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1 TUMOR MARKERS IN LIVER TUMORS
R. Lamerz¹, P. Stieber²

¹Medizinische Klinik II, ²Inst. f. Klinische Chemie, Campus-Grosshadern, Klinikum der Ludwig-Maximilians-Universität, München, Germany

On suspicion of liver tumors by symptoms or altered laboratory values, the detection of one or more liver foci by ultrasound represents a serious initial situation. This requires multi-fold procedures to ascertain diagnosis and, in case of cancer, the differential diagnosis between primary liver cancer or secondary metastases. Among primary liver tumors, hepatocellular carcinoma (HCC) represents the most frequent tumor with typical high-risk prior diseases such as chronic liver diseases due to hepatitis B and C and chronic alcohol abuse. Among the recommended, e.g. semi-annual, follow-up procedures, imaging by steadily improving technology is increasingly superior to other procedures such as tumor markers. In an overview, the multiple investigated tumor markers in HCC, including the most relevant markers (AFP, AFP-L3 lectin subtype, DCP, glypican-3), are presented and described as adjuncts for HCC screening in high-risk patients, for prognosis, diagnosis, aftercare, detection of recurrence and efficient control of therapy response, according to recommendations by EASL, EGTM and NACB.

2 ARE TUMOR MARKERS USEFUL FOR PREDICTION OF SURVIVAL RATE AFTER EXPLORATIVE LAPAROTOMY FOR LIVER MALIGNANCIES?
V. Liska¹, L. Holubec Jr.², V. Treska¹, T. Skalicky¹, A. Sutnar¹, S. Kormunda³, O. Topolcan²

¹Department of Surgery, ²Department of Oncology, University Hospital Pilsen; ³Section of Medical Statistics, Medical Faculty Pilsen, Charles University Prague, Czech Republic

Objective: Tumor markers are used for prediction of relapse and the effects of postoperative or postoncological therapy as a standard of follow-up. The metastatic process of the liver and primary malignancies of the liver and gall bladder are very common diseases in Europe.

Materials and Methods: The log-rank test and Wilcoxon test were used for statistical evaluation. Survival analysis was computed by the Kaplan-Meier method. Serum levels of tumor markers conventionally used in routine clinical praxis (CA19-9, CEA, C724) were studied. Also taken into account were markers offering information about the proliferation activity of malignancies (TK, TPA, TPS). Cut-off values were obtained from previous studies (reference group included patients with clinical remission).

Results: One hundred and nine patients, who underwent explorative laparotomy without any surgical therapy between September 1999 and June 2005, were studied (16 patients with hepatocellular carcinoma, 5 with cholangiocellular carcinoma, 25 with gall bladder carcinoma, 51 with colorectal liver metastases, 6 with metastases of mammary gland carcinoma, 2 with metastases of melanoblastoma, 1 with metastases of stomach carcinoma and carcinoid, 2 patients with metastases of unknown origin). One hundred explorative laparotomies and 9 laparoscopic operations were performed. Twenty patients had undergone other liver operations before and belonged to the group planned for reoperation for relapse of disease.

Conclusion: The results of this pilot study suggest the importance of tumor markers for prediction of short survival rate. These markers could be used as a suitable supplement to classic clinical, laboratory and radiodiagnostic parameters. They seem to be very helpful for planning of palliative oncological therapy for patients with liver malignancies, that could not be treated by surgical therapy. The correlation between early recurrence and increased proliferative activity of liver malignancy in the preoperative period and an increased tendency of colorectal liver metastasis to earlier recurrence than non-colorectal liver metastasis was proved.

3 VALUE OF p53 AUTOANTIBODIES AND CA 242 IN PATIENTS WITH PANCREATIC CARCINOMA
Ch. Hansen, J. Raedle, H. Sauer-Eppel, G.M. Oremek
Zentrum Innere Medizin, Klinikum der Johann Wolfgang Goethe Universität, Frankfurt am Main, Germany

In human pancreatic carcinoma (PCa), mutations in the p53 tumour suppressor gene are present in up to 50% of cases. Conformational change and cellular accumulation, together with subsequent release of mutant and normal p53 protein from transformed cells, may initiate a B-cell response with the generation of circulating autoantibodies to p53 protein (anti-p53). The sera of 85 consecutive patients with acute pancreatitis (n=19), chronic pancreatitis (n=33) and PCa
(n=33) were analyzed to evaluate the specificity of autoantibodies to p53 protein (anti-p53) as a serological marker for PCa. Detection of anti-p53 was performed using an ELISA system with immobilized recombinant wild-type p53 protein. Autoantibodies to p53 were detectable in 1/19 patients with acute (5.3%) and in 4/33 patients with chronic pancreatitis (12.1%). All anti-p53-positive patients with acute or chronic pancreatitis were carefully examined and no underlying malignant disease was found.

During follow-up (range: 281 – 647 days; mean: 472 days) none of these patients showed any evidence of subsequent development of PCa or any other malignant disease. In patients with PCa, anti-p53 was detected in 8/33 cases resulting in a sensitivity of 24% with a specificity of 90%. In contrast to anti-p53, detection of serum carbohydrate antigen (CA 242) resulted in a sensitivity and specificity of 66% and 80% (CA 242<27 U/ml) and 80% and 90% (Ca 242>100 U/ml) for the detection of PCa, respectively.

Taken together, the sensitivity of anti-p53 formation was low in patients with PCa (24%). Furthermore, the detection of anti-p53 was not specific for malignancy, indicating that severe inflammatory processes may also induce anti-p53 formation.

4 FECAL PYRUVATE KINASE M2 (TUMOR-M2PK) MEASUREMENT: A NEW SCREENING CONCEPT FOR COLORECTAL CANCER (CRC) WITH PROMISING PERSPECTIVES


Third Medical Department, Giessen University Hospital, Germany

Colorectal cancer (CRC) is a disease with major impact on both public health and public health costs. Colonoscopy is currently supposed to be the best screening tool for CRC. However, acceptance in the population is very poor: although it has been included in screening programs in the German health system since 2002, only about 1.7% have been enrolled. Therefore, the evaluation of additional screening tools is of great interest. Recently, testing for fecal occult blood (FOBT), genetic alterations or alterations in tumor metabolism (e.g. tumor-M2PK) have been under investigation.

FOBT has been included in screening programs around the world and proved a certain benefit, however, the performance of this marker is rather poor. Testing for genetic alterations (K-ras, p53 etc.) is very interesting, however DNA isolation requires very fresh stool samples and, to date, the tests are limited in their clinical usefulness. The most promising results have been observed with Tumor-M2PK measurements. Concerning CRC, the overall sensitivity of M2PK was around 77.9% in several clinical studies. The overall sensitivity for adenomas was 45.9%, increasing to 61.1% for adenomas >1 cm. Specificity ranged from 74.3-83.3% in recent reports in patients undergoing colonoscopy. However, according to results obtained in a health care check up setting of more than 1000 persons, the specificity must be expected to be about 95%.

Conclusion: The measurement of Tumor-M2PK in stools seems to be the most promising tool for CRC screening at present. In combination with colonoscopy for positive test results, this marker can characterize patients at high-risk at reasonable cost. This test should, therefore, be recommended for screening programs.

5 DISEASE PROGRESSION AND TUMOR STAGE IN COLORECTAL CANCER CORRELATE WITH CEA AND EGFR GENE EXPRESSION IN DISSEMINATED TUMOR CELLS

V. Zieglschmid, C. Hollmann, W. Albert, O. Böcher

AdnaGen AG, Langenhagen, Germany

Disease progression and tumor stage in colorectal cancer patients was correlated with tumor-associated gene expression in disseminated tumor cells (DTC) to evaluate the clinical utility of DTC detection and the expression of tumor-associated genes with respect to individual patient prognosis and monitoring of therapy.

Materials and Methods: A DTC detection assay, consisting of immunomagnetic enrichment and expression analysis of the tumor-associated genes CEA, EGFR and GA733-2, was developed to analyze a total of 196 peripheral blood samples collected from 76 patients with Dukes’ A, B, C and D tumor stage in a multicenter study. Tumor-associated gene expression in metastatic patients was compared with the results in patients with Dukes’ A, B and C tumor stage assessed prior to surgery and post surgery. CEA mRNA expression was compared with CEA serum protein levels.

Results: Prior to surgery, EGFR and CEA expressions were assessed in 88% (p=0.001) and 0% (p=0.002), post surgery in 66% (p=0.001) and 20% (p=0.002), as well as in 15% (p<0.0001) and 66% (p<0.0001) of blood samples collected from metastatic patients in a comparison analysis, respectively. Prior to surgery and in follow-up samples, an expression of tumor-associated genes in DTC indicated an ongoing metastatic process. There was no correlation between DTC expressing CEA and elevation of CEA serum protein levels.
Conclusion: A statistically significant correlation between EGFR and CEA gene expression and disease progression and tumor stage was assessed. The detection of CEA expression in DTC might have a predictive value in colorectal cancer and may help to identify patients at a greater risk of relapse.

6 CLINICAL USE OF RISK FACTORS ASSESSMENT FOR THE PREVENTION AND PROGNOSIS OF COLORECTAL CANCER

S. Svobodova, M. Matoulek, L. Holubec Jr., O. Topolcan, S. Svacina, M. Martiník, Z. Rusavý, V. Visokai, L. Lipska

1st Medical School, 3rd Department of Medicine, Prague, Medical School, 2nd Department of Medicine Pilsen; Outpatient Internal Medicine Clinic, Hradec Králové; Charles University, 1st Medical School, University Thomayer’s Hospital, Department of Surgery, Prague, Czech Republic

Some GI cancers (especially colorectal cancer) and other malignancies have already been described in relation to the higher prevalence rate encountered alongside certain metabolic disorders (diabetes, obesity etc.). The aim of this multicenter prospective study was to identify the parameters of the potentially most threatened subgroup of patients with a metabolic syndrome presenting a higher risk of GI cancer development. This project was based on the experimental and literature data. The clinical impact of the existing risk factors on colorectal cancer incidence is discussed. The following parameters were discussed: metabolic factors, conventional tumor markers, angiogenic factors, adhesive molecules and proteases. The authors discuss the possibility of using risk factor assessment for colorectal cancer prevention and for prognosis estimation in the case of colorectal cancer development.

7 TUMOR MARKER CEA IN THE AFTER-CARE OF COLORECTAL CARCINOMA

R. Lamerz, P. Stieber

1Medizinische Klinik II, 2Inst. f. Klinische Chemie, Campus-Grosshadern, Klinikum der Ludwig-Maximilians-Universität, München, Germany

After-care following curative primary therapy in patients with colorectal tumors aims at the early detection of relapse in an asymptomatic state, thus making curative treatment still feasible. This claim and the necessary procedures are critically questioned today due to the economical restraints. This also has led to restricted recommendations of follow-up care by the DGVS. Yet, more recent randomized studies and meta-analyses have demonstrated the value of after-care for a significant survival advantage. Furthermore, the relevance of investigative procedures such as physical examination, laboratory investigation, imaging and endoscopic procedures have been more critically re-evaluated. In addition, the tumor biology (TNM), therapeutic results (R0 resection) and patients’ compliance, as well as the consideration of quality of life, have resulted in more individualized after-care. The most important results of studies and meta-analyses are reviewed with respect to the role of the tumor marker CEA as adjunct for the prognosis, after-care, detection of relapse and efficiency control of secondary therapy, including the actualized German guidelines (DGVS) in comparison with those of EGTM, NACB, ASCO and NCCN.

8 TUMOR MARKERS IN PATIENTS WITH RELAPSE OF COLORECTAL CARCINOMA

V. Visokai, L. Lipska, M. Levy, S. Kormunda

Department of Surgery, Thomayer’s Teaching Hospital, 1st Faculty of Medicine Charles University, Prague, Czech Republic

This was a large retrospective study to evaluate the value of tumor markers in the follow-up period of colorectal cancer.

Materials and Methods: A total of 1090 patients were operated for colorectal cancer in the years 1992-2004. Disease relapse was diagnosed in 122 cases (20%): 74 patients were indicated for re-operation. Second R0 resection was performed in 43 patients. Both CEA and CA19-9 were examined in 75 patients with disease relapse. The tumor markers were assessed using the AxSYM instrument (Abbott).

Results: Both markers were within the normal range in 31% of the patients with disease relapse at the time of the relapse diagnosis. Elevated levels of CEA were observed in 61% of the patients and elevated levels of CA 19-9 were observed in 33% of the patients. Comparing the preoperative levels of CEA with the levels at the time of disease relapse, elevated levels were found in 77% of the patients prior to primary surgery; the relapse was again accompanied with elevated levels of CEA and CA19-9 (64% cases). In the subgroup with normal pre-operative levels of CEA, 48% of the patients had a normal level of CEA at recurrence and 79% in case of CA19-9.
Conclusion: It is necessary to assess tumor markers of colorectal cancer prior to surgery and during the follow-up period of the disease. Based on these results, it can be concluded that the monitoring of the tumor markers is valuable, mainly in those cases where pre-operative values were elevated.

9  PROGNOSTIC SCORE COMBINING CLINICAL PARAMETERS AND SEROLOGICAL BIOMARKERS IN COLORECTAL CANCER


Institute of Clinical Chemistry, 1Department of Surgery and 3Department of Oncology, University Munich, Germany; 2Department of Clinical Chemistry, University of Helsinki, Finland

It was investigated whether tumor markers have additive prognostic value to tumor stage in 450 patients with non-metastatic colorectal cancer (136 UICC I, 175 UICC II, 139 UICC III). Preoperative serum levels of CEA and CA 19-9 had been assessed in a prospective way. In addition CA 242, CA 72-4, CYFRA 21-1, hCGβ, S100 and HGF were analyzed using retrospective samples. For evaluating disease-free survival (DFS) and overall tumor-related survival (OTS), patients were divided into two prognostic groups: the good prognosis group (GPG) consisting of patients with colon cancer stage I-II or rectal cancer stage I and the bad prognosis group (BPG) consisting of the remaining patients with an indication for adjuvant treatment. Multivariate Cox regression analysis was performed. Evaluating tumor marker linearity was tested first. In the case of non-linearity, a cut-off value was determined. Log (CEA) showed linearity in the BPG and GPG. CA 19-9 (cut-off 55 U/ml) and CA 242 (cut-off 45 ng/ml), HGF (cut-off 1900 pg/ml) were significant predictors in the BPG, whereas in the GPG only CEA, HGF and S100 were significant factors. No other tumor marker achieved significance in predicting disease-free survival. In multivariate analysis together with T4, N2 and site (rectal), CEA and CA 19-9 were independent predictors in the BPG, but also HGF and CA 242 or HGF and CA 19-9. In the GPG, T stage (T3 or T4) and CEA or S100 (cut-off 0.03 ng/ml) were significant predictors. From the models, prognostic scores could calculated and these led to considerable overlap of the two initial prognosis groups. Defining the indication for adjuvant therapy besides tumor stage and site, CEA and CA 19-9 (or CA 242), respectively, should be included in a prognostic score. The high prognostic relevance of S100 and HGF needs to be confirmed in large trials.

