

Volume 2 Suppl 1, 2007

Meeting abstracts

35te Tagung der Pathologen am Oberrhein/35th Meeting of Pathologists of the Upper Rhine Region (PATOR)

The Institute of Pathology, University Hospital Freiburg, Germany

1 July 2006

Published: 14 March 2007

ORAL PRESENTATION

S1 HPV E6/E7 mRNA transcripts as predictors of high-grade epithelial cervix dysplasia

J Möckel¹, A Clad², A-S Endres³ and V Schneider⁴

¹Institut für Pathologie, Klinikum Lahr, Germany

²Universitäts-Frauenklinik Freiburg, Germany

³Institut für Virologie, Charite Universitätsmedizin, Berlin, Germany

⁴Pathologisches Institut Dr. Schneider, Freiburg, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S1

Aims: Detection of E6 and E7 mRNA transcripts has been shown to be of higher prognostic value for the evaluation of the precursor lesions of cervical carcinoma than the detection of HPV DNA in a number of pilot studies [1, 2, 3]. In particular in low grade lesions, HPV DNA testing has poor discriminating power as to the progression of CIN, thus leading to considerable overtreatment with ensuing costs to the health care system. We tested an E6, E7 mRNA detection system in a multicenter study in Germany for its usefulness in clinical practice.

Methods: We recruited 334 high-risk ambulatory patients in five clinics with cervical lesions ranging from low-grade intraepithelial lesions to invasive cervical carcinoma. Colposcopy, conventional cytology, HPV-DNA testing (Hybrid Capture II, Digene Corp.), HPV-mRNA-testing (PreTect HPV-Proofer, NorChips AS, Norway) and histologic sampling (biopsies and conisations) were performed.

Results: There were 140 patients WNL, 64 patients with CIN I/II, 98 patients with CIN III and 32 patients with invasive carcinoma. HPV-DNA testing was positive in 24%, 78%, 92% and 97%, respectively. E6/E7 mRNA positivity rate were 8%, 41%, 64% and 94%.

Conclusion: The mRNA-based test showed a higher prognostic value than DNA-based testing in a high-risk population of several dysplasia clinics in Germany. Thus, gene expression profiling of the viral oncogenes E6 and E7 showed superiority to DNA testing in triaging patients with cervical carcinoma precursors.

References

1. Kraus I, Molden T, Erno LE, Skomedal H, Karlsen F and Hagmar B: **Human papillomavirus oncogenic expression in the dysplastic portio; an investigation of**

biopsies from 190 cervical cones. *Br J Cancer* 2004, **90**:1407-1413.

2. Cuschieri KS, Whitley MJ and Cubie HA: **Human papillomavirus type specific DNA and RNA persistence-implications for cervical disease progression and monitoring.** *J Med Virol* 2004, **73**:65-70.
3. Lie AK, Risberg B, Sandstad B, Delabie J, Rimala R, Hagen B, Onsrud M and Thoresen S: **DNA versus RNA based methods for HPV testing in Norway. Evaluation of Hybrid Capture II and PreTect HPV-Proofer, a validation study.** *21st International Papillomavirus Conference: February 20 - 26, 2004; Mexico City, Abstract.*

S2 Immunohistochemical detection of micrometastasis and disseminated tumor cells in microscopically tumor free lymph nodes

H Adams and S Dirnhöfer

Institut für Pathologie, Universität Basel, Switzerland

Diagnostic Pathology 2007, 2(Suppl 1):S2

Frequency and clinical relevance of micrometastasis (pN1 mi) and disseminated tumor cells (pN0i+) in carcinoma patients are controversially debated or unclear. Thus we immunohistochemically examined the lymph nodes of 422 pN0 classified patients (conventional light microscopy) between November 2004 and March 2006, using the cytokeratin (CK)-22 antibody. In 13 cases (3%), we found previously undetected micrometastasis (pN1 mi) and 84 cases (20%) were positive for disseminated tumor cells, discovered by immunohistochemistry. In all cases in which micrometastasis was found, it was only present in one of the lymph nodes examined, whereas isolated tumor cells were found in up to five nodes from one patient. Cases with such findings are mainly breast and colon cancers (pN0 i+ in colorectal carcinomas: n = 39, breast: n = 25, other entities together: n = 19; micrometastasis (pN1 mi): colorectal carcinoma: n = 3, breast: n = 5, other entities together: n = 5). While the meaning of micrometastasis in axillary lymph nodes is undebated and thus integrated in the TNM system, the prognostic value of this finding for other entities remains unclear. However, the WHO suggests a classification scheme for these.

We will now start to collect follow-up results for all patients in this study to conclude how to deal with these findings in future.

S3**COX-2 expression in thymomas and thymic carcinomas: a novel therapeutic target?**

RJ Rieker¹, PhA Schnabel¹, G Mechtersheimer¹,
M Thomas², H Dienemann², P Schirmacher¹
and MA Kern¹

¹Institut für allgemeine Pathologie, Universitätsklinikum
Heidelberg, Germany

²Thoraxklinik Rohrbach am Universitätsklinikum Heidelberg,
Germany

Diagnostic Pathology 2007, 2(Suppl 1):S3

Aims: The treatment of advanced stage thymomas and thymic carcinomas is multimodal and includes surgery as well as radiochemotherapy. New therapeutic targets such as EGFR and c-kit are currently under investigation. A number of studies have shown a protumorigenic potential of Cyclooxygenase-2 (COX-2), an enzyme of the prostaglandin metabolism, in a variety of human malignancies, but so far it is unknown whether COX-2 is expressed in epithelial tumors of the thymus.

Methods: Using tissue microarrays, the expression of COX-2, microsomal-PGES-1 and -PGES-2 (mPGES-1 and mPGES-2), as well as EGFR was evaluated in thirty-four cases of different subtypes of thymoma and thymic carcinomas. Furthermore, twenty-seven additional cases of thymomas and thymic carcinomas were analysed by COX-2 western immunoblot analysis and compared with six normal thymi from young children.

