinal (GI) adenocarcinomas treated by neoadjuvant chemotherapy (CTX) between 1989 and 2009, which all were consistently worked up and evaluated by a standardized tumor regression grading system (TRG) in our institution.
Method: One hundred eight esophageal adenocarcinomas (EA), 175 adenocarcinomas of the esophagogastric junction (AEJ) and 306 gastric carcinomas (GC) treated by a cisplatin/5-FU-based CTX were included. TRG was determined using a four-tiered system based on the estimation of the percentage of residual tumor in relation to the previous tumor bed.
Results: In total, 25 patients (4%) had a complete tumor regression (TRG1a) and 111 patients (19%) had a subtotal regression (TRG1b, 1–10% residual tumor). Partial tumor regression (TRG2, 11–50%) was observed in 151 cases (26%); 302 patients (51%) had minimal or no regression (TRG3, >50%). Tumor regression was significantly associated with post-treatment ypT, ypN, ypL category, R status and survival ($p<0.001$) and was shown to be an independent prognostic factor for survival ($p=0.02$). Some characteristics relating to the tumor site were, in particular, the higher frequency of total or subtotal tumor regression in EA and AEG ($p<0.001$) and the association of pretherapeutic tumor grading and Lauren's classification with tumor regression ($p=0.001$) in GC.
Conclusion: We present our 20 year's experience of a highly standardized evaluation of upper GI adenocarcinomas after neoadjuvant CTX demonstrating highly objective and prognostic relevant information from a very large collective. We recommend the implementation of a standardised TRG system in every pathological report of these tumours.

0381

Fibroelastotic changes of the gastrointestinal tract as polyp-causing lesions

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Objective: Fibroelastotic changes (FEC) of the gastrointestinal tract (GIT) are rare lesions. Only 35 cases have been reported in the literature. Their role as an own entity is unclear and is not generally accepted.
Method: After initial recognition of an elastotic lesion in a hemicolectomy specimen, special attention was paid to amorphic accumulations in H&E-stained slides of GIT specimens. Elastica-van-Giesson staining was performed to verify the elastotic origin.
Results: Within 3 years, a total number of 21 polypoid lesions were collected. One lesion was found in the ileum and six lesions occurred in the stomach. The 14 remaining cases were colon specimens. In five cases, FEC were associated with neoplastic lesions. In 16 cases, however, the accumulation of elastotic fibres was the only histomorphological finding. One patient received intensive short intervals surveillance because of a highly suspicious endoscopic finding in the stomach. In two cases, a clinical relevant stenosis was caused by a submucosal thickening of the bowl wall with massive accumulation of elastotic fibres. Right hemicolectomy was performed in both cases.
Conclusion: Fibroelastotic changes are not as exceedingly rare as thought before. They are the only histological finding in a certain number of polypoid lesions in the GIT.

0382

Expression of p53, VEGF and collagen IV in gastrointestinal stromal tumors and its relationship with clinicopathological parameters

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Objective: Gastrointestinal stromal tumors (GISTs) have a spectrum from minimal indolent tumors to sarcomas. The aim of this study was to assess the expression of p53, VEGF and collagen IV in GISTs and to establish immunohistochemical correlations with clinicopathological parameters.
Method: Twenty-four cases diagnosed as GIST were examined by both light microscopy and immunohistochemistry (IHC). We used IHC markers such as p53, VEGF and collagen IV. All tumors were classified by the risk grade system by Miettinen et al.
Results: Of the tumors, 8/33.3% were classified into low risk grade (LR), 6/25% in the intermediate risk (IR) and 10/41.7% in the high risk group (HR). Focal necroses (50%, 50% and 50%, respectively) and microhemorrhages (37.5%, 50% and 90%, respectively) were present in GISTs of all groups. A p53 expression was correlated with a high risk grade ($r=0.48$, $p=0.0335$). GISTs of all groups showed VEGF expression (62.5%, 66.7% and 60%, respectively).

Collagen IV expression, as staining of the continuous basement membranes of vessels, varied from 25% in LR GISTs to 16% to 10% in IR and HR tumors, respectively. Gamma's correlation test revealed correlation between microhemorrhages and grade (r=0.70, p=0.0019), tumor necroses and p53 (r=0.55, p=0.0245), tumor necroses and collagen IV (r=−0.55, p=0.0107) and VEGF and collagen IV (r=−0.49, p=0.0213).
Conclusion: This study showed that both high p53 expression and the presence of tumor microhemorrhages are possibly related with unfavorable prognosis. In addition, we found that a tumor necrosis was correlated with a p53 and collagen IV.

0383
Smooth muscle tumors with CD117/CD34 positivity cells
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Objective: Immunohistochemically, smooth muscle cells are characterized expression of SMA and desmin. The aim of this study was to describe the clinicopathological and immunohistochemical features of smooth muscle tumors (SMT) with CD117/CD34 positivity cells.
Method: Eleven cases diagnosed as SMT were examined by both light microscopy and immunohistochemistry (IHC). I used IHC markers such as CD117, CD34, smooth muscle actin, desmin, vimentin, NSE and S100.
Results: The patients (M/F=4:7), with age ranging between 18 and 70 years (mean = 48.3), had SMT. A tumor size varied from 1.5 to 17 cm (mean = 8.9 cm). Tumors were localized in the esophagus (5/45.5%), stomach (1/9.1%), colon (1/9.1%), retroperitoneum (1/9.1%) and in the pelvis (3/27.3%). Histologically, tumors are composed of elongated or ovoid cells with pale nuclei and eosinophilic cytoplasm which is distinctly fibrillar. Immunohistochemically, cells of SMT were positive for SMA (100%), desmin (100%), vimentin (100%) and NSE (72.7%), but they were negative for CD117, CD34 and S100. I found that the scattered cells of the tumors were characterized by the expression of CD117 (11/100%), CD34 (7/63.6%) and S100 (5/45.5%). These cells were located diffusely or focally in the SMT. The immunophenotype of these cells was similar to immunohistochemical features of the interstitial cells of Cajal or the gastrointestinal stromal tumor (GIST).
Conclusion: Although the scattered tumor cells revealed CD117 positivity, the histological finding was similar to SMT and was different from GIST. SMT colonized by CD117/CD34-positive cells should not be confused with GISTs.