10  PROGNOSIS OF COLORECTAL CANCER

V. Visokai, L. Lipska, O. Topolcan, L. Holubec, M. Karlikova, M. Levy, S. Svobodova, S. Kormunda

Department of Surgery of Thomayer’s Teaching Hospital, 1st Faculty of Medicine Charles University, Prague, Czech Rep.

The aim of the study was to find the clinical value of cytokeratins, TK, adhesive molecules, cytokinines and angiogenic factors in colorectal cancer.

Materials and Methods: Serum levels of cytokeratins were assessed by IRMA methods (TPS: IDL Sweden, TPA: Biomedica, Italy, Cyfra 21-1: Schering, France). Simultaneously, other tumor markers were examined: CA 19-9 and CEA using chemiluminescence (Beckmann, USA). Serum levels of adhesive molecules, cytokinines and angiogenic factors were assessed by a multiplex analysis using assays kits (Linco, USA). Serum levels of the studied parameters were preoperatively examined in 44 patients with colorectal cancer. The results of the tumor markers were correlated with the surgical findings (pTNM, grading).

Results: Cytokeratins were significantly elevated in patients with clinical stage III and IV. These levels were in significant correlation with both CEA and CA 19-9 (<0.001). The correlation coefficients were low (r=0.4 – 0.7), proving the parameters independence. TK and adhesive molecules had low sensitivity and specificity. Mainly IL-6, IL-8 and VGEF correlated with colorectal cancer prognosis.

Conclusion: This pilot study demonstrates the possibility of improving the prognosis colorectal cancer based on the combination of traditional tumor markers and markers of the biological activity of the tumor. Verification of the pilot study in a multicenter clinical study seems to be justified.


C. Weissenberger1, M. Geißler2, F. Otto3, A. Barke1, K. Henne1, G. von Plehn1, A. Rein1, C. Müller, M. Henke1

1Department of Radiotherapy, University Hospital of Freiburg, 2Department of Internal Medicine II, Division of Gastroenterology, 3Department of Internal Medicine I, Division of Oncology, Germany

This retrospective study was conducted in order to evaluate the long-term outcome of standard 5-FU-based adjuvant or
neoadjuvant radiochemotherapy and to identify predictive factors. In particular, anemia before and after radiotherapy, as well as hemoglobin increase or decrease during radiotherapy, were analyzed.

Materials and Methods: Two hundred and eighty-six patients with UICC stage II and III rectal adenocarcinomas, who underwent resection by conventional surgical techniques (low anterior or abdominoperineal resection), had received either postoperative (n=233) or preoperative (n=53) radiochemotherapy from January 1989 until July 2002. Overall survival (OAS), cancer-specific survival (CSS), disease-free survival (DSF), local-relapse-free (LRS) and distant-relapse-free survival (DRS) were evaluated using Kaplan-Meier, log-rank test and Cox's proportional hazards as statistical methods. Multivariate analysis was used to identify prognostic factors. The median follow-up was 8 years.

Results: Anemia before radiochemotherapy was an independent prognostic factor for an improved DFS (risk ratio 0.76, p=0.04). The univariate analysis revealed that anemia was associated with impaired LRS (better local control), but improved DFS (fewer distant metastasis). In contrast, hemoglobin decrease during radiotherapy was an independent risk factor for DFS (risk ratio 1.97, p=0.04). Among the patients who were treated with postoperative radiochemotherapy, anemia was more frequently seen in R0-resected patients compared to R1- or R2-resected patients (60% versus 55.6%, not significant). However, during radiotherapy, only 30.8% of R0-resected patients suffered from hemoglobin decrease compared to 55.6% (p=0.04). Further, it could be proved that stage, grading, R status (free radial margins), type of surgery, CEA levels and gender were independent prognostic factors for survival or local recurrences. Five-year OAS, CSS, DSF, LRS and DRS were found to be 47.0%, 60.0%, 41.4%, 67.2% and 84.3%, respectively. There was no significant difference between preoperative and postoperative radiochemotherapy (OAS: 43.9% and 47.6%, respectively).

Conclusion: The predictive value of stage, grading, R status (free radial margins), type of surgery, CEA levels and gender were established. If there is any hemoglobin effect on the outcome of rectal cancer, it is completely biased by the extent of surgical intervention, a important predictor for outcome, as has been proved. Thus, anemia before radiochemotherapy or hemoglobin decrease during radiotherapy do not imply any predictive value for the outcome of rectal cancer. It should be considered as a consequence of age, stage, type of surgical resection, or performance status.

S. Langbein1, M. Zerilli2, A. zur Hausen3, P. Alken1, G. Stassi1, P. Schubert4, J.F. Coy4

1Department of Urology, University Hospital Mannheim, Germany; 2Department of Surgical and Oncological Sciences, University of Palermo, Italy; 3Institute of Pathology, University Hospital Freiburg; 4R-Biopharm AG, Darmstadt, Germany

Tumors ferment glucose to lactate even in the presence of oxygen (aerobic glycolysis; Warburg effect). The pentose phosphate pathway (PPP) allows glucose conversion to ribose for nucleic acid synthesis and glucose degradation to lactate. To assess the influence of the rate-limiting enzyme of the PPP, the expressions of transketolase-like 1 (TKTL1) and 2 were analyzed as well as transketolase-like 1 and 2 (TKTL1/TKTL2) at the mRNA level in invasive/non-invasive colon and urothelial carcinomas. Additionally, TKTL1 protein expression was investigated by IHC.

Materials and Methods: Fifty-five men/women (60±15 years) with colon adenocarcinoma underwent colectomy. Thirty % of colon adenocarcinomas were diagnosed as non-invasive, whereas 70% were invasive at the time of diagnosis. Of 64 patients (median age 67.5 years) with urothelial carcinoma, 59 underwent surgical treatment. Fourteen % of the patients had lymph node metastasis at the time of surgery. Twenty-eight tumors were classified as non-muscle-invasive (pTa, pT1 and carcinoma in situ) and 31 were classified as muscle-invasive (≥ pT2).

Results: Up-regulation of a mutated transketolase transcript (TKTL1) was detected in human malignancies, whereas TKT and TKTL2 transcripts were not up-regulated. Non-neoplastic colon, ovarian and urothelial tissue and non-invasive colon or pTa superficial urothelial carcinomas did not reveal TKTL1 expression, whereas 100% invasive colon and 97% of invasive urothelial and ovarian carcinomas showed mostly intense TKTL1 staining. Strong TKTL1 protein expression was correlated to invasive colon, urothelial and ovarian tumors and to poor patient outcome.

Conclusion: We propose that TKTL1 up-regulation in tumors leads to enhanced, oxygen-independent glucose usage and a lactate-based matrix degradation. Since inhibition of transketolase enzyme reactions suppress tumor growth and metastasis, TKTL1 could be the relevant target for novel anti-transketolase cancer therapies. We suggest an individualized cancer therapy based on the determination of metabolic changes in tumors that might enable the targeted inhibition of invasion and metastasis.

12 EXPRESSION OF TRANSKETOLASE TKTL1 PREDICTS COLON AND UROTHELIAL CANCER PATIENT SURVIVAL: WARBURG EFFECT INTERPRETED

13 NOVEL CONCEPTS IN SURGERY FOR CHRONIC PANCREATEITIS
Intractable abdominal pain is the most important indication for surgical intervention; complications related to adjacent organs, such as duodenal obstruction, common bile duct (CBD) stenosis, recurrent pseudocyst formation in conjunction with ductal pathology and otherwise not amenable internal pancreatic fistula represent further, undisputable indications for surgery. Duodenal and CBD obstruction require radical, "extended" excision of the pancreatic head. Whipple resection should be reserved for patients with irreversible duodenal stenosis, irrespective of an extrinsic inflammatory mass, or for those in whom the duodenum cannot be entirely freed from its surrounding scarring tissue without damaging its vascular supply. In patients with concomitant extrapancreatic portal hypertension (EPH), which may be confined to either the splenic or the superior mesenteric vein or involve the entire splenomesentericportal axis, the indication for surgery is debatable. In the author’s experience, no difference in morbidity and mortality between resectional (e.g., duodenum-preserving pancreatic head resection described by Beger, classic Whipple, PPPD) or draining procedures (longitudinal pancreaticojejunosotomy combined with a limited excision of the pancreatic head according to Frey) is found. What are the therapeutic options in patients with pain recurrence after surgery? Treatment failure is reported to account for 10% to 40%. These patients require accurate diagnostic work-up. Clinical experience shows that only in a paucity of patients is no morphological correlate of their complaints identifiable. In most instances, either an incomplete resection of an inflammatory cephalic pseudotumour or an insufficient drainage of a dilated duct system, or even both, are found. "Second-line" salvage procedures should address these individual findings. Surgical rescue options, therefore, mainly encompass: a) radical resection of a recurrent (or, more probably, residual) inflammatory tumour ("Redo"-DPPHR, Whipple procedure, PPPD); and b) consequent drainage of a dilated duct system, thereby bypassing possible ductal strictures localized in the pancreatic body or tail, respectively. As an ‘ultima ratio’, total pancreatectomy may be taken into consideration as the final therapeutic option for patients in which diagnostic imaging does not prove any pathology for the patient’s complaints. In any event, the approach to chronic pancreatitis with associated CVPT is multidisciplinary, tailoring the various therapeutic options to meet the individual patient’s needs.

**14 NOVEL ASPECTS OF LIVER SURGERY AND ABLATION TECHNIQUES**

X. Rogiers

Department of Hepatobiliary Surgery, University Hospital, Hamburg, Germany

Over the last 20 years, liver surgery has become a safe procedure in patients with a healthy liver. Accordingly, the indications for liver resection with curative intent for patients, especially those with colorectal metastases, have progressively been extended. It has been demonstrated that liver resections with resection of localised peritoneal or omental disease yield results close to those of primary resection. Lymph node disease, as long as the level of the coeliac axis is not involved, is also no longer considered a contraindication. Furthermore, a safety margin of 2 mm is now considered sufficient, with smaller resections preserving as many segments as possible. Manipulations of the segmental liver volumes by partial portal vein embolisation have become an important tool in terms of resectability. An important development has been the advent of novel, more active, chemotherapeutic agents. On the one hand, inoperable metastases can successfully be rendered operable, by neoadjuvant therapy. On the other hand, the hepatotoxicity of these drugs can pose a considerable risk factor for later surgery. The interplay between surgery and these new drugs needs to be redefined. Ablative techniques, although very successful for the treatment of small hepatocellular carcinoma, have proved disappointing for the treatment of colorectal metastases. However, they are increasingly being used in combination with resection in surgical strategies to conserve liver segments.
EXOCRINE PANCREATIC CANCER – CHEMOTHERAPY AND SUPPORTIVE TREATMENT

R. Klapdor
ZeTDT GmbH, Hamburg, Germany

During recent years, no more than 20-30 % of pancreatic cancer patients have been detected early enough to allow resective surgery. More than 90 % of these resected patients suffered from tumor recurrence. Consequently, more than 95 % of these patients died from tumor disease within 5 years. The overall survival for locally advanced and metastasized tumor disease after the start of palliative chemotherapy ranged from 5-8 months. However, therapeutic nihilism should not have a place in the clinical management of pancreatic cancer patients. Recently, prospective randomised trials demonstrated that adjuvant chemotherapy with 5FU/FA as well as with gemcitabine may significantly improve the clinical outcome of these patients. New studies, like CAPRI, are actually open for patients. Some cytostatics such as gemcitabine and 5FU/FA, or combinations with oxaliplatin or irinotecan, seem to prolong survival significantly in the case of sequential efficacy-orientated application. Our own experience over about 7 years suggests that survival significantly increases in relation to the number of effective treatment sequences. A sensitive follow-up by short-term determination of serum tumor markers (e.g. monthly) and modern imaging methods (e.g. bi-monthly) nowadays allows timely change to second- or third-line treatment in the case of further or new progression of a pancreatic cancer. Radiotherapy as well as locoregional application of cytostatics may be involved in treatment strategies. New targeted therapy concepts, based on the rapid progress in the field of molecular technologies, might offer additional possibilities in the near future. Active vaccination, using an aminoterminal gastrin-peptide sequence, seems to have improved the outcome of these patients in initial clinical trials. Active and competent tumor therapy, however, also requires close cooperation with surgeons specialized in resective surgery, with specialists in the local therapy of liver metastasis and with a competent endoscopic unit, for example for non-surgical treatment of stenoses of the biliary tract system, of the duodenum, or of a gastroenterostomy or the colon. In addition, experience in supportive treatment modalities is needed, such as enteral/parenteral nutrition via PORT systems, pain therapy, supportive treatment of bone metastasis etc. Some typical case reports will underline this modern treatment approach for exocrine pancreatic cancer.

RADIOCHEMOTHERAPY FOR LOCALLY ADVANCED EXOCRINE PANCREATIC CANCER

Department of Radiation Oncology, Münster University Hospital, Germany

Combined radiochemotherapy has been established as the standard treatment in locally advanced carcinoma of the exocrine pancreas. Unfortunately, the majority of patients continue to succumb to the disease. Recently, there has been a resurgence in clinical trials utilizing gemcitabine as a single agent, in combination chemotherapy regimens and with concurrent radiation therapy. The tumor marker CA 19-9 has been established as a useful tool in therapy monitoring and follow-up in pancreatic cancer patients.

Materials and Methods: Between January 1994 and February 2005, a total of 142 patients with locally advanced irresectable pancreatic adenocarcinoma, histologically proven, were included in the study. The inclusion criteria were no distant metastasis, a Karnofsky performance status >70%, age >18 and <75 years, normal blood counts, renal and liver function. The median age was 61 years (range: 26-75 years); 86 patients were male and 56 were female. Two different treatment schemes were used consecutively. Between January 1994 and December 2001, all patients (n=110) received a combined radiochemotherapy, consisting of hyperfractionated accelerated conformal radiotherapy and simultaneous application of 5-fluorouracil (5-FU) and folinic acid (FA). Conformal radiotherapy was carried out under megavoltage conditions with linear accelerators. A total tumor dose of 44.8 Gy was applied relative to the 90% isodose in two daily fractions of 1.6 Gy, resulting in ten fractions per week. On the first three days of radiotherapy, 600 mg/m² of 5-FU and 300 mg/m² of FA were given intravenously. Chemotherapy was repeated monthly in all cases of no progressive disease after evaluation of the radiological tumor response. From January 2002 to June 2005, in another 32 consecutive patients, the chemotherapy regimen was changed to gemcitabine (Gem) (300 mg/m²) and cisplatinum (Cis) (30 mg/m²), followed by Gem (1000 mg/m²) every two weeks in all non-progressive patients. There were 21 male and 11 female patients. Regular follow-up examinations included abdominal ultrasound, CT-scans, as well as repeated measurement of the serum CA 19-9 levels.