Results: COX-2 was expressed in all thymoma- and thymic carcinoma subtypes. When measuring the optical color intensity, no significant differences between the subtypes could be detected. A weak correlation between the expression of COX-2, mPGES-1 and mPGES-2 as well as EGFR was found. Western blot analysis of COX-2 expression revealed an up-regulation compared with normal thymus.

Conclusion: COX-2 is expressed in all subtypes of thymomas and thymic carcinomas and represents therefore a potential novel therapeutic target beside EGFR and c-kit. A combined therapy using COX-2 inhibitors in addition to the evolving anti-EGFR antibody therapy may be considered as treatment option, especially when there is no response to established chemotherapeutic schemes, since this combination has a positive impact on the treatment of other malignancies.

S4**Paraffin tissue microarrays constructed with evenly long paraffin tissue core biopsies**

U Vogel and B Bültmann

Institut für Pathologie, Universität Tübingen, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S4

Aims: Paraffin tissue microarrays (PTMAs) are blocks of paraffin with up to 1,000 paraffin tissue core biopsies (PTCBs). The construction of PTMAs consists of putting PTCBs from so-called donor blocks into preformed holes of so-called recipient blocks, the later PTMAs. Normally, paraffin tissue blocks of daily pathological work are used as donor blocks. However, these blocks have been already cut and contain tissues of different thickness. Therefore, the PTCBs punched out of these paraffin blocks are of different length. In consequence, the

sections of the deeper portions of the PTMA don't contain all of the desired PTCBs thereby diminishing the incredible efficacy of the PTMA technique.

Methods: To overcome this drawback and to cut the PTCBs to a certain length, we manufactured a cutting board out of lucent polystyrene glass with a thickness of 4 mm. Holes were drilled into this board which were filled completely by at least one PTCB. The excess length of those PTCBs which stand over the surface of the cutting board were cut with a sharp knife. These cut parts were installed again in other holes of the cutting board or stored in microtubes for further PTMAs or other investigations like morphological-independent PCR. Then the PTCBs were injected from the cutting board into the holes of a PTMA using a stylet.

Results and conclusions: Thereby a PTMA was constructed with evenly long PTCBs, ensuring that the first and the last section of the PTMA contained nearly all of the PTCBs. Using a Beecher paraffin tissue punch with a countersink, it seems also possible to inject the PTCBs from the cutting board into the Beecher punches and to construct PTMAs with the widely distributed Beecher system.

S5**Glypican 3 expression in human normal and neoplastic tissue: a tissue microarray analysis on 4338 tissue samples**

D Baumhoer, LM Terracciano, S Stadlmann and L Tornillo
Institut für Pathologie, Universität Basel, Switzerland

Diagnostic Pathology 2007, 2(Suppl 1):S5

Aims and methods: Glypican 3 (GPC3) belongs to the glypican family of GPI anchored heparan sulfate proteoglycans, which play a crucial role in cellular growth, cell migration and cell differentiation. Several studies have shown GPC3 to be a highly specific marker for hepatocellular carcinoma (HCC) and for differentiating non- and pre-neoplastic liver disease. To systematically investigate the epidemiology of GPC3 expression in non-neoplastic, pre-neoplastic and neoplastic tissues, we used tissue microarray (TMA) technology to analyze the immunohistochemically detectable expression of GPC3 in 3,678 tissue samples from 132 different tumor categories and 31 non-neoplastic and pre-neoplastic tissue types.

Furthermore, GPC3 expression was investigated in an additional TMA containing 405 non-neoplastic, pre-neoplastic and neoplastic liver samples.

Results: GPC3 expression was found in 23% of non-neoplastic (liver cirrhosis), in 37% of pre-neoplastic (low- and high-grade dysplastic nodules) and in 64% of neoplastic liver disease. Furthermore, testicular non-seminomatous germ cell tumors (55%), squamous cell carcinoma of the lung (54%), liposarcoma (52%), cervical intraepithelial neoplasia (CIN) III (41%), melanoma (29%) and schwannoma (26%) also revealed consistent expression of GPC3.

Conclusion: This study provides a comprehensive overview on the expression of GPC3 in normal and cancerous tissue. Among neoplastic tissue, our data underline the role of GPC3 in hepatocellular carcinogenesis and suggest a potential role of GPC3 as a therapeutic target in these tumors. Moreover, several non-hepatic tumors can also show GPC3 positivity.

S6**Diagnosis of Burkitt's lymphoma in due time: a practical approach**SB Cogliatti¹, U Novak², S Henz³, U Schmid¹, P Möller⁴ and TFE Barth⁴¹Department of Pathology, Kantonsspital, St. Gallen, Switzerland²Institute for Cancer Genetics, Columbia University, New York, NY, USA³Department of Internal Medicine, Kantonsspital, St. Gallen, Switzerland⁴Department of Pathology, University Hospital, Ulm, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S6

Aims: The quick diagnosis of Burkitt's lymphoma (BL) and its clear-cut differentiation from diffuse large B-cell lymphoma (DLBCL) is of great clinical importance since treatment for these two disease entities differ markedly and should promptly be initiated in BL. However, these two tumours are difficult to distinguish using the current WHO classification, particularly in regard to BL variants, i.e., BL with plasmacytoid differentiation and atypical Burkitt's/Burkitt's-like lymphomas.

Methods: We studied 39 cases of highly proliferative blastic B-cell lymphoma (HPBCL) to establish a practical differential-diagnostic algorithm. Characteristics set for BL were a typical morphology, a mature B-cell phenotype of CD10⁺, Bcl-6⁺ and Bcl-2⁻ tumour cells, a proliferation rate of >95%, and the presence of *C-MYC* rearrangements in the absence of *t(14;18)(q32;q21)*. All cases were selectively negative for cyclin D-1, CD5, CD23, LMP-EBV, CD34 and TdT, and there were no cases of endemic or immunodeficiency-associated Burkitt's lymphoma.