0385
Updates of TNM classification: for the better or worse?
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Objective: For the prognostic evaluation of tumours, the TNM classification is updated periodically. The use of this classification for colorectal cancer (CRC) has caused some problems in the assessment of metastatic lymph nodes. In this study, we have investigated the effect of the updates on metastatic lymph node interpretation and, hence, staging.
Method: H&E slides of 83 cases of CRC were reevaluated microscopically to assess the number of metastatic lymph nodes according to the criteria of TNM5, TNM6 and TNM7. Clinical parameters such as gender and age of the patients, tumour location, grade, and stage were retrieved from the patients' files. Mann–Whitney, Kruskal–Wallis, Wilcoxon signed ranks and multiple comparison tests were used for statistical analysis.
Results: There were 48 men (57.8%) and 35 women (42.2%) with a mean age of 63.14 years (ranging from 31 to 87). The tumour was located in the right colon in 11 cases (13.3%) and 72 cases in the left colon (86.7%). There were three grade 1 (3.6%), 58 grade 2 (69.9%), and 22 grade 3 tumours (26.5%). The number of metastatic lymph nodes was significantly higher in grade 3 tumours compared to grade 2 tumours in TNM5, TNM6 and TNM7. According to TNM5, significantly more metastatic lymph nodes were determined than TNM6 and TNM7 (p<0.05 and p<0.001, respectively), whereas TNM6 revealed significantly more metastatic lymph nodes in comparison to TNM7 (p<0.001).
Conclusion: Our results show that there is significant variation between updates of TNM in terms of metastatic lymph node yield which will inevitably cause variation in the TNM classification.

0386
Metastatic ileal gastrointestinal stromal tumor of the mandible
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Objective: Metastatic lesions to the mandible are rare, comprising <1% of all malignancies. An 82-year-old woman presented with a painful swelling in the right mandible growing progressively for 3 months.
Method: Computed tomography (CT) showed intraosseous expansile and destructive lesion in the corpus of the right

mandible 23 × 21 mm in size expanding to the soft tissue laterally. After administration of contrast material, moderate enhancement was seen throughout the mass. The biopsy showed stromal tumor immunopositivity for CD117. The patient had a medical history of ileal tumor resection 5 years ago at another institute which was diagnosed as gastrointestinal stromal tumor (GIST) and classified as intermediate risk group for potential of malignancy.
Results: The mandibular tumor was considered as a metastatic gastrointestinal stromal tumor. No tumor was seen on positron emission tomography (PET)/CT other than the mass in the right mandible. The histopathology of the ileal tumor was seen and the diagnosis was confirmed. Imatinib treatment was started at a dose of 400 mg daily. Resection of the mandibular mass was planned by plastic and reconstructive surgeons.
Conclusion: Liver and peritoneum are usual metastatic sites for gastrointestinal stromal tumors. To the best of our knowledge, metastasis of ileal gastrointestinal stromal tumor to the mandible has not been previously reported in the English literature.

Friday, 3 September 2010, Basement

PS-16 Poster Session Head and Neck Pathology

0387
Mycobacterium tuberculosis infection within Warthin's tumor of the parotid gland

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Objective: Warthin's tumor is the second most common neoplasm of the parotid gland accounting for 6–30% of parotid neoplasms. However, parotid gland is an extremely rare site for extrapulmonary tuberculosis.
Method: Between 2003 and 2010, 30 cases of Warthin's tumor were diagnosed at the Pathology Department of City Hospital Timisoara, but only one case of *Mycobacterium tuberculosis* infection within Warthin tumor was analysed. A 47-year-old man with a history of pulmonary tuberculosis was admitted at the Maxillofacial Department with a slow-growing right parotid mass. A 2.2/1.5-cm diameter mass was excised and formalin-fixed paraffin-embedded tissue samples were cut at 4 μm and stained using hematoxilin and eosin (HE).
Results: On the HE stain, pathological examination revealed cystic spaces lined by proliferative oncocytic basal and luminal columnar epithelium and markedly lymphocytic infiltration. A very large part of the section was occupied by chronic granulomatous inflammation with caseous necrosis, epithelioid granulomas and multinucleated giant cells; some were of the Langerhans' giant cell type.
Conclusion: Tuberculosis of the parotid gland is extremely unusual and constitutes about 2.5% to 10% of the parotid lesions. To our knowledge, only six cases of tuberculosis within Warthin's tumor have been reported in the English language literature. In this report, we present a new patient with parotid gland tuberculosis within Warthin tumor.

0389
Androgen receptor status in salivary gland cancers

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Objective: The purpose of this study was to assess the immunohistochemical (IHC) status of androgen receptor (AR) in various salivary gland cancers.
Method: The study group consisted of 61 cases of primary salivary gland cancers from 61 patients (F/M=32:29, age 12–78 years). These were 15 adenoid cystic carcinomas (AdCC), 12 adenocarcinomas NOS (AdC), 12 mucoepidermoid carcinomas (MEC), 10 acinic cell carcinomas (ACC), 5 carcinomas ex pleomorphic adenomas (CaexPA), 5 salivary duct carcinomas (SDC), 1 basal cell adenocarcinoma (BCAC) and 1 large cell neuroendocrine carcinoma (LCNEC). IHC reactions with monoclonal mouse anti-human androgen receptor antibody (Dako, clone AR441) were done on 4-μm-thick sections cut from representative archival paraffin block. In each positive case, the revealed strength of AR expression was assessed as total score (TS) according to Allred score.
Results: The AR expression was found in five AdC NOS, four CaexPA, four SDC, four ACC, three MEC, and one AdCC and BCAC, and calculated TS ranged from three to eight points. TS had eight points in six cases, seven in three cases, six in one case, five in three cases, four in two cases and three in four cases.
Conclusion: AR expression was found in 22 of 61 (36%) primary salivary gland cancers. The strength of AR expression differed in various salivary gland cancers, and it was most frequent in CaexPA (4/5) and SDC (4/5) and less frequent in AdC (5/12) and ACC (4/10), whereas only found incidentally in others.