Results: The median overall survival, analyzed by the Kaplan-Meier method, of the 5-FU/FA group was 10.3 months. The actuarial 1-year survival was 46.6%, the 2-year survival 20.1% and the 3-year survival 15.5%. The median time to progression was 8.6 months. The progression-free
Nevertheless, surgical resection offers the only possibility of prognosis, even after surgery with curative intent. Pancreatic cancer still remains a disease with a poor outcome and not generally recommended. Adjuvant radiochemotherapy is being controversially discussed in the light of recent controlled-randomized trials, the use of radiotherapy treatment volume and Gem dose must be found. Based radiochemotherapy schemes, a feasible combination of perspective. Because of the narrow therapeutic index of Gem-based multimodality treatment may give a more promising outlook. In locally advanced pancreatic cancer is an effective and well tolerated treatment, but the long-term efficacy is still limited. The integration of new chemotherapeutic agents like Gem, as well as prognostic and monitoring factors like CA 19-9 in the multimodality treatment may give a more promising perspective. Because of the narrow therapeutic index of Gem-based radiochemotherapy schemes, a feasible combination of radiotherapy treatment volume and Gem dose must be found. In the light of recent controlled-randomized trials, the use of adjuvant radiochemotherapy is being controversially discussed and not generally recommended.

17 NOVEL CONCEPTS IN Pancreatic CANCER SURGERY

J.R. Izbicki

University Medical Center, Department of General, Visceral and Thoracic Surgery, Hamburg-Eppendorf, Germany

Pancreatic cancer still remains a disease with a poor prognosis, even after surgery with curative intent. Nevertheless, surgical resection offers the only possibility of a long-term cure. Furthermore, in high volume centers, the morbidity and mortality associated with pancreatic surgery have declined significantly in the past two decades. Even resections of the SMV or portal vein can be performed safely, offering a pattern of recurrence and survival comparable to a standard R0 Whipple procedure. In the case of T3 tumors, the patient will benefit greatly from R0 surgical resection, even in the case of en bloc resection of the transverse colon or the portal vein, which can be reconstructed without vascular grafting in most instances. In the majority of cases, only exploratory laparotomy will ultimately ensure surgical resectability, since a CT-scan, MRI, ultrasonography or angiography often offer diagnostic problems with peritumoral pancreaticitis possibly mimicking vascular invasion. Only in the event of complete vascular encasement of the mesenterico-portal axis or celiac trunk is a laparotomy unnecessary. If liver metastases are found unexpectedly during exploratory laparotomy, surgical palliation should be considered (bilio-digestive anastomosis or gastro-enterostomy), since these procedures do not lead to significantly longer hospital stays and are not associated with marked morbidity or mortality. Pain control can be ensured using morphine analogs, CT-guided sympathectomy or thoracoscopic sympathectomy. To date, which option offers the best pain control and quality of life is unclear. There is an ongoing debate on palliative Whipple’s procedure even in the case of single liver metastases, since it is associated with limited mortality (well below 5% in high volume centers) and ensures excellent pain control. In high volume centers, however, there is a justification to perform a palliative pancreaticoduodenectomy in highly selected patients, although further evaluation in randomised trials is required to confirm this.

18 INHERITED PREDISPOSITION TO Pancreatic CANCER

M. Sina-Frey

Institut für Humangenetik and Institut für Klinische Genetik, Philipps-Universität Marburg, Germany

Pancreatic ductal adenocarcinoma is the fifth leading cause of cancer death in Germany. It is an almost uniformly fatal disease, despite advances in medical treatment. At diagnosis, however, only one-third of patients have a chance of potentially curative resectability of pancreatic cancer (PC). The mortality rate follows closely that of the incidence. Ninety % of carcinomas of the pancreas appear to be sporadic, while a number of anecdotal case reports and case control studies suggest that about 10% of all cases...
of PC are hereditary. Several well-defined genetic syndromes have been shown to predispose affected family members to the development of PC, including hereditary pancreatitis, ataxia telangiectasia, HNPCC, familial atypical multiple-mole melanoma, Peutz Jeghers syndrome and hereditary mammary and ovarian cancer. The gene or genes responsible for the familial aggregation of PC are on the whole unknown, but germline mutations in the BRCA2 gene and in the CDKN2A gene have been shown to predispose to PC, although probably with incomplete penetrance. Effective screening tests are not yet available. The survival rate for PC will not improve significantly until new tests are developed to screen for the disease before the patients become symptomatic.

19 EXAMINATION OF THE PANCREAS AND UPPER ABDOMEN: MRI - CT TODAY

Th. Broemel
Conradia, Radiologie im Fleethof, Hamburg, Germany

In patients with pancreatic adenocarcinoma, high spatial resolution magnetic resonance imaging (MRI) of the upper abdomen is a valuable tool for staging, decision on adjuvant chemotherapy and monitoring the therapy of recurrences. The MR protocol includes sequences addressed to the d. choledochus, the d. pancreaticus (MRCP) the arterial, venous portalvenous vessels of the upper abdomen, and the liver parenchyma after administration of a liver-specific contrast media. The liver-specific contrast medium enables the detection of filiae down to 3 mm size.

In spite of the high spatial resolution of the computed tomography (CT) examinations, the MR investigation is, in nearly all cases, superior to the CT because of the much higher contrast resolution. This is especially the case if the MR examination is performed by thin slice technique (slice thickness 0.8 - 5 mm). Therefore, a high field strength gradient system and very short rising times of modern MR systems are a conditio sine qua non. Additional dynamically performed sequences during contrast media application should be performed to differentiate postoperative scars from vital tumor tissue. The second part of the contrast media is used to visualize the vessels of the upper abdomen (MR angiography). The very short examination time of the CT is of advantage in patients suffering from a low Karnofsky Index or by dyspnoa, e.g. due to lung edema, pleural effusion or ascites. For interpretation of both CT and MR examinations, an exact knowledge of the anamnesis (operation report, blood chemical parameters) and a close relationship with clinical colleagues is extremely advantageous.

Gynecology

20 TUMOR MARKERS IN BREAST CANCER: 100% TUMOR SPECIFICITY WITHIN THE REFERENCE RANGE

P. Stieber1, D. Nagel1, D. Lässig1, V. Heinemann2

1Institute of Clinical Chemistry and 2Department of Oncology, University of Munich, Germany

The ideal marker for the purpose of diagnosis in oncology would have two characteristics: it would be secreted into blood in measurable concentrations only after the cells that produce it had undergone malignant transformation; and its detection would permit conclusions as to the site of the tumor from which it arose. Despite worldwide efforts over many years, to date no tumor markers exist in the strict sense, i.e. markers with almost 100% specificity (undetectable in benign diseases and healthy persons) and 100% sensitivity (always detectable even in the early stages of a tumor. In consequence, we have to deal with a broad overlap of the tumor marker values of healthy individuals, benign and malignant diseases. Reference ranges mainly describe the 95th percentile for healthy individuals, but are interpreted by clinicians as the separation between healthy individuals and tumor patients resulting, as can be expected, in many false-positive and false-negative test results. At the time of primary diagnosis of a tumor disease, the individual baseline values of the patients before they developed the tumor are not known, thus it is difficult to escape the cut-off problems at this time. However, as soon as the tumor is completely removed (R0 resection), patients will again reach their individual "normal" values which are the basis for follow-up care. For example, breast cancer patients reach, 4 weeks after the end of adjuvant radio/chemotherapy, median values for CEA (Abbott, AxSYM) and CA 15-3 (Roche, Elecsys), which are comparable to the medians of healthy individuals (CEA: 1 ng/ml; CA 15-3: 13 U/ml). If an increase of 100% for the early detection of metastatic breast cancer is postulated, both biomarkers have a specificity of more than 99% and a sensitivity of 14% for CEA, 23% for CA 15-3 and another 27% for both, resulting in 36% false-negatives. Using fixed cut-off values (CEA: 4 ng/ml and CA 15-3: 30 U/ml) would have given 22% false-positives (specificity 78%) and a sensitivity of 8% for CEA, 29% for CA 15-3 and another 26% for both, resulting in 37% false-negatives. Using higher cut-off values where the probability of metastatic breast cancer is high (CEA: 7 ng/ml and CA 15-3: 69 U/ml), a specificity of 96% is reached but with only 8% sensitivity for
CEA and 20% for CA 15-3, another 11% for both, resulting in 61% false-negatives. Therefore, it is evident that, in follow-up care, the correct interpretation based on individual baseline values shows by far the best profile of specificity and sensitivity.

ADJUVANT ANTIBODY TREATMENT IN BREAST CANCER

H. Meden

Department of Obstetrics and Gynecology, Diacony Hospital, Academic Teaching Hospital, University, Göttingen, Germany

In most industrialized nations, and increasingly in developing countries, breast cancer represents the most frequent cancer in women and is a major health problem. In these regions, breast cancer accounts for about 4% of all deaths, 20% of all cancer deaths and 25% of all cancer cases in women. In the United States alone, approximately 180,000 women are diagnosed with breast cancer and 46,000 die from the disease. It has been estimated that the lifetime risk of developing breast cancer is 10%. Although mortality from breast cancer has generally been increasing worldwide, there have been recent reports of declining mortality, especially in Austria, Germany, Greece and the UK. A new era in the treatment of breast cancer is now approaching, as a result of our significantly improved knowledge of breast cancer biology during the past decade. Here, new therapeutic strategies based on the overexpression of the oncogene c-erbB-2 (HER2/neu) are focused on.

Clinical trials: Phase I clinical trials in breast cancer were initiated in 1991 using a monoclonal antibody directed against the HER2 protein. rhuMab was developed as the humanized version of the original mouse monoclonal antibody, 4D5. In a phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody treatment of patients with HER2/neu-overexpressing metastatic breast cancer, the efficacy and toxicity were evaluated. After preliminary clinical trials demonstrated that rhuMab HER2 could produce responses in woman with breast cancers who overexpress HER2, a worldwide clinical phase III trial was started to check whether the combination of conventional chemotherapy plus antibody treatment (rhuMab HER2) is superior to conventional chemotherapy alone in the adjuvant setting (HERA study). The first results demonstrate a significant reduction of disease-free and recurrence-free survival. Further results will be presented.

PHOSPHATIDYLINOSITOL 3-KINASE/AKT SIGNALLING PATHWAY COMPONENTS IN HUMAN BREAST CANCER: CLINICOPATHOLOGICAL CORRELATIONS

E. S. Gershtein1, A.M.Scherbakov1, V.A. Shatskaya2, N.E.Kushlinsky1, M.A. Krasil'nikov2

1Laboratory of Clinical Biochemistry, Institute of Clinical Oncology and 2Laboratory of Molecular Endocrinology, Institute of Carcinogenesis, Russian N.N.Blokhin Cancer Research Center RAMS

The phospatidylinositol 3-kinase (PI3K)/Akt signalling system controls many of the intracellular processes that are dysregulated in human cancer, and its components are now regarded as prospective molecular targets for improvement of breast cancer therapies, though the potential clinical usefulness of its measurement is unclear.

Materials and Methods: PI3K expression was analysed by Western blotting with monoclonal antibodies to its p85 regulatory subunit in a series of tumour and adjacent mammary gland samples collected at surgery from 33 breast cancer patients. In another set of 46 patients, phosphorylated Akt1 (pAkt1) expression was quantified by a direct "PathScan™ PhosphoAkt1 (Ser473) Sandwich ELISA Kit".

Results: Seventy-nine % of the investigated sample pairs were characterised by an increased level of PI3K in the tumour as compared to the adjacent mammary gland. PI3K activation was not associated with tumour steroid receptor status, histological grade or other clinicopathological features. Furthermore, immunoblotting of epidermal growth factor receptor (EGFR) in the tumours with increased PI3K and corresponding adjacent tissues revealed no association between EGFR and PI3K activation. On the other hand, only 49% of the tumours studied had an increased pAkt1 level compared to the adjacent tissue, but the pAkt1 level was significantly higher in stage Ib as compared to stage I-IIa and III-IV tumours, and the frequency of its elevation was positively associated with tumour size and malignancy grade. pAkt1 was also twice as frequently increased in PgR-negative as compared to PgR-positive tumours, while its mean level was significantly higher in ER-positive tumours as compared to ER-negative.

Conclusion: The two major components of the PI3K/Akt signalling pathway are differentially related to the pathological features of breast cancer, hence, their clinical roles might be different.

MODERATE CONCORDANCE OF THE HER-2/NEU EXPRESSION OF PRIMARY BREAST TUMOURS
AND THEIR METACHRONOUS DISTANT METASTASES: EVALUATION BY CONVENTIONAL AND AUTOMATED IMMUNOHISTOCHEMISTRY

D. Lüftner, P. Henschke, H. Dilk, C. Fichtner, R. Geppert, M. Dietel, H. Stein, K. Wernecke, K. Possinger
Medizinische Klinik II, Charité Campus Mitte, Humboldt-Universität zu Berlin, Germany

The determination of HER-2/neu overexpression, mostly tested in the tissue of primary breast cancer, is required for the selection for trastuzumab therapy in metastatic breast cancer patients. Clonal changes in the course of the disease may compromise patient selection.

Patients and Methods: In a 10-year retrospective study from 1994-2004, we searched for paraffin-embedded breast carcinomas in two university institutes of pathology. A total of 136 slides of breast carcinoma tissues obtained from 68 patients (primary tumour and one metachronous metastatic lesion each) were stained for HER-2/neu expression. The HER-2/neu tissue results of the primary tumours were correlated to the tissue results of the corresponding metastases. Two immunohistochemistry (IHC) evaluation techniques were used: the conventional IHC method by the DAKO HercepTest® and a computerized automated IHC of the same slide using the ChromaVision ACIS® system. The concordances of those HER-2/neu results were determined using the concordance index kappa (I), the McNemar test and the intraclass correlation coefficient (ICC).

Results: The tumour characteristics were distributed as follows: 71% invasive ductal, T1/2-tumours 41(34%), N0/1 staging 41(38%), G1/2 grading 10(49%), ER/PR positivity 34(35%) in the primary tumours. Metastatic lesions (78% soft tissues, 7% visceral organs, 12% bone, 3% others) were biopsied from 24-918 weeks after initial surgery. Metastases were ER/PR-positive in 62(53%). A total of 50% of the patients was HER-2/neu-positive in the primary tumour using the DAKO test (+2/3 positive), whereas only 34% were HER-2/neu-positive with the ACIS test (≥2.0). In the metastatic lesion, 59% of the patients were DAKO-positive and 34% ACIS-positive. The concordance between the HER-2/neu expression in the primary tumour and the metastatic tissue was moderate, with I = 0.53 for the DAKO test, decreased to I = 0.28 for the ACIS test. Altogether, 77 (i.e. 68%) of the patients had the same HER-2/neu status in the primary tumour and the metastatic tissue. The comparison of the metric results of the ACIS test for the primary tumour and the metastases revealed a weak correlation (ICC = 0.514, p < 0.001). The McNemar test for a change from HER-2/neu-negative primary tumours to HER-2/neu-positive metastases as compared to a change from HER-2/neu-positive primary tumours to HER-2/neu-negative metastases revealed no statistically significant differences (p = 0.210/1.000 for DAKO/ACIS, respectively). The comparison of the two IHC techniques, DAKO and ACIS, showed a moderate concordance with I = 0.49. In 73% of the measurements, the HER-2/neu status was concordant. Of note, the McNemar test demonstrated a highly significant difference (p < 0.001) in the evaluation of the HER-2/neu expression by the two tests. In 24% of the cases, DAKO-positive slides were stained negative with the ACIS test, whereas only 3% were negative with the DAKO test and positive with the ACIS test.