Results: Altogether the set BL characteristics were found in only 5/39 cases (12.8%), whereas the majority of tumours revealed mosaic features (87.2%). In a second attempt, we followed a pragmatic stepwise approach for a classification algorithm that includes the assessment of *C-MYC* status to stratify HPBCL into four predefined diagnostic categories (DC), namely DC I (5/39, 12.8%): "classical BL", corresponding to the classical variant of sporadic BL in the WHO classification; DC II (11/39, 28.2%): "atypical BL", corresponding to the atypical Burkitt's/Burkitt's-like variants of sporadic BL in the WHO classification; DC III (9/39, 23.1%): "*C-MYC*⁺ DLBCL"; and DC IV (14/39, 35.9%): "*C-MYC* HPBCL".

Conclusion: This proposal may serve as a robust and objective operational basis for therapeutic decisions for HPBCL within one week and is applicable to be evaluated for its prognostic relevance in prospective clinical trials.

S7**Sensitivity of an immunoglobulin heavy chain gene polymerase chain reaction primer system for routine diagnosis of lymphomas**R Heyny-von Haußen, C Braun and G Mall
Pathologisches Institut Klinikum Darmstadt, Darmstadt, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S7

Aims: Polymerase chain reaction (PCR) and length fragment analyses of the immunoglobulin heavy chain (IgH) gene are useful tools for clonality assessment in malignant B-cell-lymphomas and reactive lymphoid infiltrates. The present investigation analyzes the sensitivity of an IgH gene consensus primer multiplex PCR

Table 1 (abstract S7)

Lymphoma diagnosis	Detection of clonally rearranged IgH gene
B lymphoblastic lymphoma	100% (1/1)
Mantle cell lymphoma	100% (3/3)
CLL	100% (24/24)
Lymphoplasmacytic lymphoma	100% (6/6)
Marginal zone B-cell lymphoma	100% (48/48)
Plasma cell myeloma	100% (1/1)
Hairy cell leukaemia	50% (1/2)
Follicular lymphoma	55% (6/11)
Diffuse large B-cell lymphoma	82% (9/11)
Hodgkin lymphoma	50% (1/2)

system for the detection of clonality for routine diagnosis in 109 lymphomas during a period of 3 years (2003–2005) at the Institut of Pathology Klinikum Darmstadt.

Methods: We used FR2A/JH/VLJH and FR3A/JH/VLJH primer sets for detecting clonal B cell populations. Primer sequences used for PCR: Variable region FR2A consensus primer: FR2A: TGG(AG)TCCG(AC)CAG(GC) C(CT)(CT)C(AGCT)GG. Joining region (JH) consensus primer: LJH: TGAGGA GACGGTGACC, VLJH: GTGCAGGT(AGCT) CCTTGGCCCCAG-FAM. 1. PCR: FR2A/LJH and FR3A/LJH. 2. PCR: FR2A/VLJH-FAM and FR3A/VLJH-FAM. Fluorescence fragment analyses of IgH gene rearrangement were performed with FAM-labeled PCR products by high-resolution capillary electrophoresis using the ABI-PRISM 310 Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany) with a POP 6-filled capillary (Applied Biosystems) and analyzed by using the GeneScan software (Applied Biosystems). A fragment was considered to be clonal in the case of a peak-height ratio (PHR) >2. The PHR was calculated as the quotient of the highest peak divided by the mean height of the two peaks surrounding the largest peak.

Results: See Table 1.

Conclusion: The present study showed a variable sensitivity of PCR of IgH gene region with FR2A/JH/VLJH and FR3A/JH/VLJH consensus primer sets for different lymphomas. Sensitivity of our consensus primer set mainly depends on the existence of IgH gene rearrangements or the status of the IgH gene in the special lymphoma types, especially the occurrence of somatic hypermutation in variable-region genes.

S8**Molecular and functional analysis of $\gamma\delta$ T cell expansions in immunodeficient patients**Paul Fisch¹, Petros Christopoulos², Elisabeth Nikolopoulos¹, Hendrik Veelken² and Stephan Wehl³¹Institut für Pathologie, Universitätsklinikum Freiburg, Germany²Abteilung Hämatologie/Onkologie, Universitätsklinikum Freiburg, Germany³Zentrum für Kinderheilkunde und Jugendmedizin Universitätsklinikum Freiburg, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S8

Aims: Patients with various forms of immunodeficiencies frequently show expansions of $\gamma\delta$ T cells in their peripheral blood. We attempted to characterize the $\gamma\delta$ T cell subpopulations in these

patients and possibly elucidate the cellular mechanisms involved in the $\gamma\delta$ T cell expansions in some of these patients.

Methods and results: Two adult patients with thymoma and $\gamma\delta$ T cell expansions were studied by flow cytometry and T cell receptor γ - and δ -chain spectratyping. One patient suffering from leishmaniasis and thymic carcinoma showed a peculiar polyclonal $\gamma\delta$ T cell proliferation while another patient with a benign thymoma and CMV reactivation had a persistent oligoclonal amplification of $\gamma\delta$ T cells. In one pediatric patient with incomplete RAG-1 deficiency, we found a restricted variability of the expressed V δ 3, versus V δ 1 and V δ 2 chains and a seemingly monoclonal usage of the V γ 4 element. Sequencing revealed that these $\gamma\delta$ T cells showed significant junctional diversity. These data suggested selection of the $\gamma\delta$ T cells by antigens such as CMV infection. Indeed, 4 out of 5 δ T cell clones that could be derived from this patient secreted TNF α in response to CMV infected allogeneic fibroblasts.

Conclusion: Overall, studies of human $\gamma\delta$ T cells under the conditions of a limited immune system imply two non-exclusive explanations for the $\gamma\delta$ T cell predominance in immunodeficiencies: a) a developmental advantage of $\gamma\delta$ T cells, possibly by a less stringent T cell development than for $\alpha\beta$ T cells and b) a proliferative response caused by infectious or autoantigen-driven peripheral stimulations, such as CMV infections.

S9

Induction of distinct gene expression patterns in lymphoid and epithelial cells by the BARF-1 gene of Epstein-Barr virus

A Zur Hausen¹, T Wiech¹, E Nikolopoulos¹, S Lassmann¹, T Heidt¹, M Sarbia², A Walch², M Werner¹ and T Ooka³

¹Institut für Pathologie, Universitätsklinikum Freiburg, Germany

²Technische Universität München, Institut für Pathologie, Germany

³Université Claude Bernard Lyon, Laboratoire de Virologie Moléculaire, Lyon, France

Diagnostic Pathology 2007, 2(Suppl 1):S9

Background: The expression of the BARF1 gene of Epstein-Barr virus (EBV) in latent EBV infection is restricted to epithelial malignancies, e.g. gastric carcinomas (GC) or nasopharyngeal carcinomas (NPC). In addition, BARF1 is considered to be a lytic gene because it is expressed upon induction of the lytic cycle in Burkitt's lymphoma cell lines.