0390
Mena expression in normal and neoplastic salivary glands

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Objective: Recently, studies revealed that human orthologue of murine Mena (mammalian Ena), an actin regulatory protein involved in the control of cell motility and adhesion, is modulated during breast, colon and pancreatic carcinogenesis. In our previous studies, we observed that Mena was modulated during colon and cervix carcinogenesis. In this study, we analysed Mena expression in lesions of salivary glands (SG). Previous studies regarded only Maspin expression in these lesions, a protease inhibitor which is increased in benign tumors but decreased in SG carcinomas.
Method: We have analyzed Mena expression in normal SG (n=10) and also benign (n=20) and malignant (n=30) lesions of SG. For the immunohistochemical staining, we used the murine Mena antibody, provided by BD Biosciences. Mena expression was quantified in cytoplasma of tumor cells.
Results: All normal SG and their benign lesions were Mena-negative. It included ten pleomorphic adenomas and ten Warthin's tumors. Ductal adenocarcinomas (n=10), independently by their histological grade and also carcinomas with acinary cells (n=5) and squamous carcinomas (n=10), were positive. No difference of Mena intensity in positive cases was observed. All lymphomas (n=5) were Mena-negative.
Conclusion: This is the first study in literature about Mena expression in SG tumors. Our results prove that Mena plays a role in carcinogenesis of different organs, including SG, but the exact mechanism is not yet known. In accordance with different previous studies about Maspin expression in SG lesions, it seems that these two antibodies are reversely correlated. Future studies are necessary in order to elucidate their role in SG tumors.

0391
Secondary tumors of the salivary glands

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Objective: Secondary tumors of salivary glands are rare and constitute about 5% of all malignant salivary neoplasm.
Method: A study has been carried out for 7 years on 184 cases of salivary gland tumors, and only one case of salivary metastatic lesion was diagnosed. A 79-year-old female with a history of a right temporal malignant melanoma was admitted at the Plastic Surgery Department of County Hospital of Timisoara. Six months after the surgery, the patient returned to this clinic with a rapidly growing mass in the right temporal region and right preauricular region. A 2 × 2-cm diameter tumor was excised with adjacent lymph nodes. A superficial parotidectomy was performed also, preserving the facial nerve and its branches. Formalin-fixed paraffin-embedded tissue samples were cut at 4 μm and stained using hematoxylin and eosin (HE).
Results: On HE stain, the periparotid and temporal region lymph nodes were infiltrated by malignant melanoma cells. The parotid tissue also had metastatic lesions. The metastatic malignant melanoma lesion consisted of epithelioid neoplastic cells with cytologic and nuclear atypia, large nucleoli, atypic mitotic figures and abundant melanin pigmentation. Necrosis and hemorrhage were also seen.
Conclusion: The parotid gland and its lymph nodes are possible sites of metastasis from head and neck tumors, especially squamous cell carcinoma or melanoma. The rarity of salivary secondary tumors prompted us to report this case.

0392
Mucoepidermoid carcinoma of the maxilla. Report of a rare case

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Objective: Primary intraosseous mucoepidermoid carcinoma of the jaws is a rare lesion accounting for <5% of all mucoepidermoid carcinomas reported in the literature. It is a neoplasm of adult life and affects females twice more frequently than males. Histologically, it is a low-grade carcinoma usually affecting the mandible. The radiologic presentation is that of uni- or multilocular lesions. Their origin still remains controversial.
Method: We report the case of a 66-year-old female who presented with a painful swelling in the left maxilla of 2 month's duration. There was no history of previous surgical intervention. Clinical examination revealed an ulcerated swelling. Cervical lymphadenopathy was absent.
Results: The radiological examination showed a unilocular compact lesion in the maxilla, not unequivocally diagnostic of malignancy. Surgical excision of the lesion together with a small part of the maxilla was performed. Grossly, it was a solid mass of relatively soft and only focally bony consistence measuring 1.5 cm. On brushing cytology of the surgical specimen, groups of mucous cells without any significant atypia intermingled with aggregates of squamous cells. The histologic diagnosis, confirmed by histochemical and immunohistochemical methods, was that of low-grade mucoepidermoid carcinoma of the maxilla. The postoperative period was

uneventful. The patient is on regular follow-up and is disease-free after 4 years.
Conclusion: Our case is reported because of its rarity, and at the same time, the literature is reviewed and speculations about the pathogenesis of mucoepidermoid carcinomas are attempted.

0393

Odontogenic myxoma (OM) in the maxilla: a case report and review of the literature

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Objective: Odontogenic myxoma is an intraosseous tumor of the jaws, relatively benign with locally aggressive behaviour. Most of the OMs are slowly growing with no symptoms. Large tumors cause painless expansion. It occurs in patients over the age of 10 and is mostly located in the mandible.
Method: A 45-year-old man was presented with a progressively enlarging mass in the maxillary area. The imaging findings revealed a large, rather well-circumscribed tumor mass in the body of the maxillary bone. The mass was excised by peripheral ostectomy.
Results: The gross examination revealed a grey-white mass with translucent mucinous appearance measuring 7×4.5×4 cm. The sections were examined with H+E and mucin stains and followed by IHC study for MIB-1 (ki-67). The morphologic, histochemical and IHC data were consistent with an odontogenic myxoma.
Conclusion: Our study comments on the imaging findings of macroscopic and microscopic features and the differential diagnosis of this tumor. The current literature is reviewed.