Conclusion: A clonal change of the HER-2/neu expression, tested by IHC, can be noticed more often than is generally assumed. Clinical selection of metastatic breast cancer patients for trastuzumab therapy requires an individual weighing of the evaluation method of the HER-2/neu status. HER-2/neu testing in metastatic tissue could possibly improve the probability of therapeutic effects. In a further study, our data will be verified by testing the tissue slides for HER-2/neu-amplification using the fluorescence in situ hybridisation (FISH) technique. Moreover, serum HER-2/neu results at the time of metastatic disease will be included.

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EXPRESSION OF E-CADHERIN IN HUMAN DUCTAL BREAST CANCER CARCINOMA IN SITU, INVASIVE CARCINOMAS, THEIR LYMPH NODE METASTASES, THEIR DISTANT METASTASES, CARCINOMAS WITH RECURRENCE AND IN RECURRENCE

U. Jeschke¹, I. Mylonas¹, C. Kuhn¹, N. Shabani², C. Kunert-Keil², C. Schindlbeck³, B. Gerber³, K. Friese¹

¹First Department of Obstetrics and Gynecology, Ludwig-Maximilians-University, Munich; ²Ernst Moritz Arndt University of Greifswald, Institute of Pathophysiology, Karlsburg; ³Department of Obstetrics and Gynecology, University, Rostock, Germany

Breast cancer cells can invade and generate metastasis via either lymphatic or blood vessels. E-cadherin mediates tumor cell-cell adhesion. Partial or complete loss of E-cadherin expression correlates with poor prognosis in breast cancer patients. In this study, the expression of E-cadherin in mammary ductal carcinoma in situ, invasive breast carcinomas without metastasis, invasive carcinomas with their lymph node and distant metastases and invasive carcinomas with local recurrence in breast cancer tissue were analysed.

Materials and Methods: Paraffin-embedded slides of carcinoma in situ (8 DCIS), invasive carcinomas without lymph node metastases (9 invasive ductal carcinomas
infiltrating), invasive carcinomas (7 invasive ductal carcinomas) with corresponding lymph node metastases, invasive carcinomas (8 invasive ductal carcinomas) with corresponding recurrence and invasive carcinomas (5 invasive ductal carcinomas) with corresponding distant metastases were investigated for E-cadherin expression.

Results: A strong expression of E-cadherin in carcinoma in situ was demonstrated. The expression of E-cadherin was moderate in invasive carcinomas without metastases. However, a very weak expression of E-cadherin was detected in primary carcinoma with lymph node metastases. E-cadherin expression was elevated in lymph node metastases compared to the primary tumor.

Conclusion: Analysis of a tumor antigen involved in adhesion of breast cancer cells showed that there are significant differences of expression of E-cadherin in primary breast cancer cells and their metastases. Evaluation of this marker, involved in cell adhesion, could be a useful method for evaluating the metastatic risk in breast cancer patients.

25 IMPORTANT OF THE PROGNOSTIC FACTORS OF PRE-MENOPAUSAL FEMALES WITH BREAST CANCER

J. Finek, L. Holubec Jr., O. Topolcan, L. Elgrova, A. Skalova, L. Pecen

Departments of Oncology, Nuclear Medicine and Pathology, University Hospital, Pilsen, Academy of Science, Prague, Czech Republic

Basic conventional prognostic factors for breast cancer include the age of the patient, tumor grading, regional lymph node status, and estrogen and progesterone receptors. Positivity of the HER-2 receptor (c-erbB-2) seems to be a new prognostic and predictive factor. Prognostic factors appear to be more important in the high-risk group of pre-menopausal females. Individual prognostic factors and their impact on disease-free survival (DFS) and overall survival (OS) during the 5-year follow-up period were evaluated.

Patients and Methods: Forty-two patients were monitored after standard oncology treatment during the 5-year follow-up period (minimum follow-up 5 years). The statistical significance of the levels of the individual prognostic parameters was evaluated (age, histology, TNM classification, ER, PR, CA 15-3, CEA, HER-2) in relation to the time prior to progression (disease-free interval = DFI) and to the OS.

Results: The following parameters found to be statistically significant prognostic parameters for DFS: PgR positivity ($p=0.0036$), proliferative marker MiB1 ($p=0.0108$), pre-operative level of CA 15-3 ($p=0.0425$) and ER ($p=0.570$). The immunohistochemical positivity of c-erbB-2 was not statistically significant ($p=0.6361$). The following parameters were evaluated as statistically significant prognostic parameters for OS: PgR positivity ($p=0.0003$), MiB1 ($p=0.0005$), ER ($p=0.0440$) and the pre-operative level of CEA ($p=0.0495$). The immunohistochemical positivity of c-erbB-2 was not statistically significant ($p=0.9323$).

Conclusion: A statistically significant prognostic importance of the levels of the tumor markers CA 15-3 and CEA for prognosis estimation of breast cancer in pre-menopausal females was proved. To date, these factors have been underestimated. ER, PgR and MiB1 proved to be statistically significant prognostic parameters, but no prognostic importance was confirmed for c-erbB-2 positivity. This factor can not be evaluated in pre-menopausal females separately from the other prognostic factors due to the predictive value in relationship to the adjuvant therapy (patients with HER-positive, ER-positive, PgR-negative).

26 THE RISK OF NON-SENTINEL METASTASES IN PRIMARY BREAST CANCER

I. Bauerfeind, G. Sorokina, I. Ruehl, S. Kahlert, I. Himsl, A. Lebeau, R. Linke, K. Friese, M. Untch

Department of Gynaecology and Obstetrics, Department of Nuclear Medicine, Institute of Pathology, LMU Munich, Clinic Grosshadern, Germany

The Sentinel Node Biopsy (SNB) is established as the standard of surgical care in primary breast cancer. In sentinel node (SN)-negative patients, further axillary dissection (ALND) is not necessary, although in SN-positive breast cancer further ALND is warranted. Different studies have demonstrated that the SN is the only site of nodal metastasis. Therefore, this analysis aimed at evaluating the associated risk factors for additional nodal-positive non-sentinel lymph nodes in case of the finding of a positive SN.

Materials and Methods: This was a retrospective analysis of all patients who underwent SNB between October 1999 and July 2005. The indications for SNB were: (a) tumours not larger than 3 cm and (b) no clinical evidence of positive axillary lymph nodes.

Results: 402 patients underwent SNB. The detection rate for SN was 93%, the false-negative rate 2.75%. 214 patients (51%) had SNB with consecutive ALND, while in 197 patients (49%) the SNB was the only axillary intervention. In patients with ALND, 109 (27%) had axillary metastases. In 41 patients the SN was the only site of nodal metastasis. There was a clear association between tumour size and non-sentinel metastasis.
Micrometastases, as well as other histological factors, were not associated with increased risk of non-sentinel involvement. 

Conclusion: The size of the primary tumour is associated with positive non-sentinel nodes in case of positive SN. Lymph node dissection remains a diagnostic procedure with questionable value in terms of overall survival. Even in cases of an SN involvement, there is a substantial number of patients who will not profit from an additional ALND.

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THE REMAINING FOLLICLE HYPOTHESIS (RFH) – A NEW HYPOTHESIS FOR THE ETIOLOGY AND PATHOGENESIS OF OVARIAN CANCER

W. Jäger, S. Krämer
Frauenklinik der Städtischen Kliniken, Düsseldorf, Germany

Physiological stimulation of the ovary by FSH stimulates the aromatase in the primordial follicle cells, which leads to estradiol production and the growth of one single follicle. LH then induces ovulation, by inducing meiotic division. Prolonged pharmaceutical stimulation of the ovary by FSH, as done during in vitro fertilization, can lead to clinical symptoms resembling those observed in ovarian cancer, e.g. increase of ovarian size with multiple cyst formation, ascites, pleural effusion and massively elevated CA-125 serum levels. In rat experiments, this kind of hyperstimulation with FSH also leads to the formation of benign ovarian stromal tumors. However, as soon as LH is continuously administered for a prolonged period of time, these tumors invariably turn to malignant tumors, which can metastasize and induce tumors in other animals after transplantation. Little was known about the composition of the fluids in human ovarian tumors. While benign cysts never contain LH or FSH, both gonadotropins were always found in ovarian cancer cysts. LH and FSH can not be produced by ovarian cancer cells in culture – therefore, it must be assumed that they are derived from the serum, probably by diffusion, which is strengthened by nearly identical concentrations in the serum and in the cyst. Cancer cysts also contain estradiol and, surprisingly, sometimes also hCG, i.e. these cancer cells have aquired synthetic functions which are usually exerted by granulosa cells and the syncytiotrophoblast. The postmenopausal ovary consists of an epithelial layer at the surface, stromal cells and sometimes remaining primordial follicles (RPF). These RPF appear as granulosa and theca cells gathered around an ovum. They had been described previously by various authors as "hormone-active" stromal cells. Could it be that these cells take over the above-mentioned synthesis of estradiol and hCG and, therefore, are responsible for cancer development? The ovum is the omnipotent human stem cell with all this information. Additional genetic and epigenetic changes can lead to cell division and proliferation and the possibility of leaving a cell cluster and growing at a different place (metastasis), which is considered to be "malignant". The continuous stimulation of an ovum with LH/hCG is only observed during pregnancy, therefore this continuous stimulation of the ovum with LH could be described as a state of "pseudo-pregnancy". It would be a logical step if the ovum then behaves as a stem cell and starts to produce all the factors which are needed for implantation, i.e. growth factors and factors for neoangeogenesis. The assumed associations between the "remaining follicle" and ovarian cancer lead to a number of questions and offer the chance to test this hypothesis in the future. Even if the remaining follicle hypothesis is falsified some day, knowledge of ovarian physiology will have advanced.

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WIRKUNGSWEISE VON PHYTOÖSTROGENEN AUF HORMONABHÄNGIGE TUMOREN-LIGNANE UND IHRE EINFLUSS AUF DAS WACHSTUMSVERHALTEN HUMANER KARZINOMZELL-LINNEN DES ENDOMETRIUMS

S. Vincevic, C. Bergemann, D.-U. Richter, W. Ruth, V. Briese
Universitätsfrauenklinik Rostock, Germany


**Ergebnisse:** In unserer Arbeit zeigte sich, dass der Leinsamenrohextrakt nicht zytotoxisch wirkt. Hohe Konzentration (1 mM) des Rohextraktes hatten einen proliferationssteigernden Effekt, niedrige Konzentration (30 μM) einen hemmenden Effekt im MTT-Assay. Im BrdU-Assay zeigten umgekehrt niedrige Konzentration (30 μM) des Rohextraktes einen proliferationssteigernden und hohe Konzentration (1 mM) einen hemmenden Effekt. Bei MFE-280 hemmten beide Konzentrationsformen die Proliferation.

**Schlussfolgerung:** 1. Unsere Ergebnisse zeigten sowohl dosisabhängige als auch tumorabhängige Wirkungen der Lignane auf das Proliferationsverhalten der Zellkulturen. 2. Die unterschiedlichen Ergebnisse (MTT versus BrdU) deuten auf verschiedene Wirkungsebenen der Lignane auf die Tumorzellen hin. Es kann vermutet werden, dass der Leinsamenrohextrakt über den Rezeptorweg auf DNA-Ebene wirkt als auch auf receptorunabhängigen Weg die Mitochondrienaktivität beeinflusst. Entsprechende Nachweise sind Gegenstand weiterführender Forschungsarbeiten, so dass Dosisempfehlungen für die Klinik derzeit noch nicht gegeben werden können.

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**EXPRESSION OF HORMONAL RECEPTORS (ESTROGEN RECEPTORS α AND β, AND PROGESTERONE RECEPTORS A AND B) IN HUMAN MUCINOUS CARCINOMA OF THE ENDOMETRIUM**

N. Shabani1,2, I. Mylonas2, U. Jeschke2, A. Thaqi3, C. Kuhn2, T. Puchner1, K. Friese2

1 Landes-Krankenhaus Schärding, Abteilung für Frauenheilkunde und Geburtshilfe, Schärding, Austria; 1,2 Frauenklinik der Ludwig-Maximilians-Universität, Institut für Medizinische Informatik, Biometrie und Epidemiologie der Ludwig-Maximilians-Universität3, München, Germany

Endometrial carcinoma is the most common female pelvic genital malignancy. The strong association between the development of endometrial cancer and influence of steroid hormones (especially estrogen) was demonstrated in many studies. Mucinous carcinoma is an uncommon type of endometrial carcinoma. Most cancers are low grade and have an excellent prognosis. The expressions of one type of estrogen-(ER) and progesterone receptor (PR) are well documented. Recently, another 2 types of receptors (ER-β and PR-B) were demonstrated. Our aim was to demonstrate the expressions of all 4 types of steroid receptors (ER-α, ER-β, PR-A and PR-B) in human mucinous carcinoma of the endometrium.

**Materials and Methods:** An immunohistochemical hormone receptor assay, using specific monoclonal antibodies against estrogen receptors (ER-α, ER-β) and progesterone receptors (PR-A and PR-B), was used to study formalin-fixed and paraaffin-embedded slides of 12 cases, diagnosed as primary endometrial mucinous carcinoma of different histological grades (G1 n=9; G2 n=3; G3=0).

**Results:** Three types of steroid receptors (ER-α, PR-A and PR-B) were frequently expressed in mucinous adenocarcinoma. ER-β was weakly expressed in only one case analyzed. The immunohistochemical expression of PR-B demonstrated a statistically significant decrease in G1 neoplasm in comparison to G2 (p<0.001).

**Conclusion:** The expressions of 4 different steroid receptors in mucinous endometrial carcinoma were demonstrated. No significant difference between the different histological grades of tumors in the case of ER-α and ER-β expressions were found. Interestingly, a statistically significant decrease in the expression of PR-A in G1 neoplasm compared to G2 was demonstrated. The lower expression of PR-A in G2 tumors suggests a substantial function of progesterone, and thus the progesterone receptor, in the malignant transformation of mucinous endometrial cancer. Therefore, PR-A expression might be utilized as a tumor marker to distinguish between G1 and G2 mucinous tumors. However, additional studies are necessary to evaluate whether these parameters could be used as tumor markers for endometrial cancer.

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**INHIBIN/ACTIVIN SUBUNITS ARE IMMUNOHISTOCHEMICALLY EXPRESSED IN COMPLETE AND PARTIAL HYDATIDIFORM MOLES**

I. Mylonas, J. Vogl, C. Kuhn, S. Schulze, U. Jeschke, K. Friese

Ludwig-Maximilians-University, 1st Department of Obstetrics and Gynaecology, Munich, Germany

Inhibins are dimeric glycoproteins, belonging to the TGF-b, that are composed of an a-subunit (INH-a) and one of two possible b-subunits (bA or bB; INH-bA and INH-bB). Additionally two further b-subunits (bC or bE; INH-bC and INH-bE) have been cloned, although their function still remains unclear. The immunohistochemical detection of inhibin/activin subunits has been recently proposed as a
ET1 overexpression of tumor cells was highly significantly correlated to early stages. No correlations could be shown for ETAR or ETBR concerning TNM stages. High staining levels for ETBR within tumor tissue were significantly related to tumor progression and delayed metastasis, and low staining levels to relapse-free survival. A significantly reduced overall survival and reduced disease-free survival could be shown to be associated with the low stages (pT1-2).