Aims: To analyze the gene expression patterns of a BARF1-transfected epithelial and lymphoid cell line in order to identify cellular genes regulated by BARF1.

Methods: Gene expression of a BARF1-transfected lymphoblastoid (Louckes+) and a BARF1-transfected epithelial (HaCaT+) cell line were compared by cDNA microarray analysis using Affymetrix UI33A chips. Of each group, 6 genes were confirmed by Realtime PCR. In addition, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) was performed on a tissue microarray (TMA) of 181 gastric carcinomas for selected genes, including 11 EBV-associated gastric carcinomas (6.1%) as tested by EBER-RNA in situ hybridization.

Results: The Louckes+ cells revealed 730 genes downregulated and 545 genes were upregulated, whereas the number of upregulated genes in epithelial HaCaT+ was 10-fold lower and the number of downregulated genes comparable with Louckes+.

Among others, cyclin D1 expression was significantly upregulated in HaCaT+ by BARF1, but not in Louckes+. Analyzing cyclin D1 expression on a TMA of 181 gastric carcinomas revealed a significant overexpression of cyclin D1 in EBV-associated gastric carcinomas ($p < 0.012$) but not in EBV-negative gastric carcinomas as tested by IHC. Cyclin D1 FISH showed that cyclin D1 overexpression was not due to gene amplification.

Conclusion: Expression of BARF1 induces the expression of distinct sets of cellular genes in epithelial and lymphoblastoid cells underlining the restricted BARF1 expression patterns in latent epithelial EBV infection and lytic EBV replication in lymphoblastoid cell lines. In addition, the overexpression of cyclin D1 in EBV-associated gastric carcinomas is induced by BARF1 and not due to cyclin D1 gene amplification. Next to cyclin D1, other genes have been identified in this study by cDNA microarray analysis which might help to understand the role of EBV in gastric carcinogenesis.

S10

Intrahepatic cholangiocellular carcinoma associated with von Meyenburg complexes: case report and review of literature

A Zimpfer¹, B Nebel², L Terracciano³, S Stadelmann³ and H Koch¹

¹Gemeinschaftspraxis für Pathologie Prof. Koch, Dr. Hellerich, Dr. Venske, Freiburg, Germany

²Kreis Krankenhaus Emmendingen, Abteilung Anästhesie und Intensivmedizin, Emmendingen, Germany

³Institut für Pathologie, Universität Basel, Switzerland

Diagnostic Pathology 2007, 2(Suppl 1):S10

Introduction: Intrahepatic cholangiocarcinoma (ICC) arising in bile duct hamartoma (BDH), also referred to as "von Meyenburg complexes", is very rarely seen. Here, we report a case of an ICC in direct association with BDH. The case is discussed and the literature reviewed.

Case report: A 73-year-old obese male patient (BMI 46 kg/m²) was admitted to hospital due to cardiac failure and septicaemia. Tracheotomy and assisted artificial respiration were performed due to cardiorespiratory failure. A subacute myocardial infarction was diagnosed and chronic pneumonia suspected. Additionally, on ultrasonography, a hepatic mass was revealed. After death, an autopsy was performed and approved the clinical diagnosis. The liver displayed a nodular surface and signs of chronic congestion. There was a scirrhous, gray-white tumor of the right liver lobe (4 × 4 cm). Macroscopically, a primary tumor of the colon and pancreas was excluded. On histology, surprisingly, multiple dilated bile duct, some containing bile plugs, were found. The tumor itself showed small glandular units lined by cuboidal cells with marked atypia. The neoplastic glands were embedded in a dense desmoplastic stroma and invaded the liver parenchyma. Twice a direct transition of normal cuboid bile duct epithelium of the BDH in a neoplastic tubular epithelium was seen. The tumor displayed immunoreactivity for CK7.

Conclusion: Occasionally, ICC was reported in association with multiple BDHs. In this case a clear transition of non-neoplastic bile duct epithelium to the neoplastic invasive glands could be demonstrated. Also, this rare case underlined the importance of autopsy, as the presence of multiple BDHs in this case, as well as the tumor entity, was clinically not known.

S11**Pigmented neuroendocrine carcinoma of the hepatic duct: a case report**

U Schneider¹, V Ihle², B Kohler³, D Meier¹ and H Frenzel¹
¹Institut für Pathologie, Städt. Klinikum Karlsruhe, Germany
²Chirurgische Klinik, Fürst-Stirum-Klinik Bruchsal, Germany
³Medizinische Klinik, Fürst-Stirum-Klinik Bruchsal, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S11

Background: Neuroendocrine tumors of the gallbladder or the extrahepatic bile ducts are rare. We describe a pigmented neuroendocrine carcinoma of the common hepatic duct with a regional lymphnode metastasis. We reviewed the literature for these tumors and discuss the nature of the pigment.

Methods: The clinical and macroscopic appearance was documented together with a preoperative cytology. Paraffin-embedded tissue was used for histological, histochemical and immunohistochemical analysis. An electron microscopy analysis was performed.

Results: A pigmented tumor measuring 2.9 cm was located in the common hepatic bile duct with lumen stenosis reaching to the bifurcation of the hepatic ducts. The preoperative cytology showed atypical and single pleomorphic tumor cells with pigment granules and an immunocytological positive reaction for cytokeratin. The histological examination revealed an unencapsulated neoplasm infiltrating the biliary wall including the mucosa. The neoplastic cells were organized in solid, trabecular structures or cords in a condensed stroma. They were argyrophil in the Grimelius and non-argentaffin in the Fontana-Masson silver impregnation, while the pigment granules were argentaffin. The tumor cells exhibited a diffuse strong staining for chromogranin A, neuron-specific-enolase, a lesser staining for synaptophysin and neurofilament, and a weak nuclear reaction for S-100 protein. Cytokeratin was positive and HMB45 completely negative. No S-100 positive sustentacular cells could be found. The proliferation-index Ki-67 (MIB1) was low (2%). The electron microscopy revealed electron-dense neuroendocrine granules.