0397

Comparison of MMP-2, MMP-9, KAI1 expression in oral squamous cell carcinomas and in adjacent marginal tissue

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Objective: The role of interactions between MMP-2, MMP-9 and KAI1 protein expression as markers involved in aggressive behaviour of tumours is still under investigation. The study aimed to evaluate and compare MMP-2, MMP-9 and KAI1 expression in primary oral squamous cell carcinomas (OSCC) and adjacent marginal normal squamous cell epithelium and to estimate their association with each other in relation to clinical variables.
Method: MMP-2, MMP-9, and KAI1 expression was evaluated on primary OSCC (n=54) and adjacent marginal normal tissues (n=32) using immunohistochemistry.
Results: Significant differences between MMP-9 and KAI1 expression was found in OSCC and marginal normal tissue (P=0.01, P=0.001, respectively). In normal tissue, MMP-2, MMP-9 expression was found in basal cell layers and also frequently was observed in stromal tissues with inflammatory component. KAI1 protein was not observed in stromal tissues. No significant differences were observed between MMP-2, MMP-9, KAI1 expression and tumour grade and stage. Inverse correlation between MMP-2 and KAI1 expression was found in OSCC (P=0.006). KAI1-negatve/MMP-2-positive cases were observed mainly in high tumour grade and in advanced stage of tumours.
Conclusion: Inverse correlation revealed between KAI1 protein and MMP-2 expression in OSCC could affect the function of KAI1 suppressor protein and influence the metastatic potential of the tumour cells. Our results suggest that accumulation of MMP-2 and MMP-9 in the surrounding tissue may enhance local invasion in OSCC.

0398

Expression of MMP-2 and PTEN in laryngeal squamous cell carcinoma—biological predictors?

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Objective: Reliable predictors of laryngeal squamous cell carcinoma (LSCC) biology are still missing. The aim of this study was to investigate two newly discovered factors (MMP-2 and PTEN) in LSCC patients with correlation to the stage of the disease.
Method: We used 41 specimens from surgical resections selected from 25 patients and divided into controls (12 samples) and LSCC (29 samples). We used standard immunohistochemistry for MMP-2 and PTEN expression studies with EnVision system and DAB as a chromogen. Antigen expression was classified as followed: 0—no positive cells, 1—<10%, 2—10–50%, 3—above 80% positive cells. All results were statistically evaluated using Mann–Whitney U test and ANOVA, with statistical significance at $p<0.05$.
Results: In patients with LSCC, the expression PTEN was detected in the cytoplasm of 20% tumor cells and 30% stromal area ($p<0.05$) while in controls only in 5% of

stromal area ($p<0.05$ vs LSCC). PTEN expression was decreased in tumor cells in N+ vs N0 cases (10% vs 30%). In LSCC, MMP-2 was found in 3.5% of the tumor cells, but in 45% of stromal area ($p<0.05$). There was no MMP-2 expression in controls, while expression level (0–3 scale) was higher in tumor cells versus stromal compartment. MMP-2 stromal expression in N0 and N+ cases was 50% versus 38% of the area, respectively.
Conclusion: We might conclude that decreased PTEN expression in tumor cells predicts lymph node involvement, which then is accompanied by decreased expression of MMP-2 in stromal area in the main lesion.

0401
Mucosal large cell neuroendocrine carcinoma (mLCNEC) of the head and neck regions: a new clinicopathologic entity

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Objective: Large cell neuroendocrine carcinoma (LCNEC) is well known as a subtype of lung cancer, but it is extremely rare in the head and neck regions. Our objective was to establish mucosal LCNEC (mLCNEC) as a new entity in the head and neck regions.
Method: We reestimated 814 surgically resected specimens of the primary mucosal carcinoma in the head and neck regions, including 24 basaloid squamous cell carcinoma, during 2002–2009. The immunostainings for neuroendocrine (NE) markers such as CD56, chromogranin-A and synaptophysin were performed. In the cases which were positive for two or three NE markers, we re-diagnosed as "mLCNEC".
Results: Only eight cases (0.98%) were re-diagnosed as mLCNEC. All cases were male and their mean age was 64.6 years. Three cases occurred in the tongue base, four cases in the larynx and one case in hypopharynx. Although seven cases showed numerous regional lymph node metastases, only one case showed death of disease. Histologically, mLCNEC showed the sheet-like, trabecular, organoid pattern growth of relatively large basaloid cells in which the central necrosis, rosette formation, peripheral palisading, and high mitotic figures were sometimes seen. Immunohistochemically, mLCNEC indicated to be positive for two or three NE markers. Only three cases of mLCNEC were immunopositive for TTF-1, whereas all cases except one case were only focally immunopositive for p63. All cases showed high proliferating activity.
Conclusion: We propose that mLCNEC can occur in the head and neck regions and is a new clinicopathological entity. The prognosis of mLCNEC remains unclear.

Friday, 3 September 2010, Basement

PS-17 Poster Session Paediatric Pathology

0402
Massive ovarian edema et fibromatosis

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Objective: Massive ovarian edema is an unusual cause of ovarian enlargement in young women. It is due to the accumulation of edema fluid and in some cases is associated with mature cystic teratoma. The patients are young (average 21 age), with present abdominal pain, menstrual abnormalities or evidence of hypergonadism. The ovarian enlargement is unilateral in 90% of cases.
Method: We report a case of a 12-year-old girl with tumor of the left ovary. Grossly, the enlarged ovary's greatest dimension was 18 × 20 cm (1,900 g), composed of solid mass with large gelatinous and hemorrhagic foci. Paraffin sections from 20 samples were stained with HeEo and immunostained for vimentin, S100 protein, SMA, desmin and CD34.
Results: Histological findings were identical in all slides. Ovarian stroma cells were scattered within abundant edematous fluid, and in some foci, hypocellular stroma surrounded follicles. In the cortex, follicles were dilated in fibromatous stroma with rare luteinized cells. CD34 confirmed the presence of many vessels with thrombi composed of fibrin-causing hemorrhagic necrosis. In some areas of necrosis, we found foci of dystrophic calcification suggesting an older process.
Conclusion: Massive ovarian edema is a lesion which can be misdiagnosed as a neoplasm. The cause is unclear. It has been attributed to intermittent torsion of the ovarian pedicle and interference with its lymphatic drainage. The fibromatosis and massive edema represent two ends of the same disorder.