Conclusion: These results predict that, in addition to known histological risk factors and TNM classification criteria, measurement of ET-R, especially ETBR expression, by means of a simple immunohistochemical analysis, might contribute to predicting the prognosis of patients with vulvar squamous cell carcinoma.

CA 125-SERUMBESTIMMUNGEN BEI PATIENTINNEN MIT EINEM OVARIALKARZINOM

S. Markmann, B. Gerber, V. Briese
Universitätsfrauenklinik Rostock, Germany


Ergebnisse: In 20% ergab sich präoperativ ein normaler CA 125-Serumspiegel (<35 U/l). In allen Fällen ergab sich in der Summe keine Korrelation zwischen präoperativen CA 125-Konzentrationen und dem FIGO-Stadium (Pearson correlation coeff. rprrior = 0.444, p<0.001; rafter treatment =0.476, p<0.001; rafter treatment =0.244, p<0.05). Allerdings ergab sich für Patientinnen mit CA 125-Werten oberhalb von 100
U/l eine signifikant geringere 3-Jahres-Überlebensrate ($p<0.05$). Bei 33 Patientinnen kam es postoperativ zu einem signifikanten Abfall der CA 125-Serumkonzentrationen ($p<0.001$). In der Gesamtbetrachtung ergab sich für CA 15-Serumkonzentrationen eine Signifikanz für einen Prognosefaktor hinsichtlich der Überlebensrate ($p<0.01$).

**Schlussfolgerungen:** CA 125 im Serum bei Patientinnen mit einem Ovarialkarzinom stellt einen signifikanten Prognosefaktor dar. Ein CA 125-Wert präoperativ oberhalb von 100 U/l weist auf eine ungünstige Prognose.

**PATTERN OF RELEASE OF VARIOUS SEROLOGICAL BIOMARKERS IN BENIGN AND MALIGNANT DISORDERS OF THE OVARY – A MULTIVARIATE APPROACH**

A. Burges$^1$, C. Kümper$^1$, D. Nagel$^2$, S. Holdenrieder$^2$, K. Hofmann$^2$, P. Stieber$^1$

$^1$Department of Gynecology and $^2$Institute of Clinical Chemistry, University, Munich, Germany

Diagnostic oncology mainly focuses on the follow-up care of tumor diseases using a single marker with the best profile of specificity and sensitivity for this clinical indication. Increasingly it has become evident that malignant diseases lead to a significant release or non-release of multiple biomarkers and that, despite the lack of organ and tumor specificity of each marker, the sum of this information may lead to an increase of diagnostic, differential diagnostic or prognostic capacities.

**Materials and Methods:** The sera of 120 patients suffering from ovarian cancer at the time of diagnosis before first treatment and of 442 patients with various benign gynecological disorders were investigated using the following parameters: CEA, AFP, CYFRA 21-1, NSE, CA 19-9, CA 15-3, CA 72-4, CA 125, S100, PSA, fPSA and βHCG (Elecsys, Roche). A logistic regression model was used to combine the diagnostic capacity of these markers. Evaluating the biomarker results, linearity was tested first; in case of non-linearity, a cut-off value was determined.

**Results:** In the benign gynecological diseases, some biomarkers showed no increased release as compared to the frequency distribution in healthy individuals (AFP, S100, PSA, NSE), some showed slightly increased release (CYFRA 21-1, CEA, CA 15-3) and some significant increase (CA 125, CA 72-4, CA 19-9, βHCG). In ovarian cancer patients, the highest release was found for CA 125 (median 286 U/ml, 95th percentile 3357 U/ml) and CYFRA 21-1 (median 3 ng/ml, 95th percentile 69 ng/ml). CA 72-4, CYFRA 21-1 and age showed linearity and could be included in the multivariate analysis as logarithmic functions. From the model, a diagnostic score was calculated. This score led to a significant increase of the AUC as compared to the single markers, especially at a very high specificity between 85 and 100%.

**Conclusion:** The combined analysis of the frequency and the extent of release and non-release of oncological biomarkers revealed a high potential for diagnosis and differential diagnosis.

**DEZIDUA-PROTEIN GLYCODELIN A (GDA) BEIM OVARIALKARZINOM**

C. Richter, M. Baethje, U. Jeschke, D.-U. Richter, V. Briese

Universitätsfrauenklinik Rostock, Germany


**Ergebnisse:** Die Vergleichsgruppe (Uterus myomatosus) ergab eine Übereinstimmung GdA negativ (PCR, IHC) in 81% (13/16). Bei den Ovarialkarzinomen ergab sich eine Übereinstimmung GdA positiv (PCR, IHC) in 52% (14/27). Bei den anderen Karzinomen wurde eine Übereinstimmung GdA positiv (PCR, IHC) in 33% (7/21) nachgewiesen.

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HIGH RECURRENCE RATE OF CERVICAL DYSPLASIA AND PERSISTENCE OF HPV INFECTION IN HIV-INFECTED WOMEN

A. Gingelmaier, T. Grubert, R. Kaestner, I. Mylonas, T. Weissenbacher, K. Friese

I. Universitätsfrauenklinik München, Germany

Carcinoma of the cervix is an AIDS-defining disease according to the CDC-classification. The aims of this study were: a) evaluation of the recurrence rate of cervical dysplasia after operative treatment and the rate of HPV persistence; b) the influence of an antiretroviral therapy on recurrence.

Materials and Methods: In a retrospective analysis, the following data of HIV-positive women visiting our gynecological out-patient clinic over recent years were analyzed: results of every cervical cytology, cervical HPV detection (Hybrid Capture® 2 from Digene or Reverse line blot from Roche molecular systems), cervical biopsy and/or other histology e.g. conization, patient history of dysplasia, antiretroviral therapy etc.

Results: 388 women had a mean follow-up of 2.7 years and a mean of 8.7 out-patient visits. 197/344 patients (57.3%) showed at least one positive result regarding HPV (high-risk). 136 women had 4 or more times HPV results, which showed that 84 of them (61.8%) had a persistent HPV infection. 157/388 had cervical dysplasia and 70 of them needed operative therapy (>50% carcinoma in situ). 41/70 patients (58.6%) received more than one operation because of a recurrent dysplasia; all of them had persistent HPV. Two women developed a cervix carcinoma. Antiretroviral therapy had no influence on the recurrence rate.

Conclusion: The rate of recurrence of cervical dysplasia in HIV-positive women after operative treatment was found to be very high as well as the associated long-term persistence of the HPV infection. HPV persistence represented an excellent marker for relapsing cervical dysplasia. Therefore, HIV-infected women should be monitored frequently using cervical cytology and HPV.

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EXPRESSION OF HPV AND INHIBIN/ACTIVIN SUBUNITS IN DIVERSE PARTS OF ADENOSQUAMOUS ENDOMETRIAL CARCINOMA

S. Gutsche, I. Mylonas, N. Shabani, C. Kuhn, S. Kunze, U. Jeschke, K. Friese

I. Frauenklinik, Klinikum Innenstadt der Ludwig-Maximilians-Universität München, Deutschland

Objective: Adenosquamous endometrial carcinomas are rare carcinomas of the endometrium. The aim of this study was to determine the possible pathogenetic factors and molecules relevant for therapy of these tumors. Eight adenosquamous endometrial carcinomas were tested for HPV, steroid receptors and inhibin/activin subunits.

Materials and Methods: Eight adenosquamous endometrial carcinomas were immunohistochemically analyzed with specific monoclonal antibodies for HPV (polyclonal anti-HPV and monoclonal anti-HPV 18), estrogen receptor (ER)-alpha and ER-beta, progesterone receptor (PR)-A and PR-B and the inhibin/activin subunits Inhibin-alpha, -betaA, -betaB, -betaC and betaE.

Results: HPV 18 and the polyclonal HPV antibody were detected in all adenosquamous endometrial carcinomas, both in the endometroid (n=7/8) and squamous (n=8/8) parts of the tumor. Neither ER-alpha or ER-beta were detectable in any tumor, in contrast to the PR-A and PR-B, which were detected in about half of these tumors (PR-A: n=5/8 and PR-B: n=2/8). Inhibin-alpha and -betaB were not detected, Inhibin-betaA and -betaE were expressed in all adenosquamous carcinomas and Inhibin-betaC in nearly all tumors (n=7/8).

Conclusion: The carcinogenesis of adenosquamous endometrial carcinomas could be correlated with an HPV 18 infection. Adenosquamous endometrial carcinomas seem not to be controlled by estrogens. The missing expression of the Inhibin-alpha subunit suggests a tumor-suppressive function in adenosquamous endometrial tumors. The missing expression of the Inhibin-betaB subunit, which is probably a marker of differentiation, might indicate the malignancy of these tumors. The other inhibin subunits, Inhibin-betaA and -betaE, were expressed in all and Inhibin-betaC in almost all, adenosquamous tumors. It remains to be seen whether it will be possible to use these parameters as tumor markers.

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UNTERSUCHUNGEN ZUR INHIBINEXPRESSION IN DER ENDOMETRIUM-KARZINOMZELLLINIE RL-95-2 UNTER STIMULATION DURCH HYDROCORTISOL UND ESTRADOL

J. Vogl, A. Höing, S. Schulze, Ch. Kuhn, I. Wiest, U. Jeschke, I. Mylonas, K. Friese

I. Universitätsfrauenklinik München, Germany

Inhibine sind dimere Glykoproteine, die sich aus einer alpha-Untereinheit und einer von zwei möglichen Inhibin-beta-Untereinheiten zusammensetzen. Das ebenfalls zur TGF-B zählende Aktivin besteht dagegen aus zwei Inhibin-beta-Untereinheiten (betaA- oder betaB-Untereinheit). Ovarielle
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EXPRESSION PATTERN OF OSTEOPONTIN (OPN) IN ENDOMETRIAL CARCINOMA – CORRELATION WITH EXPRESSION OF THE ADHESION MOLECULE CEACAM1

J. Briese1, H.M. Schulte2, T. Lönnning1, A.M. Bamberger1

1Department of Pathology, University Clinic and 2Endokrinologikum, Hamburg, Germany

Background: OPN and CEACAM1 have diverse biological functions in the uterus throughout the estrous cycle and have been shown to interact with integrin beta3. OPN is a glycoprotein of the extracellular matrix, which has been shown to mediate cellular migration and invasion and to contribute to tumorigenesis in several types of cancers. CEACAM1 is an adhesion molecule of the carcinoembryonic antigen family, which we have recently found to be expressed in endometrial cancer and which has been shown to be down-regulated in colorectal and breast cancer. Endometrial carcinoma is the most frequent invasive malignancy of the female genital tract in developed countries. The present study was designed to investigate the expression patterns of OPN in normal human endometrium and in endometrial carcinomas and to correlate it with CEACAM1 expression and function in tumor cell invasiveness.

Materials and Methods: Immunohistochemistry and immunofluorescence with specific antibodies were performed on a series of 20 normal endometrial samples and 40 endometrial carcinomas, to investigate the expression pattern and cell type-specific localization of OPN and to correlate it with the expression of CEACAM1. In addition, Western blot was performed on normal human endometrium and endometrial neoplasia. ACI hybridoma cells, transfected with CEACAM1 and stimulated with OPN, were studied using the Matrigel invasion assay.

Results: Strong OPN expression, with a consistent cytoplasmatic localization in epithelial glandular cells, was observed in the normal human endometrium in 80% of the samples of the proliferative and secretory phase (score 8-12). CEACAM1 showed similar results. Strong expression of OPN could be observed in 35 (87.5%) of the 40 analyzed endometrial carcinomas. Of the 40 analyzed tumors, 19 (47.5%) were in the high score (8-12) category with a strong OPN expression level, while 9 tumors out of 40 (22.5%) showed a moderate score (4-7) category. With increasing malignancy grade, increasing areas with a low OPN expression level or complete loss of OPN expression could be observed. CEACAM1 showed similar results and was found to be co-expressed with OPN in normal human endometrium and in endometrial neoplasia. To investigate a potential functional link between OPN and CEACAM1 in regulating cellular invasion, ACI hybridoma cells transfected with CEACAM1 were treated with OPN and showed increased invasiveness, indicating that CEACAM1 and OPN potentially act together to enhance the invasiveness of tumor cells.

Conclusion: In the current study the different expression patterns of OPN in endometrial tumors seem to support the biological diversity of endometrioid and serous carcinomas. It is suggested that OPN might play a different role in the pathogenesis of endometrial cancer (possibly as a functional complex with CEACAM1) and could be relevant for the invasive growth of such lesions. Using an in vitro model, increased cellular invasiveness after OPN treatment was also demonstrated. We speculate that OPN and CEACAM1 may act as a functional complex involved in the regulation of endometrial invasiveness.


Ergebnisse: Bei der mikroskopischen Auswertung zeigte sich, dass sowohl Inhibin a, als auch die betaB-Untereinheit durch Zugabe von Hydrokortisol vermindert exprimiert wird, die βA-Untereinheit dagegen durch Hydrokortisol vermindert exprimiert wird. Unter Zugabe von 0,01 mikromol Estradiol stieg die Expression der BB-Untereinheit an, die Expression der α- und βA-Untereinheit war unverändert.

DAS EXPRESSIONSVERHALTEN DES PANKARZINOMANTIGENS THOMSEN-FRIEDENREICH (TF) BEI DER PARTIELLEN BLASENMOLE

Universitätsfrauenklinik Rostock, Germany


Ergebnisse: Es gelang uns das TF-AG und das Muc 1 bei der partiellen Blasenmole nur in den Erythrozyten und in den Membranen der Chorionzotten zu verifizieren. Jedoch im Vergleich zu der partiellen Blasenmole wurde bei den induzierten Aborten nicht nur im Blut, sondern auch in den dezidualen Zellen das TF-AG u. das Muc 1 detektiert.