Conclusion: Neuroendocrine tumors of the extrahepatic bile ducts represent only 0.1 to 0.2 percent of all neuroendocrine tumors of the gastrointestinal tract. In the English literature, nearly 40 cases are reported. Pigmented neuroendocrine tumors reported previously are mainly broncho-pulmonary carcinoids. In our review of the literature, a pigmented neuroendocrine tumor was not described for the extrahepatic biliary ducts before. The pigment in these tumors is considered as melanin pigment, lipofuscin or neuromelanin. In some cases, the nature of the pigment could not be designated. Pigmented neuroendocrine tumors are an important differential diagnosis in pigmented tumors because the black pigment suggests the diagnosis of metastatic melanoma. In the present case, the preoperative documented immunocytological positivity for cytokeratin prevented the diagnosis of metastatic melanoma in the frozen section.

S12**Cap polyposis: a rare colorectal disease**

A Spiethoff¹, J Hoch², R Jakobs² and MH Bohrer¹
¹Institut für Pathologie, Klinikum Ludwigshafen, Germany
²Medizinische Klinik C, Klinikum Ludwigshafen, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S12

Aims: Cap polyposis is a rare and benign colorectal disease first described in 1985. It is characterized by multiple

inflammatory polyps with a cap of granulation tissue near to the surface. We report the case of a 71-year-old man with cap polyposis who required surgical intervention. A review of literature is given.

Methods: Our patient suffered from severe bloody diarrhoea. Endoscopy revealed multiple sessile polypoid lesions of the mucosa in the rectum and the sigmoid colon. Anorectal manometry did not show any evidence of mucosal prolapse.

Results: Initially, in several endoscopic sessions a complete polypectomy was intended. Since the disease was only temporarily responsive to antiproliferatives and abdominal symptoms worsened, a left hemicolectomy including part of the rectum had to be performed. Examination of the resected gut and of biopsies showed typical histological features of cap polyposis: sessile polyps with elongated crypts and a granulation tissue 'cap' near to the surface of the partly ulcerated mucosa. The epithelium of the crypts had saw-tooth configurations like in hyperplastic polyps without evidence of adenomatous changes. In the lamina propria signs of fibromuscular obliteration were found: moderate hyperplasia of smooth muscle fibers and mild fibrosis. Between the polyps the mucosa was normal.

Conclusion: Pathogenesis of cap polyposis which affects both genders of a wide range of age remains still unclear. In many patients abnormal colonic motility and straining at defecation seems to be responsible for shearing stress and prolapse of the mucosa resulting in the development of polyps with fibromuscular obliteration of the mucosa, which can be found in other gastrointestinal conditions of prolapsing mucosa as well. Infection has also been discussed as a possible cause. Until now no infective organisms have been detected in the affected gut but there are four reported cases of cap polyposis that were cured by eradication therapy for *Helicobacter pylori* found in the stomach.

S13**Life-threatening endocrinologic paraneoplasia in an 83-year-old patient with solitary fibrous tumor of the pleura: a case report**

U Oehler

Institut für Pathologie, Hegau-Bodensee-Hochrheinkliniken, 78207 Singen, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S13

Case report: An 83-year-old male patient with progressive alterations in behavior and aggressiveness was suspected of psychiatric disease. Loss of conscience led to hospital admission. The blood glucose was reduced to 11 g/100 ml and insulinoma could be clinically excluded.

5 months before, the patient had refused to be operated on for a large (20 cm) pleural tumor. Punch biopsy had revealed a solitary fibrous tumor. The hypoglycemia was regarded to be paraneoplastic due to IGF-II production, which is known to occur in about 5% of patients with this disease. As the condition got rapidly worse (the patient had to be wakened every hour at night due to hypoglycaemia), the patient finally agreed to surgery. During the operation, the blood glucose level rose to 200 mg/100 ml, and the patient is well since. The clinically problematic hypoglycemia, in this case, appears to be a matter of size of the tumor which had clinically grown during the past 5 months, whereas the lesion had been incidentally detected on

a chest x-ray 37 years before in 1969, when the patient had also refused surgery.

The pathologist should be aware of this although rare complications in an otherwise non malignant tumor and inform the clinician.

S14

On the pathology of malignant pulmonary tumors after percutaneous radiofrequency ablation

S Kröber¹, S Clasen², A Böhmches³, P Pereira², H Aebert³ and B Bültmann¹

¹Institut für Pathologie, Universitätsklinikum Tübingen, Germany

²Radiologische Klinik, Universitätsklinikum Tübingen, Germany

³Klinik für Herz- Thorax- und Gefäßchirurgie, Universitätsklinikum Tübingen, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S14

Aims: Systematic study regarding pathological findings and correlation of the effectiveness of radiofrequency (RF) ablation-treated pulmonary tumors from different primary sites.

Methods: RF ablations of ten pulmonary malignancies in eight patients were performed under computed tomography (CT) guidance. The primary tumors were adenocarcinoma of the lung, rectal and colonic carcinoma, sarcoma, melanoma and adrenocortical carcinoma. Three days after RF ablation, surgical resection was performed followed by pathologic examination. Specimens were evaluated macroscopically and histologically by hematoxylin and eosin (HE) staining, immunohistochemically for cleaved caspase 3 expression, and terminal deoxy-nucleotidyl transferase-mediated nick end-labeling (TUNEL). In addition, electron microscopic investigations were performed. The pathological extent of coagulation was correlated to the helical CT immediately after the ablation procedure.