0403
Post-neonatal cardiac death associated with intimal fibroplasia of coronary and of major systemic arteries

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Objective: Intimal fibroplasia is a variant of fibromuscular dysplasia (FMD), a non-inflammatory and non-atherosclerotic arterial segmental disease, resulting in narrowing of the lumen. FMD of coronary arteries is a

rare pathology associated with cardiac and sudden death occurring during infancy, childhood and young adulthood.
Method: We describe the case of a female infant born prematurely (gestational age 33+4/7) presenting a mild supravalvular aortic stenosis associated with an atrial septal defect and cardiac valvular dysplasia. During the first weeks of life, she developed a progressive obstructive cardiomegaly and she died at day 55 of a cardiogenic shock.
Results: The autopsy confirmed a marked cardiac hypertrophy and the aforementioned congenital cardiac anomalies. Histologically, we observed a myxomatous thickening of the aortic, pulmonary and mitral valves and a significant segmental intimal fibroplasia of all coronary arteries, producing subtotal obstruction of the right coronary artery. The myocardium presented multiple areas of infarction of various ages and foci of interstitial fibrosis with calcifications. Sampling of the aorta and its major branches showed mild to moderate intimal fibroplasia of the aorta, celiac trunk, superior mesenteric and left renal arteries.
Conclusion: The pathogenesis of FMD is unclear: Mural ischemia, hormonal, genetic and mechanical factors have been suggested. This case supports the theory of a congenital origin and emphasizes the importance of an extensive examination of coronary arteries when performing autopsy in paediatric patients as FMD is a segmental disease, and it must be considered in the etiologic differential diagnosis of cardiac and sudden death in paediatric age.

0404
Increased amount of chorionic disc extravillous trophoblasts (EVT) in placental hypoxia

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Objective: Increased amount of EVT in the maternal floor was found in preeclamptic placentas. This analysis intends to retrospectively prove that the EVT is increased in the whole chorionic disc also in other than preeclampsia clinical conditions at risk for fetal and placental hypoxia.
Method: Frequencies of clinical and placental parameters of 189 consecutive cases with more than five cell islands per full-thickness paracentral section of grossly unremarkable placenta (study group, SG) were compared to all remaining 1,006 placentas (control group, CG).
Results: The numbers of placental septa/cell islands were statistically significantly ($p<0.05$) higher in the SG than in the CG in association with preeclampsia, chronic hypertension, diabetes mellitus, oligohydramnios, intrauterine growth restriction, induction of labor, cesarean sections, low 5-min Apgar score, small placentas, placental infarction, massive perivillous fibrin deposition, decidual arteriolopathy, diffuse placental hypoxia, microscopic chorionic pseudocysts, and maternal floor clusters of multinucleate trophoblastic giant cells. The reverse was seen in the premature rupture of membranes, perinatal mortality, abnormal umbilical cord, acute chorioamnionitis, chronic villitis of unknown etiology, and histological placental meconium staining.
Conclusion: The amount of chorionic disc EVT is increased in association with clinical conditions and placental lesions known to be associated with hypoxia, but is decreased in "non-hypoxic" clinical conditions and placental lesions. Counting the placental septa and cells islands can serve as a surrogate test of placental hypoxia, not only in preeclampsia.

0405
Pseudo-TORCH and the cerebro-costo-mandibular syndrome

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Objective: While the posterior rib gaps and Pierre Robin sequence are well documented, components of the cerebro-costo-mandibular syndrome (ccms), the "cerebral" part thereof is more variable, including mental retardation in survivors, hydrocephalus, severe hypoplasia of frontal and occipital lobes, abnormal olfactory bulbs, cerebral heterotopias, or gliosis.
Method: This is a case report of unusual brain findings in ccms, to our knowledge found for the first time at postmortem examination.
Results: Because of fetal hydrops and congenital anomalies found on prenatal ultrasound, a 23-week pregnancy was terminated to reveal a fetus with multiple rib gaps, dysmorphic facial features, prominent micrognathia, high-arched palate, club feet, dysplastic organ of Corti, incomplete visceral rotation, pseudoglandular transformation of the pituitary gland, mucosal eosinophilia of the stomach and intestines, microcalcifications of liver, and extensive, diffuse, predominantly perivascular and not only periventricular brain calcifications. There was no laboratory evidence of intrauterine infection either clinically or by in situ hybridization.
Conclusion: This case illustrates a not yet described pseudo-TORCH, congenital infection-like, presentation of the ccms with extensive brain calcifications and mucosal eosinophilia of the gastrointestinal tract. Extensive brain calcifications were described in other genetic syndromes such as Baraitser–Reardon syndrome, Aicardi–Goutieres syndrome, and mitochondrial encephalopathies, but not the

ccmc. Calcium metabolism can have genetic background in the ccms and explain cerebral dysfunction in at least a subset of the syndrome.

0406

Alveolar capillary dysplasia/misalignment of pulmonary veins: report of four cases

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Objective: Alveolar capillary dysplasia (ACD) with or without misalignment of pulmonary veins (MPV) is an uncommon congenital cause of persistent pulmonary hypertension of the newborn characterized by a lack of alveolar and vascular development. Most are sporadic, but familial ACD/MPV has been reported, linked to the gene FOXF1 on chromosome 16 q24.1-q24.2. It is universally fatal and diagnosis is entirely dependent on the pathological examination of surgical lung biopsy specimen. Four cases of ACD, three with MPV, are reported.
Method: The four neonates were full term, without associated congenital anomalies. They were admitted to the paediatric intensive care unit for hypoxia and severe pulmonary hypertension unresponsive to maximal cardiorespiratory support, including high-frequency ventilation, inhaled nitric oxide and extracorporeal membrane oxygenation.
Results: Surgical lung biopsies performed in three cases and postmortem lung examination in one case showed pathological findings of ACD, characterized by poor capillary apposition and density and medial arterial hypertrophy. These lesions were associated with misalignment of pulmonary veins in three cases. The four infants died of refractory hypoxemia during the first 2 months of life.
Conclusion: These observations emphasize the importance of considering ACD with or without MPV in all newborn infants who present a persisting severe pulmonary hypertension without anatomical cause. Histological diagnosis based on early surgical lung biopsy may prevent from using costly, invasive and ineffective treatments and procedures such as extracorporeal membrane oxygenation.