Urology

CAPILLARY ELECTROPHORESIS COUPLED TO MASS SPECTROMETRY AS A TOOL TO DEFINE POTENTIAL PROSTATE CANCER BIOMARKERS IN URINE

H. Mischak, D. Theodorescu, S. Wittke, E. Eltze, O. Bettendorf, C. Wülfing, A. Semjonow
Urology, Univ.-Klinik, Münster, Germany

The use of capillary electrophoresis (CE) coupled with mass spectrometry (MS) to identify single polypeptides and patterns of polypeptides specific for prostate cancer in human urine is described. Using improved sample preparation methods, that enable enhanced comparability between different samples, samples from >100 patients who underwent prostate biopsy were examined. Of this group, >30 patients each, who had either benign pathology (BP) or prostate cancer (CaP), were used to define potential biomarkers which allow discrimination between these two states. In addition, CE-MS data from these urine samples were compared to 41 controls, without known or suspected clinical CaP, to further confirm the polypeptides indicative for CaP. These analyses led to the definition of several urinary polypeptides that could serve as potential biomarkers for disease. When these were utilized in a disease model, correct classification of the CaP patients with >90% sensitivity and specificity was possible in the training set. An additional 474 samples from patients with renal disease, enrolled in other studies, were analyzed and it was found that 14 (3%) had polypeptides suggestive of CaP, possibly indicating that they harbor clinical CaP. In an ongoing study aimed at the validation of these biomarkers, samples from patients scheduled for prostate biopsy are prospectively collected and examined in a blinded fashion. The
Early prostate cancer (PCa) detection is considerably enhanced by measurement of the prostate-specific antigen (PSA). However, PSA lacks specificity, since elevated PSA concentrations are also found in patients with benign prostatic diseases. Especially in the 2-10 ng/mL PSA "gray zone", this serum test alone cannot distinguish between PCa and benign prostatic hyperplasia (BPH). Measurement of free PSA (fPSA) and the use of its ratio to PSA (%fPSA) has been shown to improve the specificity over total PSA (tPSA) alone. Approximately 20-25% of unnecessary biopsies can be avoided. The combined use of %fPSA and artificial neural networks (ANNs), with inclusion of clinical data like prostate volume and status of digital rectal examination (DRE), can further eliminate unnecessary prostate biopsies by 11-49%, as shown in different studies. To further improve ANNs, various new serum markers were tested within different ANNs. Additionally, we also compared our routine IMMULITE PSA-based ANN (iANN), named "ProstataClass", with newly PSA assay-adapted ANNs (nANNs) in 5 different populations (4480 men) and in 798 men, where tPSA and fPSA were parallel measured by 5 different assays to verify the impact of assay-specific changes within ANNs. Regarding new tumor markers, 3 newly developed serum assays for human kallikrein 11 (hK11), macrophage inhibitory cytokine (MIC-1) and macrophage migration inhibitory factor (MIF) were measured within a PSA- and %fPSA-based ANN. "Leave one out" ANN models with these variables and prostate volume were constructed and compared to logistic regression and all single parameters. The discriminatory power of MIC-1, hK11 and MIF was less than that for PSA, despite significant differences in BPH compared to PCa patients. At 90% sensitivity, the ANN was only significantly better than %fPSA if the prostate volume was included, but the improvement from 30% to 80% specificity was substantial. Another kallikreins, hK2, and the ratio of hK2 to fPSA has been shown to enhance the discrimination between PCa and BPH. The combined use of hK2 within a %fPSA-based ANN was first reported by our group. In 475 patients within the PSA range 1-20 ng/mL, ANN models with the variables PSA, %fPSA, hK2, hK2/%fPSA and hK2/%fPSA were constructed. Whereas hK2 was not significantly different between BPH and PCa patients, the ratios %fPSA, hK2/%fPSA and hK2/%fPSA and the hK2-based ANN outputs were always significantly different between PCa and BPH patients. Only in the 1-4 ng/mL PSA range did the hK2-based ANN perform better than %fPSA regarding the area under the curve (AUC) and at 90 and 95% sensitivity. Thus, hK2-based ANNs showed improved PCa detection only at lower tPSA ranges. In a total of 4480 patients with serum PSA concentrations in the range 2-10 ng/mL, different ANNs were generated from 5 databases with PSA, fPSA (assays from Abbott, Beckman Coulter, Diagnostic Product Corp., Roche or Wallac), age, prostate volume and DRE status. Data were tested with the iANN "ProstataClass" and compared with outputs of the nANNs for each PSA assay using AUC analysis and cut-off calculations at 95% specificity or sensitivity. In 15 different comparisons, the AUC in different PSA ranges for the nANN were always significantly larger than the AUC for %fPSA or PSA. In 14 out of 15 evaluations, the nANN also performed significantly better than the iANN. Therefore, for each patient population, PSA assay-specific ANNs should be used to optimize the ANN outcome in order to reduce the number of unnecessary biopsies. Use of a trained iANN for other PSA assays can be only partially recommended. Sera from 798 untreated and histologically proven men (468 PCa and 330 BPH) with tPSA concentrations between 0.49 and 25 ìg/L were parallel measured with tPSA and fPSA (cPSA) assays from 5 different manufacturers (Abbott, Buyer, Beckman Coulter, Diagnostic Product Corp. and Roche). Different nANN models and the iANN "ProstataClass" ANN were compared. The median percent free PSA (%fPSA) values indicated large differences (up to 10%) in BPH and PCa patients. ROC analysis always showed a significantly better performance of the iANN "ProstataClass" program compared to %fPSA, regardless of the assay used. However, new ANN models built with data from the respective assay further slightly improved the outcome of the ANNs. The large heterogeneity of %fPSA values and, thus, also ANN outcomes, leads to difficulties by making these assay-specific ANNs freely available. In the future, inclusion of other new serum markers like bPSA or proPSA (Beckman Coulter) or the PSA velocity in ANNs may further improve the general outcome of %fPSA-based ANNs.

43 PERFORMANCE OF COMPLEXED VERSUS TOTAL PROSTATE SPECIFIC ANTIGEN USING DISCORDANCE ANALYSIS CHARACTERISTICS (DAC)

F. Oberpenning1, O. Bettendorf2, G. De Angelis3, T. Keller4, A. Semjonow5

1Department of Urology, University of Bonn; 2Prostate Center, Institute of Pathology; 3Department of Clinical Chemistry and 4Prostate Center, Department of Urology, University of Münster; 5ACOMED Statistik, Leipzig, Germany

Previous analyses of C-PSA versus T-PSA for the early diagnosis of prostate cancer (PCA) rendered discrepancies, partly due to conflicting results from ROC-curve comparisons, where some bias results from the determination of PSA ranges and cut-offs investigated. To overcome this bias, the method of DAC has recently been proposed. DAC focuses on analyzing individuals, who are discordantly categorized by the 2 tests investigated, for iterated cut-offs with identical sensitivity.

Materials and Methods: In sera from 401 men (T-PSA range 2-30 ng/ml), T- and F-PSA (Access) as well as C-PSA (Bayer) were determined prior to prostate biopsy and the results related to biopsy outcome using DAC analysis.

Results: Histology yielded 199 PCA and 202 men without PCA (nPCA). In clinically relevant ranges, DAC showed, among discordantly tested patients, that C-PSA used as a single test detected men with PCA with an over 3-fold better specificity than T-PSA. At >90%/>26% sensitivities, a T-PSA range of 4.1 to 10 (n=255) corresponded to a C-PSA range of 2.9-7.2 (n=262). Within these gray zones a 0.20 F/T-PSA matched with a 0.33 F/C-PSA at >90% sensitivity. Applying gray-zone-triggered 2-step diagnostics (T-PSA and F/T-PSA vs. C-PSA and F/C-PSA), the first-line use of C-PSA triggered 7 additional F-PSA determinations, however it detected 2 additional cancers while saving 7 unnecessary biopsies.

Conclusion: C-PSA seems moderately superior to T-PSA as a first-line test in prostate cancer diagnostics. The DAC method gives additional information to ROC analyses of subgroups when evaluating 2 markers.
STANDARDIZATION OF PSA

B.G. Blijenberg

Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, The Netherlands

Since the discovery of prostate-specific antigen (PSA) in the early 1970s, this protein has gained a huge amount of attention, both clinically and methodologically. The importance of PSA as a tumour marker was clearly shown in early clinical studies that were published between 1980 and 1990. Around 1990, several papers pointed out the wide variation (more than 100%) in results between the then available commercial and laboratory assays for PSA.

As a result of two conferences held in 1992 and 1994 in Stanford (USA), organized by Stamey, two calibrators for total and free PSA were described and developed by the National Committee for Clinical Laboratory Standards (USA). These were recognized as international standards by the World Health Organization (WHO) in 1999. Since 2005, these calibrators, WHO 96/670 for total PSA and 96/668 for free PSA, have been incorporated by most leading manufacturers of PSA methods in their assay systems, leading to a much better comparability between various PSA assays. However, despite all positive developments including improvements of PSA methodology and the availability of international standards, it still has to be accepted that a variation of at least 15% exists, depending on the concentration of PSA. Surveys in The Netherlands and Germany, as part of quality assessment schemes used in these countries, show this clearly. It must also be accepted that this situation will not change easily in the near future because of the heterogeneity of PSA in blood.

THERAPIE DES HORMONREFRAKTÄREN PROSTATAKARZINOM

M. Graefen

Martini-Klinik, Prostata-Zentrum, UKE, Hamburg, Germany


TREOSULFAN IS AN EFFECTIVE CYTOTOXIC AGENT FOR THE PROSTATE CANCER CELL LINES LNCAP, DU145 AND PC3

G. Feil1, S. Feyerabend1, J. Krug1, A. Anastasiadis1, A. Kassen2, A. Stenzl1

1Department of Urology, Eberhard-Karls University, Tuebingen; 2Medac, R&D Urooncology, Wedel, Germany

Treosulfan (TS) is a cytotoxic substance that has proved its indication in the treatment of ovarian carcinomas. The active metabolites are mono- and diepoxybutane derivates which are formed out of TS under physiological conditions by non-enzymatic, spontaneous internal nucleophilic substitution. The aim of our study was to evaluate a possible cytotoxic effect of TS on prostate cancer cell lines in vitro.

Materials and Methods: The human prostate cancer cell lines LNCaP (ATCC, USA), DU145 (I.A.Z., Munich, Germany) and PC3 (I.A.Z.), deriving from prostate cancer metastasis of
lymph node, brain and bone, respectively, were seeded at densities of 5x10^4 cells/ml and cultured in specific media supplemented with fetal calf serum, at 37°C and 5% CO₂. Each cell line was treated with various TS concentrations (Ovostat®, Medac, Wedel, Germany) from 0.5 - 500 μM for 24, 48, 72 and 96 hours. Cytotoxic activity was determined with the WST-1 assay (Roche, Penzberg, Germany) and quantified with a microplate reader at a wavelength of 450 nm.

**Results:** TS led to highly significant inhibition of proliferation and cell death in all the cell lines at concentrations ≥10 μM. After 24 hours, 10 μM TS inhibited cell proliferation and viability 2.6-fold in LNCaP (p<0.001), 1.2-fold in DU145 (p<0.0001) and 1.3-fold in PC3 cell cultures (p<0.00001). The maximum cytotoxicity was measured for LNCaP cells, with 250 μM TS after 72 hours and with 500 μM TS after 48 hours and for DU145 cells, with 90 μM TS after 96 hours, 100 μM TS after 72 hours and 500 μM TS after 24 hours. For PC3 cells, maximum cytotoxicity was determined with 150 μM TS after 96 hours, 200 μM TS after 72 hours, 300 μM TS after 48 hours and with 500 μM TS after 24 hours.

**Conclusion:** TS inhibited the proliferation of well-differentiated, hormone-sensitive (LNCaP), as well as of poorly-differentiated, hormone-refractory (DU145, PC3) prostate cancer cell lines in vitro. The maximum cytotoxicity of TS was dose- and time-dependent for each cell line. The significant sensitivity of prostate carcinoma cell lines to the genotoxic activity of TS suggests a treatment option for patients with prostate cancer.

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**POLYMORPHISMS OF HUMAN ESTROGEN RECEPTOR (ER) GENE ALPHA AND BETA IN PROSTATE CANCER CELL LINES PC-EW AND PC-OR**

C.C. Bergner¹, F. St. Krause¹, V. Zugor¹, T. Rith¹, K.M. Schrott¹, S. Endele², D.G. Engehausen¹

¹Department of Urology and ²Institute of Human Genetics, University of Erlangen, Germany

Prostate cancer is the leading cause of death among men in Western countries. Genetic alterations of the estrogen receptor gene present a higher risk of this disease. The estrogen receptor gene is found as two subtypes, α and β. In this study, the estrogen receptor α and β genes were tested in 2 human prostate cancer cell lines, the hormone-sensitive PC-EW and the hormone-independent PC-OR.

**Materials and Methods:** Genomic DNA was isolated from 2 cell lines from metastatic prostate adenocarcinoma in heterotransplanted male athymic nude (nu/nu) Balb/c mice. Mutation screening was performed by sequencing of exons 1–9 of the human estrogen receptor gene.

**Results:** No point mutations were detected in the ER gene subtypes of both cell lines. We found polymorphisms of ER-α in exons 1, 3, 4, 5, 6 and 8, and polymorphisms of ER-β in exons 3 and 9.

**Conclusion:** Point mutations of ER-α and -β are not necessary for metastatic prostate cancer; alterations in different areas of the ER genes are more often found. These polymorphisms are a part of many genetic influences that cumulate to contribute to the overall risk of developing prostate cancer.

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**RENALZELLKARZINOME BEI PATIENTEN UNTER 35 JAHREN**

V. Zugor, S. Friedel, D. G. Engehausen, G. E. Schott

Urologische Universitätsklinik mit Poliklinik der FAU Erlangen-Nürnberg, Germany


**Material und Methoden:** In einer retrospektiven Analyse der letzten 40 Jahre wurden 34/2510 Nierentumorpatienten (1.4%), die zum Zeitpunkt der Diagnose unter 35 Jahre alt waren, berücksichtigt. Die Gesamtüberlebensrate wurde über einen Zeitraum von 5 und 10 Jahren postoperativ mit Hilfe von Kaplan-Meiers Überlebensanalyse beobachtet.

**Ergebnisse:** Das mittlere Alter der Patienten lag bei 29 Jahren, Median 30.9. Das Patientenkollektiv bestand zu 76.5% aus Männern und zu 23.5% aus Frauen. Histologisch zeigten sich bei allen Patienten Renalzellkarzinome, 31 klarzellig und 3 nicht klarzellig. Zum Zeitpunkt der Diagnose waren 55.9% der Patienten symptomatisch. 29.4% der Patienten hatten Begleiterkrankungen. 67.6% der Patienten wurden durch transabdominale Nephrektomie mit systemischer LD operativ versorgt. Es wurde ein lokal fortgeschrittenes Tumorstadium (>pT3a) bei 14.7%, pN+ bei 14.7% und Fernmetastasen bei 8.8% nachgewiesen. Die 5 Jahres-Überlebensrate lag bei 79.4%. Die 10 Jahres-Überlebenszeit betrug 73.5%. 8 Patienten verstarben tumorbedingt und 2 nicht tumorbedingt.

**Schlussfolgerung:** Auch bei jüngeren Patienten muss bei RF der Niere in der Regel von einem RCC ausgegangen werden. Die biologische Aggressivität, histologisches Muster und die Prognose unterscheiden sich nicht von älteren Patienten, wobei der Anteil an symptomatischen Tumoren höher ist.
EARLY DETECTION OF BLADDER CANCER IN HEMATURIA PATIENTS

P. Oehr

University Bonn, Germany

Due to lack of easy to handle point of care (POC) assays and no significant changes in routine follow-up of hematuria patients with suspected cancer, the number of cases with early detected urinary bladder cancer has remained unchanged during recent decades. The objective of the presented study was to investigate the diagnostic power of the new Matritech NMP22-BladderChek® POC assay.