Results: Pulmonary CT-guided RF ablations were technical successful in all cases. Histology revealed a preserved tissue architecture outside the coagulation zone. A zone of interstitial hemorrhage occurred at the outer boundary of the central zone of coagulation. Some specimens showed tumorous lymphatic spreading outside the RFA zone. On high-power view, despite an irregular homogenization of chromatin, tumor cells showed signs of thermal fixation and characteristics of vital cells especially with mitotic figures. DNA of tumor tissue and the adjacent lung tissue was characterized by double-strand fragmentation as determined by TUNEL. Ultrastructurally apoptotic bodies were found, indicating apoptotic cells. Immunohistochemistry for active caspase-3 gave no conclusive results. Due to the DNA fragmentation and the results from electron microscopy, the tumor tissue was supposed to be completely ablated in nine cases. The extent and shape of coagulation correlated to so-called ground-glass opacities on postinterventional CT.

Conclusion: In this first systematic study of RF ablation of human pulmonary malignancies, RF proved as a locally effective treatment verified by histology, electron microscopy, immunohistochemistry and molecular studies. However, classic criteria for tissue necrosis were not fulfilled by standard histological staining (HE) especially showing preserved tumor tissue architecture and microscopic cellular details indicating greyzone of tumor regression between apoptosis and necrosis.

S15

EGFR-gene analysis on cytological specimens of non-small-cell lung cancers

S Savic, B Grilli, A Ruffe, M Bihl, A Barascud, M Herzog, C Tapia, L Terracciano, G Feichter and L Bubendorf
Institute for Pathology, University Hospital Basel, Switzerland

Diagnostic Pathology 2007, 2(Suppl 1):S15

Aims: The diagnosis of lung cancer is often based on cytology alone. The relative paucity of tumor cells in these specimens is a challenge for the analysis of epidermal growth factor receptor (EGFR) gene mutation and EGFR gene copy number to select for treatment with EGFR-tyrosine kinase inhibitors. Here, we tested whether such EGFR gene analyses are feasible on cytological specimens of non-small-cell lung cancers (NSCLC).

Methods: We analyzed 87 Papanicolaou stained cytological specimens from patients with NSCLCs (51 adenocarcinomas, 27 not further defined NSCLCs, 8 squamous cell carcinomas and one neuro-endocrine carcinoma). The carcinoma cells were selectively dissected from the cytological specimens under visual control using laser microdissection in combination with a laser pressure catapulting system (PALM®). We sequenced the exons 18–21 of the EGFR gene. EGFR gene copy number was evaluated by fluorescence in situ hybridization (FISH) under visual control using relocation software. A FISH positive result was defined according to the criteria defined by F. Cappuzzo *et al.* on biopsies of NSCLCs [1]. FISH results of cytological specimens were compared with the FISH results on corresponding biopsies.

Results: DNA sequencing was successful in 79 of the 87 specimens (91%). Three adenocarcinomas showed EGFR-gene mutations (3.8%). 44 of 65 cancers (68%) were FISH positive on the cytological specimens as compared with only 2 of 9 biopsies (24%).

Conclusion: EGFR gene sequencing and FISH are well applicable to cytological specimens from lung cancers in diagnostic routine using laser microdissection and automated relocation. The high FISH positive rate of 68% suggests that the criteria for a FISH positive result suggested by Cappuzzo *et al.* need to be adjusted for cytological specimens.

Reference

1. Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, Haney J, Witta S, Danenberg K and Domenichini I, *et al*: **Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer.** *J Natl Cancer Inst* 2005, **97**:643–655.

S16

Primary sebaceous adenoma of the salivary glands – a rare differential diagnosis: report of a case

C Bersch and W Back

Pathologisches Institut, Universitätsklinikum Mannheim, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S16

We report a case of an unusual tumour of the salivary glands. The sebaceous adenoma of the submandibular gland is a very rare benign neoplasm sometimes also localized in the parotid gland. More men than women are affected. The origin of the sebaceous differentiation in salivary glands is questionable.

Typically sebaceous tumors of the salivary glands occur in a fibrotic stroma. Sebaceous adenomas and sebaceous lymphadenomas are benign tumors that will not recur if adequately excised. Sebaceous carcinoma and sebaceous lymphadenocarcinoma are low malignant tumors that have the ability to recur locally. Linkage for instance to the Muir-Torre syndrome is not evident.

S17

Synovial sarcoma of the left tonsil in a 31-year-old patient: report of a rare case

U Vogel, M Wehrmann and B Bültmann
Institut für Pathologie, Universität Tübingen, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S17

Aims: Synovial sarcoma (SS) is a mesenchymal spindle cell tumor with variable epithelial differentiation and a specific chromosomal translocation t(X;18)(p11;q11). Despite the name, SS is unrelated to synovium and may occur at any site of the body, mostly in the deep soft tissue of extremities. Around 5% arise in the head and neck region. Although the WHO textbook already describes the tonsils as an unusual site for the occurrence of SS, only two case reports could be found in the literature. Because of this rarity, we dare to present the following case.

Case report: A hitherto healthy 31-year-old male Turkish patient was admitted to hospital due to a left-sided sore throat accompanied by increasing dysphagia, which developed within 3–4 months. Preoperative computer tomography disclosed a 4.2 × 2.6 × 2.3 cm encapsulated tumor in the left tonsillar region expanding to the hypopharynx and the epiglottis.

Methods: Intraoperatively performed quick frozen sections detected an encapsulated malignant “small, round and blue” tumor, which was removed completely. Histologically, a malignant undifferentiated spindle-cell shaped component prevailed that stained immunohistochemically for CD99, bcl2, CD10 and calponin. At multiple sites, an additional pancytokeratin (AE1/AE3)- and EMA-positive epithelial differentiation of the tumor was detected, partly with glandular differentiation. Nuclear ki-67 expression was present in about 50% of the spindle cells and in about 15% of the epithelial compartment. The presence of the SYT(SS18) rearrangement indicative for the t(X;18) translocation and characteristic for synovial sarcoma was demonstrated by fluorescence in situ hybridization.

Results: Based on the histological, immunohistological and molecular pathological findings, the tumor was classified as biphasic SS with poorly differentiated areas, qualifying for grade III.

Conclusion: The diagnosis of SS should be kept in mind even in such unusual sites as the tonsils.