0407

A case of complete trisomy 9: autoptic, cytogenetic and radiologic findings

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Objective: An autopsy of a female fetus of the 22+2nd gestational weeks was performed after an induced abortion due to multiple intrauterine malformations suspecting a chromosomal disorder.
Method: Prior to the autopsy, the 220-g female fetus was extensively X-rayed. We performed an autopsy including histological examination of all organs. Cytogenetic analysis was performed using both chorionic trophoblast and tendon fibroblasts.
Results: The fetus was characterized by pronounced global hydrops, craniofacial dysmorphy and malformations of both hands and feet. We found a complex cardiac malformation, unilobular lungs, dysmorphic liver and spleen, malrotated and doubled right kidneys, and an aplasia of the left kidney. The parenchymatous organs showed inconspicuous histomorphological architecture. In the cerebrum, no corpus callosum was found and immature neuronal rosettes in both optical nerves were detected. X-ray showed vertebral clefts, a slight hypertelorism, skeletal dysplasias of both hands, and confluent frontal and occipital fontanelles. Cytogenetics turned out a complete trisomy 9 (GTG-Banding).
Conclusion: We present radiological, autoptic and cytogentic findings in a case of a very rare chromosomal aberration which mostly occurs as a mosaic or partial trisomy 9. This chromosomal abnormality usually induces early abortion. Therefore, a fetus with trisomy 9 and a gestational age of 22+2 weeks is very uncommon. We found multiple malformations of the skeleton and organs, mostly located in the midline.

0409

Holoprosencephaly with cyclopia: presentation of two fetuses with multiple congenital malformations and investigation of sonic hedgehog (Shh) expression

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Objective: Holoprosencephaly is the commonest forebrain developmental anomaly occurring in 1 of 16,000 live borns. Sonic hedgehog is the major gene implicated in holoprosencephaly.
Method: Two fetuses of 27-week and 32-week pregnancies, terminated due to multiple congenital malformations, were autopsied, and paraffin-embedded tissue sections were examined microscopically. Shh expression was investigated immunohistochemically in orbital and CNS sections.

Results: Twenty-seven-week fetus: karyotype 46XX; alobar holoprosencephaly, true cyclopia, proboscis formation, pituitary gland anterior lobe agenesis, atretic anus and vagina, hypertrophic clitoris, female internal genitalia, right hand oligosyndactyly, left-sided overriding fourth and fifth toes, ventricular septal defect, hypoplastic left heart complex, truncus arteriosus, asplenia, irregular liver lobation, left kidney agenesis, right kidney cystic dysplasia, hypoplastic right ear auricle, external auditory meatus atresia. Thirty-two-week fetus: karyotype 46XY; alobar holoprosencephaly, synophthalmia, proboscis formation, hypoplastic right heart complex, patent foramen ovale, ostium secundum defect, tricuspid valve and pulmonary artery atresia, pulmonary arteries analogous branching from the aorta. No teratogenetic factors and consanguinity were reported. Histological examination revealed scattered rosette-like structures in areas of dysplastic retina in both cases. Immunohistochemical expression of Shh was negative.
Conclusion: The absence of Shh immunostaining suggests a potential causative role in the pathogenesis of the above phenotypes. Even though both karyotypes were normal, the authors could not exclude the possibility of the existence of chromosomal alterations not detectable by routine karyotypic analysis. Finally, to our best knowledge, the above phenotypes do not seem to match any known clinical syndromes, and we therefore consider their origin unknown at present.

0412

The impact of nutritional colourant tartrazine (E102) on morphological state of thymus of rat descendants

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Objective: Synthetical colourant of yellow colour tartrazine (E102) whose application is prohibited in some countries of EU got widespread use. The aim of the work was the assessment of influence tartrazine to thymus of infant rat descendants 1 month from birth from mothers who had been taking tartrazine during gestation and feeding.
Method: It was histologically discovered that density of cortical and medullar thymocytes was much higher than in the control group. Mitosis is often diagnosed in the subcapsular zone. The most part of thymocytes of this zone is apoptosis amenable, cells with low concentration of DNA in the nucleus, with the sings of dystrophy and necrosis often met. Thymus medullary substance generally consists of fine lymphocytes. The small bodies of thymus, which are situated in medullary substance, are few in number and fine.
Results: Immunohistochemical investigation with MCA defined the signs of thymocyte-interrupted maturation of depletion of CD4 and CD8 population and macrophage ED1 and B lymphocytes CD45RA amount rising.
Conclusion: Thymus morphological specification of infant rats with tartrazine intoxication (during 1 month) indicates frank hyperplasia of thymus lymphoid with underlying maturation retention, and component lymphocyte immunological differentiation points to antigenic activation, which influenced the test animals.

0413

Pleuropulmonary blastoma: a challenging congenital tumor of fetal lung

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Objective: Pleuropulmonary blastoma (PPB), a rare tumor arising during fetal lung development, can be part of an inherited cancer syndrome. About 20% of children with PPB have a family history of cystic nephroma and rhabdomyosarcoma. It is related to DICER 1 mutations on 14q32 and consists in malignant mesenchymal cells and cysts lined by benign epithelium.
Method: We report our experience in a paediatric pathology center. Two girls of 1 and 39 months presented pulmonary lesions, the first purely cystic and the second purely solid, corresponding histologically to early type I and type III PPBs.
Results: The first patient underwent an upper lobectomy with an unremarkable postoperative outcome. The second patient presented a thoracic relapse 3 years after initial treatment consisting in upper lobectomy, pre- and postoperative chemotherapy. Tumoral karyotype revealed complex clonal chromosomal aberrations, dominated by interstitial 14q32, 7q22 deletion and trisomy 8. No familial history of neoplasia. She is disease-free 14 years after presentation.
Conclusion: PPB must be taken into account in the evaluation of cystic and even solid pulmonary lesions in children under 6 years. A correct histologic diagnosis is needed to identify early PPB, which may be difficult to differentiate from congenital pulmonary airway malformation (CPAM), to appropriately manage patients and to identify an inherited predisposition to PPB. Recent data showed that DICER1 on 14q32, a gene implicated in miRNA synthesis, plays a crucial role in lung development.