Materials and Methods: This qualitative POC assay is based on a 30-min chromatographic analysis of 4 drops of freshly voided urine at room temperature, including antigen detection by anti-NMP22-(nuclear matrix protein) antibodies. Excluding patients with urocystitis, stones, urinary tract infections and incorporated catheters, and prior to endoscopy, 212 hematuria patients in 16 urologic practitioners sites were investigated.

Results: Hematuria did not influence the test results. There were 18 true-positive, 186 true-negative, 4 false-positive and 4 false-negative results. This led to 82% sensitivity, 98% specificity, as well as to 82% for the positive and 98% for the negative predictive value. In a subgroup of 113 patients (14 tumor and 99 tumor-free), NMP22-BladderChek® and cytology were determined and evaluated simultaneously (10 urology sites). NMP22-BladderChek® had 86% sensitivity and 98% specificity compared to cytology with 57% and 97%, respectively. When both tests were positive, every patient turned out to have urinary bladder cancer (8 out of 14 cases).

Conclusion: The superior sensitivity of NMP22-BladderChek® over cytology, at a specificity of 98% and a negative predictive value of 98%, substantiate that it is an easy to handle cancer screening assay. However, this does not exclude professional cytology by well-trained personnel. In such a case, the combined use of these diagnostic procedures can result in a highly reliable non-invasive screening result for a further subgroup: under the condition "both tests are positive", no false-positive results arose, making this assumption a 100% tumor inclusion criterion in 67% of the patients who had developed an, as yet undetected, urinary bladder cancer. In addition, a negative predictive value of 98% for NMP22-BladderChek® is an excellent exclusion criterion. Independently, NMP22-BladderChek® can detect 82% to 86% urinary bladder cancer in suspected hematuria with a specificity of 98%. These results and conclusions from the different screening criteria, with and without cytology, give evidence that NMP22-BladderChek® will change the routine patient management of hematuria patients with suspected urinary bladder cancer.

NUKLEÄRES MATRIX PROTEIN 22 (NMP22), EIN TUMORMARKER BEIM BLASENKARZINOM – EIN METHODENVERGLEICH

A. Oertl1, S. Balan2, R. Manu3, H. Sauer-Eppel2, D. Jonas1, G.M. Oremek2

Klinik für Urologie1, Klinikum der Johann Wolfgang Goethe Universität, Zentrallabor2, Zentrum der Inneren Medizin, Klinikum der J.W. Goethe Universität, Frankfurt am Main, Germany, Centrul de Chirurgie Urologica3, Dializa si Transplant Renal, Institutul Clinic Fundeni, Bucuresti

In the following study the diagnostic accuracy of the Nukleärer Matrix-Protein 22 (NMP22) was determined. Two methods were used for the determination of NMP22, the "Two-Side-ELISA" and the Bladder Chek™. Both methods showed a sensitivity dependence on grading. The sensitivity was 38% for grade 1 and 77% for grade 3. Additional the cytology was used for evaluation. We obtained the following sensitivities: for grade 1 was 29%, grade 2 was 55%, and grade 3 was 70%. This work confirms the high diagnostic value of NMP22 in the control of patients with bladder tumors. False positive results occur in inflammatory processes of the bladder or bacterial urinary infections.

ADJUVANT INSTILLATION TREATMENT WITH A STANDARDIZED MISTLETOE EXTRACT TO PREVENT RECURRENCE OF SUPERFICIAL URINARY BLADDER CANCER

C. Leiber1, U. Elsässer-Beile1, P. Bühler1, U. Wetterauer1, U. Mengs2

1Department of Urology, Freiburg; 2Madaus AG, Köln, Germany
Patients with superficial bladder cancer are mainly treated by transurethral tumor resection and adjuvant intravesical therapy with Bacillus Calmette-Guerin (BCG), which was shown to reduce tumor recurrence significantly. However, serious side-effects of this treatment promoted the search for other immunoactive substances, which, to date, have failed to show equal efficacy with BCG. Therefore, the aim of the present study was to evaluate the effect of intravesical mistletoe extract (ME) with respect to tolerability and recurrence rate.

Materials and Methods: In a phase I/II clinical trial, an aqueous ME, standardized to mistletoe lectin, was administered intravesically to 30 patients with superficial urinary bladder cancer of stages pTa G1 (n=6), pTa G2 (n=14) and pT1 G2 (n=10). After transurethral resection, each patient received 6 instillations of 50 ml of the extract at weekly intervals, with mistletoe lectin concentrations between 10 ng/ml and 5,000 ng/ml. A local historical control group consisted of 18 patients with pTa G2 (n=5) and pT1 G2 (n=13) tumors that were treated with 6 BCG instillations after transurethral resection.

Results: The tolerability of the ME was very good. None of the 30 patients had local or systemic side-effects. Comparison of the 24 study patients with pTa G2 and pT1 G2 tumors and the 18 BCG-treated local controls revealed a recurrence rate of 8/34 (33%) in the ME group and 5/18 (28%) in the BCG group, within the observation time of 12 months.

Conclusion: Standardized ME could be a potential alternative adjuvant therapy for superficial bladder cancer.
Materials and Methods: Paraffin-embedded slides of vaginal tissue (8), vulva tissue (8), penis shaft tissue (8) and penis gland tissue (8) were fixed and incubated with monoclonal antibodies against SLeX (IgM), SLea (IgM), LeY (IgM) and TF (IgM). The staining reaction was performed with the ABC reagent. The intensity of the immunohistochemical reaction on images of the slides was analyzed using a semi-quantitative score.

Results: A moderate expression of both Sialyl Lewis antigens was found in the urethra of the penis shaft and a strong expression of SLeX and SLea in squamous epithelial tissue of the vulva. A moderate expression of TF was observed in male squamous epithelial tissues of the gland and foreskin. A faint expression of Le Y was observed in male epithelial tissue.

Conclusion: Expression of SLeX, SLea, LeY and especially of the TF antigen in normal non-malignant epithelial tissue is surprising and can be explained by the immunosuppressive function of this tissue which is involved in human reproduction. In addition, TF expression seems to be exclusively restricted to the epithelial tissue of the penis gland and foreskin.

54 VERSCHIEBUNG DES F/T-PSA-QUOTIENTEN BEI NIERENINSUFFIZIENZ: AUSWIRKUNG AUF DIE FRÜHERKENNUNG DES PROSTATAKARZINOMS BEI PATIENTEN MIT TERMINALER NIERENINSUFFIZIENZ

K. Fischer, A. Hamza, A. Wicht, H. Loertzer, P. Fornara

Universitätsklinik und Poliklinik für Urologie, Halle, Germany


55 TREATMENT OF ADVANCED BLADDER CANCER BY IRINOTECAN – A USEFUL ALTERNATIVE?

S. Landsmann1, Th. Papadopoulos2, D.G. Engehausen1, K. M. Schrott1, F. St. Krause1

Departments of 1Urology and 2Pathology, FAU, Erlangen, Germany

Chemotherapy is, in spite of unsatisfactory regression rates, the therapy of choice in the palliative treatment of advanced...
urothelial carcinoma of the bladder. The new zytostatic drug irinotecan was tested in an animal experiment of poorly-differentiated urothelial carcinoma, having shown good response rates in patients with advanced colorectal cancer.

**Materials and Methods:** The effects of irinotecan were tested in single- and multi-application, in combination with docetaxel. The local tumor size and the weight of the mice were evaluated in short-term periods. The histopathology of the local tumor and of defined and suspect organs or lymph nodes was realized after death.

**Results:** Regarding the regression rate, local tumor size and age at time of death, there was no benefit over the control group (without therapy). No metastatic diseases were diagnosed. As side-effects, in all groups hepatic and bronchopulmonary diseases were documented.

**Conclusion:** Irinotecan should not be used either as a single-agent or in combination with docetaxel in the treatment of advanced urothelial cancer of the bladder.

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**Lung**

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**AUSSAGEKRAFT VON ENTZÜNDUNGSPARAMETERN UND TUMORMARKERN BEI BRONCHIALKARZINOMEN**

Ch. Hansen¹, H. Sauer-Eppel¹, R. Siekmeier²,³, G.M. Oremek¹

¹Zentrallabor, Zentrum der Inneren Medizin, Klinikum der J.W. Goethe Universität, Frankfurt am Main; ²Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM), Bonn; ³Fachhochschule Bonn-Rhein-Sieg, St. Augustin/Rheinbach, Germany


**Material und Methode:** In dieser Studie wurde die Aussagekraft von Entzündungsparametern, wie CRP, Tumornekrosefaktor und der Tumormarker CEA, NSE, SCC, Cyfra 21-1 und ProGRP an 245 Patienten überprüft. Die Konzentrationen der Tumormarker CEA, NSE, Cyfra 21-1 wurden am Elecsys 2010, SCC am IMx-Analyser und ProGRP am SLT-Spectra-Photometer gemessen.

**Resultate:** CRP und TNF waren in 75% der Fälle im pathologischen Bereich. Bei 95% Spezifität wurden Sensitivitäten für die einzelnen Tumormarker ermittelt, die zwischen 47% und 75% lagen. Bei nichtkleinzelligen Bronchialkarzinomen erwies sich Cyfra 21-1 als Marker der Wahl mit einer Sensitivität von 68%. Dagegen bei kleinzeligen Bronchialkarzinomen sind die Sensitivitäten von NSE und ProGRP fast gleich und liegen bei 49% bzw. 51%.

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**PROGNOSTIC MODELS IN NON-SMALL CELL LUNG CANCER INCLUDING 60 SERUM MARKERS AND CLINICAL VARIABLES**

St. Holdenrieder¹, J. von Pawel², H. Raith², K. Feldmann², U.-H. Stenman³, D. Nagel¹, P. Stieber¹

¹University Hospital, Grosshadern, Munich; ²Asklepios Hospital, Gauting, Germany; ³University Central Hospital, Helsinki, Finland

Currently available data concerning the prognostic relevance of biochemical markers in non-small cell lung cancer (NSCLC) are conflicting.

**Materials and Methods:** In a prospective study on 300 patients with newly-diagnosed, advanced NSCLC undergoing chemotherapy, 60 pretherapeutic parameters were investigated including clinical factors such as "classic" laboratory markers and oncological biomarkers. First, clinical parameters with independent prognostic relevance were selected by Cox regression analysis. Second, the linearity of all the biochemical markers was analyzed; for non-linear parameters, appropriate cut-offs were defined. Third, each marker was tested, with clinical factors, for its prognostic relevance. Fourth, the remaining independent variables were analyzed simultaneously by Cox regression, using both forward and backward selection in parallel. Depending on characteristic marker panels, prognostic models were established.

**Results:** The median survival of 172 patients who died was 6.3 months; the median observation time of 128 censored patients was 7.3 months. Of the clinical factors, performance score, weight loss, metastases other than lung and mode of therapy showed prognostic relevance. Fourth, the remaining independent variables were analyzed simultaneously by Cox regression, using both forward and backward selection in parallel. Depending on characteristic marker panels, prognostic models were established.

**Conclusion:** The median survival of 172 patients who died was 6.3 months; the median observation time of 128 censored patients was 7.3 months. Of the clinical factors, performance score, weight loss, metastases other than lung and mode of therapy showed prognostic relevance. By testing biochemical variables with these clinical factors, a mass remained prognostically significant. On analyzing all the prognostically relevant clinical and biochemical variables simultaneously, characteristic multivariate models were...
established, depending on the definition criteria: A) all clinical and laboratory variables and lung markers available pre-therapeutically, B) and, additionally, all other pre-therapeutic tumor markers, C) all clinical and laboratory variables and lung markers available before cycles 1 and 2, D) and, additionally, all other pre-therapeutic tumor markers. These models were applied on the various performance status groups further subcategorizing the prognostic groups.

Conclusion: The standardized procedure for establishing prognosis enables a fair comparison of clinical, "classic" laboratory and oncological parameters. The resulting multivariate models are helpful for prognostic stratification, independently of clinical factors such as performance score.

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A FUZZY-CLASSIFIER USING A MARKER PANEL FOR THE DETECTION OF LUNG CANCERS IN ASBESTOSIS PATIENTS

J. Schneider, N. Bitterlich

1Klinikum der Justus-Liebig Universität, Giessen; 2Medizin & Service GmbH, Chemnitz, Germany

The aim of this study was to evaluate the diagnostic power of a Fuzzy-classifier and a marker panel (CYFRA 21-1, NSE, CRP) for the detection of lung cancers, in comparison to asbestosis patients at high-risk of developing lung cancer. In this prospective study, a fuzzy-classifier was generated with the data of 216 primary lung cancer patients and 76 patients suffering from asbestosis. The patients and the controls were recruited from the university clinics in Giessen. At 95% specificity, it was possible to detect, with this tool, non-small cell lung cancers in 77% at stage I (n=30), in 95% at stage II (n=22), in 98% at stage III (n=56), in 92% at stage IV (n=50) and small cell lung cancers in "limited disease" status (n=21) in 90.7% and while at "extensive disease" status (n=37) in 97.3%. In contrast, single markers had a detection rate significantly far below these levels. The application of the classifier was examined on an independent collective of 38 non-small cell lung cancers and 77 asbestosis patients. The latter underwent stationary rehabilitation in clinics of Bad Reichenhall or Falkenstein, Germany. The fuzzy-classifier showed correct negative classification in 75 of the 77 cancer free-asbestosis patients. The overall sensitivity for lung cancer detection in high-risk populations was 73.6%. All large cell carcinomas could be detected. The positive predictive value was 77.7%, while the negative predictive value reached 94.8%. With the fuzzy-classifier and a marker panel, a reliable diagnostic tool for the detection of lung cancers in a high-risk population is available.

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PROGNOSTIC VALUE OF A TUMOR MARKER INDEX (TMI) BASED ON CYFRA 21-1 AND CEA IN NSCLC PATIENTS TREATED BY SURGERY

Th. Muley, H. Fetz, M. Meister, H. Hoffmann, H. Dienemann, W. Ebert

Thoraxklinik am Universitätsklinikum, Heidelberg, Germany

Recently, we proposed an algorithm by which the most important non-small cell lung cancer (NSCLC) tumor markers, CYFRA 21-1 and CEA, could be combined into a new variable for prognostic purposes. This variable was called the tumor marker index (TMI) (Anticancer Res 24: 1953-1956, 2004). We reported on a negative prognostic impact of increased CYFRA 21-1 and CEA levels expressed as TMI, especially in the early stages of operated NSCLC patients. The aim of this analysis was to reassess the prognostic impact of TMI in a larger number of patients.

Materials and Methods: 735 completely resected (R0) early stage NSCLC patients, exclusively treated by surgery between 1995 and 2002, entered the analysis. 448 patients were classified as p-stage I and 287 as p-stage II. CYFRA 21-1 and CEA were measured using commercially available tests (Boehringer/Roche, Mannheim, Germany). TMI, which represents the geometric mean of normalized CYFRA 21-1 and CEA values, was calculated as described recently. Discriminatory values, which differentiate between prognostic groups were identified by the crit-level procedure described by Abel et al. (Meth Inf Med 23: 154-156, 1984). The corresponding value for TMI was found to be 0.54. Survival was analyzed according to the method of Kaplan and Meier, while multivariate analysis was done using Cox proportional hazard model.