S18

Late effects of radiation on central nervous system

I Sinicina¹, K Bise² and H Pankratz¹
¹Institut für Rechtsmedizin, LMU, München, Germany
²Zentrum für Neuropathologie und Prionforschung, LMU, München, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S18

Aims: Primary germ cell tumors of the nervous system account for only 1% of all brain tumors. Traditionally, the treatment of intracranial germinomas has been craniospinal irradiation, which led to a long-term survival of more than 90%. The doses applied have had a wide range, with the mean dose of

approximately 36 Gy. The potential late irradiation effects include growth retardation, endocrine deficits and second malignancies. The longest reported interval for late delayed irradiation reactions was 7 1/2 years.

Methods: A 14-year-old boy presented in a pediatric clinic in 1983 with a beginning bitemporal hemianopsia. A tumour in the suprasellar region was diagnosed and classified after an incomplete resection as a germinoma. The remaining tumor mass was irradiated. After an uneventful period of 3 years, the tumour dissemination along the liquor way was suspected. The radiation encompassed the entire spinal axis and cranium and resulted in a long-term remission with a good quality of life for 17 years. In 2004, a hyperdense structure protruding in the fourth ventricle was seen in MR scans. Three months later, the patient was found dead in his bed.

Results: On post mortem examination there were no pathologic findings on the inner organs. Intoxication was ruled out. Within the dorsal part of the brain stem an exuberant formation of a collagen rich scar, which corresponded in a position to the structure seen in MR scans, was found. A marked microglia activation was evident in CR 3/34 immunostaining.

Conclusion: For the first time active persistent changes in the irradiated brain areas were described 17 years after the last radiation therapy of germinoma.

S19

Are there different vascular pathologies in non-hereditary neuropathies of axonal type?

K Mueller¹, A Nagel² and B Volk¹
¹Abt. Neuropathologie, Universität Freiburg, Germany
²Pathologisches Institut, Städtisches Klinikum Karlsruhe, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S19

Aims: This study refers to the problem of the etiological heterogeneity of primary axonal neuropathies, which appear in a relatively monotonous morphology. Investigations of the vascular parenchyma of peripheral nerve biopsies were carried out to determine possible specific vascular pathomorphological features in context of each patient's medical history.

Methods: Sural nerve biopsies of the last 3 years in our institute were studied (n = 76). Cases with primary axonal neuropathic changes were investigated with regard to the vascular pathology of epi- and endoneural vessels. Conventional histology, immunohistochemistry and electron microscopy were performed and a semiquantitative analysis of number, distribution and thickness of vessels and cellular proliferation is given.

Results: In our examination of the nerve parenchymal vessels, four groups with common patterns of vasopathic lesions could be subdivided: 1) primary endoneural microangiopathic type; 2) primary endo- and epineural angiopathic type with involvement of small arteries and heterogenous distribution of axonal atrophy; 3) subtype with fibromuscular dysplastic-like features; 4) secondary concentric stenosing type of endo- and epineural vessels after inflammatory or demyelinating diseases.

Conclusion: In view of the uniform morphological appearance of the peripheral nervous parenchyma in cases of axonal neuropathy the precise examination of the vasculature can help in elucidating the etiopathogenesis of the underlying disease. To achieve the best clinicopathological correlation full access to all clinical data is an urgent condition.

S20**Hypertrophic obstructive cardiomyopathy of an infant with neuroblastoma: a case report**H Kendziorra¹, M Kumpf², S Mackensen-Haen¹ and B Bültmann¹¹Institute of Pathology, University of Tuebingen, Germany²Department of Pediatric Cardiology, University of Tuebingen, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S20

The prognosis of neuroblastoma during infancy is not really bad. Prognostic relevant factors are the extent of metastatic sites and the existence of an amplification of the n-myc gene.

In the case of a male infant of only four weeks old, a neuroblastoma of 6 cm in diameter was found in the right adrenal gland (degree 2–3). The fate of this infant, however, depended on a massive hypertrophic obstructive cardiomyopathy, which was diagnosed on his second day of life.

Therefore, a resection of muscles of the right ventricular outflow was required. Nevertheless, the child died due to progressive heart failure.

At the autopsy, not only metastasis in both kidneys, in his bone marrow, in his liver and angiosis neuroblastomatosis in his lungs were found, but also an extended string of metastasis in his heart, precisely in his right ventricle and his septum. Close to it, there were necroses of different ages and calcification of the myocardium. There was no amplification of the n-myc gene (Ogahospital Stuttgart).

By this case, we want to show the possibility of a complication of a neuroblastoma caused by the development of a secondary hypertrophic obstructive cardiomyopathy.

The reasons for this cardiomyopathy are more likely to be seen by the existence of extended metastasis in the heart with the local production and secretion of catecholamine, together with the systematic effect of the catecholamine. Postpartal the angiosis neuroblastomatosis of the lungs could be additionally important.

S21**Cardiac fibroma: a case report**S Gross¹, C Marko¹, L Tietze¹, IC Ennker² and J Ennker²¹Institut für Pathologie, Lahr, Germany²Herzzentrum Lahr/Baden, Lahr, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S21

We report a case of a 65 year-old woman with a cardiac fibroma. Dyspnea and bend in efficiency lead to clinical examination. Ultrasound revealed a large tumor-like lesion associated with the left ventricular wall. Grossly, the resected tumor appeared greyish-white, solid and firm.

Microscopically, the tumor was composed of mainly acellular, partly hyalinized collagen with few embedded small spindle cells. The case will be presented with regard to the differential diagnosis and histogenetic aspects.

S22**Prenatal lesions of the myocardium in patients with congenital single-ventricle heart disease**S Mackensen-Haen¹, H Kendziorra¹, M Kumpf² and B Bültmann¹¹Institut für Pathologie der Universität Tübingen, Germany²Abteilung für Kinderkardiologie der Universitäts-Kinderklinik Tübingen, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S22

Today congenital heart disease with functionally only one ventricle can be remediated successfully by surgery.

We report two cases with univentricular heart. One showed a hypoplastic left ventricle (HLHS), atresia of the aortic valve and restriction of the foramen ovale (case I). The other showed a hypoplastic right ventricle (HRHS), atresia of the pulmonary valve, an intact ventricular septum and a large fistula between the left anterior descending artery and the right ventricular outflow tract (case II).

