50% of cases. Proliferation index were low (<1% of nuclei) in 24% of cases, intermediate (>1% and <10% of nuclei) and high in 57% of cases.
Depending on expression of steroid receptors and HER2 receptor in all material, 4 groups of tumors were found (15 to 29 cases for one group). Those groups were subdivided according to mitotic index.
The highest precentage (80%) of high proliferative index cases were among steroid negative and HER2 positive cancers.
The highest precentage (31%) of low proliferative index cases were among steroid positive and HER2 positive cancers.

## P-566

**HER2/neu and angiogenic factors in breast carcinoma**

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INTRODUCTION: Solid tumours up to a size of 2 mm diameters are able to obtain the necessary oxygen and nutrient supplies needed for growth and survival by simple passive diffusion. However, every subsequent increase in tumour mass must be preceded by the proliferation of new capillary blood vessels. As a result autocrine stimulation of tumour cells becomes necessary and angiogenic factors like vascular endothelial growth factor (VEGF), platelet derived–endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/TP) and other angiogenic factors are released. The antibody Herceptin™ blocks to extracellular binding domain of the HER2 receptor. Therefore, the aim of the present study was to investigate the effect of Herceptin™ treatment on the regulation of vascular growth factors.
METHODS: Paraffin-embedded specimens from 160 invasive ductal and lobular breast cancer have been tested immunohistochemically for the presence of HER2, HER1/EGF-R, VEGF and PD-ECGF/TP. Human breast cancer cells containing 13 copies of HER2/neu gene (BT474) have been treated with Herceptin™ (50 µg/ml for 72 h). After blocking of HER2-receptor with Herceptin™ the expression of VEGF and PD-ECGF/TP was studied using immunofluorescence techniques.
RESULTS: In human breast cancer tissue VEGF-expression correlated with high grade invasive ductal carcinomas. HER2/neu status was not significantly correlated with VEGF-, PD-ECGF- and EGF-R-protein expression. However, preliminary results of the cell culture experiments indicate a down-regulation of VEGF after HerceptinTM treatment.
**CONCLUSIONS**: Tumour angiogenesis in invasive breast cancer regulated via HER2 pathway might be of potential and therapeutic value.

## P-567

**Value of cytokeratin 5/6 immunostaining using D5/16B4 antibody in the spectrum of proliferative epithelial lesions in situ of the breast. A comparative study with 34βE12**

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**Introduction:** Previous studies have shown the interest of basal-type cytokeratin (CK) immunoprofile to distinguish between ductal hyperplasia (DH) and the spectrum of atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). However, the practical value of D5/16B4 immunostaining versus 34βE12 has never been investigated.
**Methods:** Immunostaining of CK5/6 and CK1/5/10/14 was performed, using respec-tively D5/16B4 and 34βE12 antibodies on serial sections of 100 breast specimens. Lesions included DH (n=31), ADH (n=5), DCIS (n=54) and LCIS (n=10). The percentage of positive cells was evaluated using a four point score: 1+=0–25%; 2+=26–50%; 3+=51–75%; 4+=76–100%.
**Results:** Staining of most of the cells (3+ or 4+) was observed in all the 31 cases of DH using both antibodies. None of the ADH had 3+ or 4+ staining by D5/16B4, with a score 1+ in 4/5 cases. Scores with 34βE12 were 1+ in 3, 2+ in 1, 3+ in 1/5 cases. Using D5/16B4, none of the DCIS had a 3+ or 4+ score, with a 1+ score in 52/54 cases. Using 34βE12, 13/54 of the DCIS showed 3+ or 4+ labeled cells. All the 10 cases of LCIS showed D5/16B4 1+ positive cells whereas 34βE12 was scored 3+ in 2/10 cases and 4+ in 6/10 cases.
**Conclusion:** Anti-CK5/6 antibody D5/16B4 is useful to distinguish these immuno-profiled proliferative lesions in situ of the breast. This antibody appears more valuable in the differential diagnosis between DH and atypical- malignant lesions than the anti-CK1/5/10/14 antibody 34βE12.

## P-568

**Myoepithelial carcinoma of the breast arising in adenomyoepithelioma. A case report**

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**Introduction:** Adenomyoepithelioma (AME), and especially myoepithelial carcinoma (MC), the malignant variant of this tumor, is a rare breast neoplasm. This report comprises the pathological features of a breast MC arising in AME with an aggressive clinical course.
**Case report:** A 48-year old woman with a palpable mass in the right breast was admitted in November 1998. A month later following FNAB, surgical excision was performed. During the follow-up period of 17 months, until the patient's death, two local recurrences developed. Despite the aggressive chemotherapy, administrated after the first recurrence, subsequently bone, pulmonary and brain metastases appeared.
**Results:** The primary tumor was an ovoid, white-gray, encapsulated mass measuring 4×3.5×1.5 cm. Microscopically, this biphasic tumor was composed of rare tubules surrounded by interlacing bundles of spindle cells exhibiting mild atypia. It was initially classified as a benign phyllodes tumor. The immunohistochemical analyses performed after the second recurrence proved the myoepithelial origin of the neoplasm (S-100, cytokeratin and alpha-SMA positive cells), with a high mitotic index (11 MF/10 HPF), and an immunophenotype indicating aggressive biological potential (steroid receptor negative, 50% Ki-67 and 30% p53 positive cells). Therefore, it was reclassified as MC arising in spindle cell type of AME.
**Conclusion:** The histopathological diagnosis of AME could be very difficult, especially when a distinction from other spindle cell

breast neoplasms must be done. Immunohistochemistry is essential to confirm the diagnosis, having in mind the reported pathological characteristics and the immunohistochemical profile of recurrent and malignant tumors of this type.
Hepatopathology