Loss of DICER1 alters miRNA-dependent regulation of diffusible growth factors that induce mesenchymal cell proliferation.

Friday, 3 September 2010, Basement

PS-18 Poster Session Infectious Disease Pathology

0415

Proliferation index measured with Ki-67 and/or topoisomerase II alpha expression is a useful prognostic factor in neuroblastoma

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Objective: Proliferation index assessed immunohistochemically with Ki-67 (PI Ki67) is the accepted prognostic factor in some types of cancer. Topoisomerase II alpha (TOPO2a) expression is another marker of cellular proliferation. The significance of both markers in neuroblastoma (NB) is not well established. The study objective was to evaluate their prognostic impact and pathoclinical relations in a series of NB tumors.
Method: Examined group consisted of 103 patients. Analyzed data included: patients' age, tumor histology, location, stage, MYCN status and overall survival. Ki67 and TOPO2a PI values were established on slides as percent of immunopositive nuclei for 100–1,000 neoplastic cells.
Results: PI Ki67 ranging 0–72% (median 18%) and TOPO2a ranging 0–58% (median 20%) were lower in children older than 18 months (>18 m, $p=0.0002$ and $p=0.01$, respectively). The two markers were strongly interrelated ($r=0.83$). Higher Ki-67 and TOPO2a correlated with higher MKI and adrenal location and inversely with increasing tumor differentiation. The cutoff values of PI Ki67 ≥ 30% and TOPO2a ≥ 25% correlated with fatal outcome of the disease. In the subgroup of patients, >18 m significant correlations between higher values of both PI markers and metastatic stage, unfavorable histology, high MKI, MYCN amplification, and adrenal localization were observed. At cutoffs Ki67 ≥ 10% and TOPO2a ≥ 25%, the markers predicted long-term unfavorable outcome by Kaplan–Meyer analysis. Cox regression analysis indentified PI (assessed jointly as Ki67 ≥ 10% + TOPO2a ≥ 25%) as the independent prognostic factor.
Conclusion: Ki67 and TOPO2a PI markers have prognostic significance and clinicopathological correlations in NB. We propose to include PI to the standard histological assessment protocol of NB tumors.

0417

Risk factors for fatal outcome in AH1N1 influenza patients

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Objective: There are few clinical studies of the systemic human pathology of the novel AH1N1 influenza infection. We decided to test the hypothesis that fatal cases of influenza occur mainly in association with important comorbidities as was stated based in previous studies.
Method: We included in our study 97 patients with fatal AH1N1 influenza. During autopsy, we largely sampled vital organs—brain, lungs, heart, liver, kidney, lymphoid organs—lymph nodes and spleen and any other diseased organ.
Results: In our group, sex ratio was male/female = 1.7:1; patients were 5 and 76 years old (mean age 42.01). Nine women were pregnant/parturient (9.28%). Other patients presented obesity, malignant neoplasia, diabetes mellitus, cardiac diseases and tuberculosis. We analyzed the prevalence of these comorbidities in the general population (see Table). However, 48 patients did not have any comorbidities or any known cause of immunosuppression.
Conclusion: Our study group was heterogeneous with a large age distribution and different associated diseases. We identified new risk factors for fatal outcome in AH1N1 patients (obesity and/or pregnancy) and exclude other diseases as risk factor for severe evolution (diabetes mellitus). We have to emphasise that in half of the cases, there were no comorbidities or special status to actuate the severity of the disease. These findings are reinforcing the statement that the novel influenza has a different behavior than common influenza.

0418

Pathologic aspects in AH1N1 virus-related deaths in Romania

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Objective: Novel influenza is an acute viral infection of the respiratory tract caused by type A influenza virus, affecting by now the entire world. Although the illness is mild and self-limited in a great majority of cases, few patients may have a severe clinical course eventually leading to death.
Method: We studied necroptic tissue samples from 97 patients submitted over a 4-month period from a total of 7,008 positive cases identified in Romania since the outbreak of this type of influenza. Data collected included demographic and clinical information.
Results: Most of the patients (65.97%) were adults between 21 and 50 years (median age, 42.01 years), male/female ration 1.7:1. The vast majority of the cases revealed diffuse alveolar damage, interstitial pneumonia and bronchopneumonia, all of them associating severe cytopathic effect in the bronchial and alveolar epithelial cells; 17 cases presented pulmonary ischemic lesions. More than half of the cases (59.79%) presented myocardial interstitial inflammatory infiltrate with full-blown picture of myocarditis in 11 patients. Of the patients, 80.41% had lymphocytic depletion in lymphoid organs. Lymphocytic meningitis, meningothelial hyperplasia, acute tubular necrosis, and liver steatosis were identified in some cases.
Conclusion: The respiratory tract is the major target of influenza A H1N1 virus. Pulmonary lesions are the main cause of death in all patients with new influenza virus infection, but most of them had significant extrapulmonary comorbidities.

0419

Role of HPV-mRNA detection in HPV infections

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Objective: HPV DNA has been identified in almost all cervical cancers, and women with active HPV infection (HPVI) express E6/E7 oncogenes. As only a small proportion of infections progress towards cancer, it is important to distinguish transient HPVIs from persistent or progressive ones.
Method: One hundred ninety-six samples were tested by conventional Pap smear, HPV-DNA test and typing, E6/E7-mRNA expression from HPV types 16, 18, 31, 33, 45. *K*-statistic value was used in order to identify possible significant associations.
Results: Pap-smear: negative (NEG), 248 (68.5%); atypical cells of undetermined significance (ASCUS), 50 (12.4%); low-grade squamous intraepithelial lesion (LSIL), 36 (9.9%); high-grade squamous intraepithelial lesion (HSIL), 28 (7.7%). One hundred ninety-two of 362 samples (53.04%) were positive to the HPV-DNA test and 80 of 362 (22.10%) to the HPV-mRNA test. The HPV-DNA test was positive in 110 of 248 (44.4%) of NEG, 37 of 50 (74.0%) of ASCUS, 22 of 36 (61.1%) of LSIL and 23 of 28 (82.1%) of HSIL; the E6/E7-mRNA test was positive in 25 of 248 (10.1%) of NEG, 17 of 50 (34.0%) of ASCUS, 15 of 36 (41.7%) of LSIL and 23 of 28 (82.1%) of HSIL.
Conclusion: The detection of HPV-mRNA shows a greater association with the degree of development of atypical or malignant lesions in comparison to the presence of HPV-DNA. The mRNA test might be a second level tool for the appropriate follow-up of ASCUS and LSIL patients with persistent or progressive HPVIs.