In case I, a modified Norwood-I operation (Blalock-Taussig anastomosis, reconstruction of the aortic arch and atrioseptectomy) was performed at the age of 4 days. This child died unexpectedly at the age of 9 days, presumably due to a thrombosis of the anastomosis.

Also in case II, a Blalock-Taussig anastomosis was constructed. The coronary fistula was thereby disconnected temporarily. This prematurely born twin died in tabula at the age of 15 days.

Using light-optical microscopy, the myocardium of both children showed severe lesions, in the case of HLHS: on the right, in the case of HRHS: on the left side. Histologically, signs of necrosis of different ages, partly presenting scars and calcification, could be observed. This necrosis had to be older than the time since delivery.

No cardiotropic virus could be detected in samples obtained from myocardium using nested-PCR on paraffin-embedded tissue.

We conclude the already intrauterine myocardial load and hypoxaemia lead to irreversible damage of the myocardium, thus limiting the prognosis of these children.

S23**Multiorgan infestation with macrophages with PAS-negative material inclusions in long-term hemodialysis: a case report**SM Shah¹, R Heyny-von Haußen¹, I Berger² and G Mall¹¹Pathologisches Institut des Klinikum Darmstadt, Germany²Pathologisches Institut der Universität Heidelberg, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S23

We report on a case of an 82-year-old female patient with long-term hemodialysis. Causes of hospitalization were a severely impaired general condition, somnolence and progredient dyspnea due to urosepsis with cardiac decompensation. No clinical improvement was obtained by treatment with antibiotics. Moreover, aggravation of neurological symptoms like seizure and progredient somnolence appeared. Cerebral hemorrhage or other lesions could be excluded by computer tomography. Due to persisting ill condition, hemodialysis was discontinued. Death occurred three days after admission in consequence of a septic toxic multiple organ involvement. Autopsy findings revealed severe chronic obstructive emphysema, liver cirrhosis, severe atherosclerosis of aorta and large arteries as well as ischemic cardiomyopathy and terminal stage kidneys. The general clinical and pathoanatomical constellation mainly suggests that a status infectiosus with cardiac/multiple organ failure constituted the cause of death. The most striking microscopic findings were multi-systemic, non-neoplastic infiltrates of CD68-positive macrophages with PAS-negative material inclusions in liver, spleen, heart, kidneys, bone marrow and generalized within fatty tissue. Fibrosis, necrosis and epitheloid cell reaction were not

found. Molecular analysis with 16S-rRNA-broad-range-bacteria-PCR and subsequent cycle sequencing showed amplification of *Proteus vulgaris* DNA in the left kidney, which most probably was the sepsis-causing agent. No bacterial DNA was found in the other organs infiltrated with macrophages. The potential etiopathogenesis of the observed infiltration with a grotesque amount of PAS-negative material and the nature of particles will be discussed.

S24

An unusual case of intracerebral Non-Langerhans cell histiocytosis with review of the literature

K Mueller¹, M Trippel², A Berlis³, W Janzarik⁴ and B Volk¹

¹Abt. Neuropathologie, Universität Freiburg, Germany

²Abt. Stereotaktische Neurochirurgie, Universität Freiburg, Germany

³Abt. Neurochirurgie, Sektion Neuroradiologie, Universität Freiburg, Germany

⁴Abt. Neurologie, Universität Freiburg, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S24

Aims: In the face of an intracerebral case of Non-Langerhans cell histiocytosis (NLCH), the diagnostic procedure and the clinical handling of this rare histiocytic tumorous lesion are demonstrated and differential diagnoses as Rosai-Dorfman or Erdheim Chester diseases are discussed.

Methods: A stereotactic serial biopsy of frontal brain was taken from a patient with unclear contrast enhancing masses around the sellar region, the hypothalamus, and a wide lining intraventricular. Smear preparations, conventional histology, immunohistochemistry and electron microscopy were done to rule out the etiology of the tumorous lesion.

Results: The morphological analysis of our case revealed a histiocytic proliferation, which, in view of the immunohistochemical and ultrastructural findings, was classified as Non-Langerhans cell histiocytosis. No infectious agents could be detected and no lymphoma-like features could be observed.

Conclusion: This case shows the unusual manifestation of a primary intracerebral Non-Langerhans cell histiocytosis, which begins as a solid mass around the sellar region and shows a

remarkable extension as a flat lining of inner and outer liquor spaces. Stereotactic brain biopsy technique today is a valuable high tech tool to clarify intracerebral tumorous lesions with a minimum of risk for the patient and with the possibility to perform all necessary tissue investigations.

S25

Counterattack – a principle of tumour cell metastasation?

N Freudenberg¹, S Göppinger², A Gold¹, C Galanos³ and MA Feudenberg³

¹Institut für Pathologie der Universität Freiburg, Germany

²Institut für Pathologie der Universität Heidelberg, Germany

³Max-Planck-Institut für Immunbiologie Freiburg, Germany

Diagnostic Pathology 2007, 2(Suppl 1):S25

Aims: The aim of the present study was to investigate whether tumour cell supernatants of 4 different malignant tumour cell lines with different aggressive behaviour show comparable pathobiological reactions.

Methods: Mature mouse (C57/Bl6) macrophages were incubated with the a.m. different tumour cell supernatants and followed by the investigation of apoptosis factors using immunocytochemistry. In addition, the experiments were performed with Fas-knock-out mouse macrophages and PCR in order to investigate the involvement of the Fas/FasL system.

Results: All tumour cell supernatants investigated induced a significant increase of apoptotic activities in macrophages compared with control groups. Interestingly, the tumour cell supernatants showed differences in their intensities of apoptosis-inducing effects on the macrophages one to each other. The Fas/FasL system has been identified as one of the involved factors from tumours which induce apoptosis in macrophages.

Conclusion: Our observation of the induction of apoptosis in macrophages due to so far undefined tumour cell factors supports the counterattack hypothesis which supposes the active destruction of the tumoricidal cell system by neoplastic cells. For now, the counterattack is an exciting new way for research in tumour immunology and probably offers a therapeutic potential.