## P-569

**Expression of MET-protein and of the EDB isoform of fibronectin in chronic HVC hepatitis.**

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*Introduction*: The c-MET proto-oncogene encodes a 190-kd transmembrane tyrosine kinase. This molecule is the receptor for the hepatocyte growth factor (HGF) which is a potent mitogen for hepatocytes in vivo as well in vitro. Serum levels of HGF vary in liver diseases, reflecting liver damage and dysfunction. Fibronectin is a major constituent of extracellular matrix and participates in adhesion processes and functions as substrate for cell migration, particularly during embryogenesis and wound healing. EDB is one of the cellular isoforms originated from alternative splicing patterns of the primary transcript of a single gene. EDB is absent in normal adult tissue, but is highly expressed in fetal and tumour tissue.
*Methods*: To clarify the potential clinical significance of c-met protein and EDB in HCV hepatitis, we have investigated their expression by immunohistochemistry in frozen sections of 38 liver biopsies, using DO-24 (Met protein) and BC-1 (ED-B) monoclonal antibody. The histology, activity and fibrosis were scored using the METAVIR system.
*Results*: The anti c-Met showed a typical membrane staining in all cases, and the signal was much stronger in biopsies with more elevated activity and/or fibrosis. The immunostaining for EDB was detected in sinusoids and in portal tracts. In these latter, EDB was correlated with the extent of fibrosis. In sinusoids, a positive correlation was noted between Met-protein staining of the hepatocytes and EDB expression in the basement membranes ($p<0,001$; R: 0,863).
*Conclusions*: Our findings suggest that expression of Met protein and EDB are upregulated during HCV-induced inflammation, and that EDB may represent an early marker of liver fibrosis.

## P-570

**Expression of schistosomiasis mansoni antigen could confirm the frequent coexitance of hepatic schistosomiasis with chronic hepatitis C. An immunohistochemical study including assessement of ICAM-1 and collagen IV among those cases in comparison to pure hepatic schistosomiasis**

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Diagnosis of hepatic schistosomiasis (HS) in liver needle biopsies is sometimes difficult without the accidental detection of a schistosomal periovular granuloma, and especially when associated with chronic viral hepatitis.The purpose of the present study is to verify or confirm the coexistence of hepatic schistosomiasis in some cases of chronic hepatitis C, in liver needle biopsies when suspected in pathological diagnosis.to be treated as mixed lesions of viral and parasitic pathogenesis and to evaluate the role of both; the soluble intercellular adhesion molecule (sICAM-1 as expressed in hepatic tissue in relation to its serological levels and the expressed interstitial collagen IV, in relation to the severity of the disease as correlated with the different grades of chronic inflammation and stages of fibrosis in the examined livers. The material for this study comprised 36 patients suffering from chronic liver disease, diagnosed and classified as 31 (86%) cases of chronic hepatitis C (CHCof which 21 (about 58% of total) were proved mixed hepatic lesions (MH); and 5 (about 14%) cases as pure hepatic schistosomiasis, beside 4 control cases. Serological markers for HCV were performed for all the patients as well as serological levels for ICAM-I and for the schistosoma mansoni antigen (SMA). Liver needle biosy specimens were obtained from all patients and processed for the histopathological assessement of the hepatic lesions including grading & staging of the disease according to Desmet et al. (1994). Unstained sections were immunohistochemically treated for the expression of (SMA), sICAM-1 and the interstitial collagen IV within the hepatic tissue . Expression of SMA was strongly positive in all cases diagnosed as pure HS and was significantly positive in 10 (47.6%) of the examined CHC cases confirming the coexistence of HS in 5 (about 24%) of them which was only suggested. SMA expression was negative in all cases of CHC; 11 (52.4%) .It was maily localized within sinusoidal cells (kupffer & endothelial cells), diffused within hepatocytic cytoplasm and in macrophages in portal areas. sICAM was more significantly strongly positive in cases of CHC of grades II and III inflammation and stages II & III fibrosis as well as in hepatic schistosomiasis, stages II & III portal fibrosis more than in cases with established cirrhosis or with advanced schistosomal fibrotic stage. sICAM was mainly detected within sinusoidal cells, hepaocytes, focal areas of hepatic necrosis and within mononuclear inflammatory cells. There was a high significant correlation ($p<0.001$) between serum levels of sICAM-1 and the grades of inflammation in the groups of CHC & MH without cirrhosis. Collagen IV expression was strongly positive at periphery of schistosomal granulomas whenever detected and increased progressively with the increased stage of the disease in all the examined groups slightly more increased with the advanced schistosomal infection,wether pure or mixed and mainly found located in stroma of portal areas and fibrous septae ,in walls of blood vessels and basement membranes of bile ductules and along perisinusoidal walls. We concluded that we can use the immunohistochemical detection of schistosomal antigen to confirm the diagnosis of hepatic shistosomiasis in needle liver biopsies whenever in question. sICAM -1 and collagen IV can be used for monitoring the disease activity.

## P-571

**Changes of hepatic sinusoidal wall structure in chronic viral hepatitis: An electron microscopic and immunohistochemical study**

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Change of the hepatic sinusoid is one of the principle manifestsations of chronic hepatitis. However, its structure in chronic viral