0420

Combination of multiple sclerosis with chronic herpetic meningoencephalitis

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Objective: Etiology of multiple sclerosis (MS) includes the virus infection which plays probably an important cofactor role in the pathogenesis of disease. Clinical and morphological analysis of the cases of chronic herpetic meningoencephalitis (CHME) and MS permitted to discover two cases of a combination of diseases: man, age 58 years (onset of MS since 18 years), and woman, age 65 years (onset of MS since 27 years).
Results: Autopsy revealed the small plaques in white matter of brain and cord and some foyers of necrosis in frontal lobe and brain stem. The histological signs of chronic inflammation were established in both cases. Leptomeninges and brain were infiltrated by lymphocytes and macrophages (perivascular cuffs). The progressive lesions of cortical neurons and proliferation of macroglial cells were constant histological findings. Etiological diagnosis of CHME was established on the base of typical intranuclear viral inclusion types I and II in the cells of brain as well as on the revealing of HSV antigens in histological slice. Laminar nonischemic necrosis was found in the cortex of frontal and parietal lobes. In the brain stem, necrosis appeared as the small foci of destruction of nervous tissue. In one case, laminar necrosis were detected in white matter of hippocampe.
Conclusion: Thus, the morphological investigation on the light microscopic level permits correctly to recognize the combination of MS and CHME.

0422

Whipple's disease associated whit Crohn's disease

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Objective: We present the first case reporting the exceptional association of Whipple's and Crohn's disease.
Method: A 59-year-old man developed since 2005 diarrhea, abdominal pain, weight loss, and polyarthralgia. Endoscopy showed aphthous ulcers in terminal ileum and colon. Histology revealed a rich collection of epithelioid granulomas in the lamina propria with chronic inflammation. A diagnosis of Crohn's disease (CD) was given and steroid therapy was administered. Diarrhea and abdominal pain quickly subsided. Perduring a severe weight loss, a gastroduodenal endoscopy was performed. Oedema with whitish spots in the duodenum was observed. Histology demonstrated lymphangiectasia and an infiltration of PAS positive foamy histiocytes in the lamina propria. Immunohistochemistry for *Tropheryma whipplei* was positive.
Results: Whipple's disease (WD) diagnosis was made and an antibiotic therapy was given. In respect to symptomatic improvement, persistence of the bacterium was demonstrated in the following performed gastric and duodenal biopsies. Immunohistochemistry in previous ileal and bowel specimens was positive in the ileum.
Conclusion: In WD, the macrophages seem to be of central importance in the development of the disease. This infection shares with CD the pathogenetic hypothesis that a defect in cellular immune response contributes to the development of the disorder. Evidences also suggest that bacteria play a role in the onset and perpetuation of CD. This case strengthens the immunological hypothesis in the pathogenesis of the disorders. The functional defect of the macrophages caused by WD can explain the amazing number of granulomas in Crohn's-affected specimens. The double immunological defect due to CD and CD may contribute to the lack of eradication of the bacteria.

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PS-19 Poster Session IT

0423

The use of digital microscopy and Internet in the pathological diagnosis—own experience

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Objective: The aim of the study was to analyze the experience obtained during pathological diagnosis using digital microscopy and telepathology.
Method: There were 594 microscopic examinations performed over the Internet using a dynamic digital microscope NICON-COOLSCOPE, scanners APERIO-SCANSCOPE and OLYMPUS dotSlide, between January 2007 and March 2010. The study included 213 intraoperative studies, 317 cytology specimens and 62 oligobiopsies. The specimens were prepared and sent electronically by four cytotechnicians and assessed by five pathologists. All diagnoses were verified through routine, light microscopy assessments.
Results: In cases of intraoperative, frozen sections studies as well as oncologically positive cytological diagnoses, above 98% agreement was noted between the diagnoses made due to evaluation over the Internet and the routine assessment. The agreement of 100% was achieved in cases of benign lesions and the assessment of bronchial surgical margins.
Conclusion: (1) The efficiency of histological slide examination by sending digital images via the Internet in cases of intraoperative frozen sections during thoracic surgery or to confirm "positive" results is comparable to the traditional assessment in a light microscope. (2) Application of this method enables surgeons at the hospitals which do not have a readily available pathologist to perform the surgical procedures.

Friday, 3 September 2010, Basement

PS-20 Poster Session Electron Microscopy

0424

The role of electron microscopy in the diagnosis of childhood glomerulopathies

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Objective: Optimum diagnosis of glomerulopathies requires light microscopy, immunofluorescence and electron microcopy. In fact, electron microscopy has a confirmatory role in glomerular diseases. In this study, the value of electron microscopy in the diagnosis of glomerulopathies in children was investigated.
Method: The contribution of electron microscopy to the final diagnosis was graded as necessary—diagnosis could not be reached without it; supportive—it increased the level of confidence in the final diagnosis; and noncontributory—the diagnosis does not need electron microscopy for confirmation.

Results: One hundred thirty-four cases of renal biopsy with some clinical data are reviewed. The contribution of electron microscopy to the final diagnosis was necessary in 51 cases (38%), supportive in 40 cases (30%) and noncontributory in 43 cases (32%).

Conclusion: It is concluded that ultrastructural study was an essential tool in the study of renal biopsy in childhood glomerulopathies, suggesting that electron microscopy still remains a useful tool in the diagnosis of glomerular disease.