Results: Patients with a TMI less than 0.54 had a significantly better 5-year survival rate (median survival) as compared to patients with a TMI above this value: 70.2% (93 months) versus 54.7% (76 months), p<0.001. The effect of TMI was most prominent in the subgroup of p-stage I NSCLC. The 3-year survival rate (5-year survival rate) was 86.3% (76.1%) for a TMI value less than 0.54 and 71.1% (58.8%) for a TMI greater than this cut-off point (p=0.001). In multivariate analysis, an elevated TMI was a significant prognostic factor in p-stage I (p=0.001) with a relative risk of 2.0 (95% CI: 1.3-3.1).

Conclusion: In patients with completely resected early stage NSCLC, the calculation of TMI based on preoperative CYFRA 21-1 and CEA levels may help to distinguish between patients with good or impaired prognosis. However, the usefulness of TMI as a stratification marker,
which assigns operated early-stage NSCLC patients to adjuvant chemotherapy and/or radiotherapy schedules, remains to be proved.

60 INCREASED TUMOR VOLUME AND TUMOR MARKER INDEX (TMI) ARE NEGATIVE PREDICTORS OF OUTCOME IN COMPLETELY RESECTED STAGE I/II NSCLC PATIENTS

Th. Muley, H. Fetzer, M. Meister, H. Hoffmann, H. Dienemann, W. Ebert

Thoraxklinik am Universitätsklinikum, Heidelberg, Germany

Although serum levels of tumor markers are thought to reflect the tumor burden in patients with non-small cell lung cancer (NSCLC), there is only a weak correlation with the stages according to the UICC classification.

Materials and Methods: The relationship between tumor volume and marker levels in 495 NSCLC patients, operated on between 1996 and 1998, were analyzed. Among them, there were 277 completely resected (R0) stage I and II patients. The tumor volume was assessed by measurement of the tumor diameters (length = l, height = h, width = w) of resected tumors and calculation of an ellipsoid body (V_path = \( \frac{4}{3} \pi \frac{l}{2} \frac{h}{2} \frac{w}{2} \)). In addition, volumes were calculated from radiographs after measurement of diameters (h, w), using an adapted formula (V_rad = \( \frac{4}{3} \pi \frac{h}{2} \frac{w^2}{2} \)). The tumor marker index (TMI), which represents the geometric mean of normalized CYFRA 21-1 and CEA values, was calculated, as recently described (Anticancer Res 24: 1953-1956, 2004).

Discriminatory values, which differentiate between prognostic groups were identified by the crit-level procedure described by Abel et al. (Meth Inf Med 25: 154-156, 1984) for each parameter. These cut-off values amounted to 13.7 cm\(^3\) for V_path and 14.1 cm\(^3\) for V_rad. The corresponding value for TMI was found to be 0.54. Spearman rank correlation was used for analysis of the relationship between the parameters; survival was analyzed according to Kaplan and Meier. Multivariate analysis was done using the Cox proportional hazard model.

Results: In contrast to advanced stages, there was a clear-cut prognostic impact of tumor volume and TMI in the early stages (I and II) of NSCLC. Patients (stage I+II, R0) with a V_path less than 13.7 cm\(^3\) had a significantly better 5-year survival rate compared to patients with volumes greater than 13.7 cm\(^3\) (76% vs. 45.5%; p=0.0001). A similar result was found for V_rad (67.9%, 51.7%, p=0.04). Patients with a TMI < 0.54 had a 5-year survival rate of 76.7%, compared to 45.7% in patients with a TMI>0.54 (p<0.0001). The correlation data were found to be r=0.7 (p<0.01) between V_path and V_rad. There was only a weak correlation of r=0.45 (p=0.01) between TMI and V_path, and one of r=0.38 (p=0.01) between TMI and V_rad. Besides age (>70 years) and gender, both V_path (>13.7 cm\(^3\)) and TMI (>0.54) retained significance in the multivariate Cox model, with a relative risk of 2.09 (95%CI: 1.27-3.44) (p=0.004) and 2.01 (95%CI:1.12-3.61) (p=0.019), respectively.

Conclusion: In conclusion, increasing values of TMI and tumor volume have a negative prognostic impact on survival in the early stages of NSCLC as opposed to advanced stages. There is only a weak relationship between tumor markers expressed as TMI and tumor volume. Additional factors, such as degree of fibrosis or necrosis in the tumor mass, may influence the marker levels.

61 PROGRP AND NSE IN THERAPY MONITORING OF PATIENTS WITH SMALL CELL LUNG CANCER

E. Wojcik, J. Kulpa, B. Sas-Korczyńska, Z. Stasiak, St. Korzeniowski

Center of Oncology, M. Skłodowska-Curie Memorial Institute, Cracow Division, Poland

Small cell lung cancer (SCLC) is a relatively common, aggressive neoplasm. Although SCLC is highly sensitive to chemotherapy and radiotherapy, cure is still not satisfactory, with only 5-10% of patients remaining alive and disease-free at 5 years. In patients with limited extent of disease, chemotherapy combined with chest and cranial irradiation causes a rise in the percentage of total regression, prolongation of remission time and overall survival. The aim of this study was the evaluation of ProGRP and NSE utility during treatment of SCLC patients.

Materials and Methods: ProGRP, NSE, CYFRA 21-1 and LDH were determined, before initial treatment, each course of chemotherapy and after restaging, in a group of 64 patients with SCLC-LD receiving combined chemo- and chest as well as prophylactic cranial radiotherapy.

Results: The elevated tumor marker levels before treatment were, respectively: for ProGRP – 76.6%, NSE – 59.4%, CYFRA 21-1 – 23.4% and LDH – 17.2%. Whereas, the abnormal levels of NSE, CYFRA 21-1 and LDH were presented by less than 7% of patients before the second course of chemotherapy, increased levels of ProGRP were still shown in 53% of them. In the subsequent chemotherapy courses, a further drop of ProGRP levels and only a slight tendency to NSE decrease were observed. Elevated ProGRP concentrations were found 4 times more
frequently than NSE, even 2 months after finishing therapy. Between the group of patients with complete and partial remission after treatment, there were no significant differences in the concentrations of tumor markers, however, the patients with clinical progression during or at the end of therapy, in comparison to those with remission, initially presented significantly higher ProGRP and NSE levels. Also assessed was the influence of performance status, weight loss, prophylactic cranial irradiation (PCI) administered and also the initial levels of tumor markers for disease-free and overall survival of SCLC-LD patients. The study showed that shorter survival was presented by the group in which PCI was not given, as well as with ProGRP higher than 410 ng/ml, NSE higher than 46 ng/ml and CYFRA higher than 3.5 ng/ml. No PCI or high levels of NSE were found to be unfavorable independent prognostic factors for those patients.

Conclusion: 1. Changes of ProGRP seem to be a more precise tool than NSE for the assessment of the reaction of SCLC-LD patients to the therapy. 2. The NSE concentration, apart from the administered prophylactic cranial irradiation, is an independent prognostic factor in such a selected group of patients.

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MESOTHELIN: A NEW MARKER FOR THE DETECTION OF MESOTHELIOMA
P. Stieber, R. Hatz1, S. Holdenrieder, J. Reinmiedl2, K. Hofmann, C. Ruedel, A. Schalhorn3
Institute of Clinical Chemistry, Departments of 1Surgery and 2Internal Medicine III, University Hospital, Munich; 3Asklepios Hospital, Gauting, Germany
Soluble mesothelin-related peptide – a 40kDa protein which is expressed and released by mesothelioma cells and detected by the antibodies OV569 and 4H3 – is supposed to be a promising marker for mesothelioma detection in blood.

Materials and Methods: Soluble mesothelin-related peptide was examined in the sera of 148 healthy individuals, 26 patients with mesothelioma, 106 patients with various benign lung diseases and 179 other benign disorders (gastrointestinal, gynecological, urological), 428 patients with malignant lung diseases (among them 195 squamous, 119 adeno-, 59 large cell and 45 small cell lung cancer) and 596 patients with malignant diseases other than lung. The Mesomark-ELISA (Fujirebio, USA; CIS Biointernational, France; Schering, Germany) was used.

Results: The median mesothelin-related peptide concentrations were lowest in healthy individuals (1.0 nM; range 0.4-8.0 nM) and other benign disorders, lung cancer (1.4 nM; range 0.2-12.7 nM), ovarian cancer (2.1 nM, range 0.3-23.7 nM) and mesothelioma (2.0 nM; 0.8-48 nM). The 95th percentiles were comparable for benign lung diseases as well as other benign and malignant diseases, other than lung and lung cancers (3.4 nM) except mesothelioma, but significantly superior for ovarian cancer (18.4 nM) and mesothelioma patients (39.6 nM). At 95% specificity for healthy individuals (1.9 nM), the sensitivities for benign lung diseases, lung cancer and mesothelioma were 25%, 25% and 50%, respectively; at 95% specificity for benign lung diseases (3.4 nM), the sensitivities for lung cancer and mesothelioma were 4% and 26%, respectively.

Conclusion: The first clinical data confirm the high specificity of soluble mesothelin-related peptide for mesothelioma in high concentrations. Further investigations, particularly including patients with benign and malignant pleural effusions and other effusions, are ongoing to show the diagnostic value of this promising marker.

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TUMOR MARKERS IN EXUDATES
L. Holubec Jr., O. Topolcan, Pesek, T. Hajek, V. Polivkova, S. Svobodova, L. Bartunek, J. Finek
University Hospital and Medical Faculty Charles University Pilsen, Czech Republic
The aim of this study was to find out the importance of tumor marker assessment in patients with cancer.

Materials and Methods: The tumor markers (CEA, CA 19-9, CA 15-3, CA 15-3, TK and cytokeratines were assessed in 30 patients with exudates of cancer origin, in 30 patients with inflammatory exudates and in 30 patients with heart failure disease.

Results: The tumor markers CA 125, TPS and TPA were elevated in all cases. The tumor markers CEA, CA 19-9, CA 15-3, TK and cytokeratines were assessed in 30 patients with exudates of cancer origin, in 30 patients with inflammatory exudates and in 30 patients with heart failure disease.

Conclusion: The assessment of the tumor markers CEA, CA 19-9 and CA 15-3, of the control group (heart failure disease) and in a group with inflammatory lung diseases, were mostly within the reference ranges. The only elevated levels of TK were observed in viral lung infections. The sensitivities of the individual tumor markers CEA, CA 19-9 and CA 15-3 were 55-70% at 95% specificity based on the cancer disease type. The sensitivity of the cytology examination was only 30 - 50% at 95% specificity.

Conclusion: The assessment of the tumor markers CEA, CA 19-9 and CA 15-3 seems to be a promising parameter for differential diagnosis of chest exudates. However, it would not be useful to assess CA 125, TPA and TPS. Elevated levels of these tumor markers reflect mesothelial cell damage, not the origin of the exudates.
64 PRECLINICAL AND CLINICAL EFFECTS OF ERYTHROPOIETIN IN THE MANAGEMENT OF ANAEMIA IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

W. Dempke
TTG Bochum, TZR, Universitätsstrasse 142, Bochum, Germany

The myelosuppressive toxicities of chemotherapy are one of the principal reasons for the overall failure of some agents to have a meaningful impact on responses and survival in cancer, and anaemia is a common side-effect of almost all cytostatic drugs used clinically. As regulators of haematopoietic homeostasis, cytokines mediate cellular proliferation, differentiation and survival. Among the various growth factors currently available, erythropoietin (EPO) is the principal factor responsible for the regulation of red blood cell production during steady-state conditions and for accelerating recovery following cytostatic bone marrow depletion. Many studies have provided evidence that EPO is able to correct and to prevent anaemia in approximately 64% of cancer patients, with subsequent reduction of blood transfusion requirements. Among the prognostic factors for survival in patients with advanced non-small cell lung cancers (NSCLC), anaemia is associated with reduced quality of life and a poorer prognosis. Recently, some studies have suggested a possible relationship between increased haemoglobin levels and survival in NSCLC patients. Furthermore, there is evidence that NSCLC patients with high haemoglobin levels have a better outcome after radio- or chemotherapy. Although the highest rate of transfusion-dependent patients (34%) has been observed in those suffering from NSCLC, there are no universally accepted guidelines addressing the most effective methods of monitoring NSCLC patients for anaemia. Thus, further randomized, controlled trials are needed to evaluate the effect of any therapeutic intervention against anaemia on survival and disease control in patients with NSCLC.

65 INFLUENCE OF IRON STATUS ON HEMOGLOBIN, FATIGUE AND PROGNOSIS IN HEAD AND NECK CANCER

T. Gorbatov1, O. Micke2, J. Büntzel1
1Department of ORL, Nordhausen; 2Department of Radiooncology, Münster, Germany

Hemoglobin has been identified as one of the most important prognostic markers in head and neck cancer, as well as the main parameter in the treatment of fatigue. The presented study aimed to analyze the impact of iron status on hemoglobin / prognosis and, secondly, the relationship between iron status and fatigue status in a group of patients with head and neck cancer.

Materials and Methods: The data of 149 patients (134 men, 15 women, median age 59 years, range 26-88) were included in the first part of the analysis. The iron status parameters were serum iron, transferrin and ferritin. The follow-up data were recorded in the patient’s files of our out-patient department. One hundred patients (88 men, 12 women, median age 59 years, range 26-85) were included in the second analysis. Fatigue was categorized through a visual analog scale and the standardized questionnaire SF 12.

Results: The subgroup of anemic HNC patients was characterized by increased percentages of decreased serum iron (60%), decreased transferrin (40%) and increased ferritin (46%), compared to the whole investigated population (decreased iron 45%, decreased transferrin 35%, increased ferritin 36%). The non-anemic patients had a significantly better prognosis. Increased rates of fatigue were seen in patients with changed iron status as well as significant anemia.

Conclusion: The results indicate that the changed iron status has an important influence on prognosis, as well as the development of fatigue, in HNC patients. Studies are necessary to investigate the influence of iron substitution on both parameters.

66 SERUM ZINC DURING HEAD AND NECK CANCER

J. Büntzel1, A. Garayev1, O. Micke2
1Department of ORL, Nordhausen; 2Department of Radiooncology, Münster, Germany

Decreased zinc serum levels are well known characteristics in patients with head and neck cancer. The aim of this study was the observation of this parameter during the tumor progression of this subgroup.

Materials and Methods: Twenty-one patients (20 male, 1 female, median age 62 years) were enrolled and blood samplings at the beginning of the disease and during the follow-up visits to our out-patient department. The zinc serum concentration was measured by atom absorption spectrometry (Parker 600). The clinical data (survival, tumor status) were recorded.

Results: At the end of the follow-up period (median 24 months, range 9-40), 11 patients had died, 2 are living with cancer and 8 are tumor-free. A continuously decreasing zinc
serum level was observed in 7 out of 11 of the patients who had died, in 2 out of 2 tumor-progressing patients and in 2 out of 8 tumor-free patients still alive. The significant reduction of zinc serum levels was measured in a median of 5 weeks (range 2-7 weeks) before a patient’s death.

**Conclusion:** The measurement of zinc levels seems to offer additional information for the palliative situation in head and neck cancer. Further studies and larger data pools are necessary to investigate this hypothesis.

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**INFLUENCE OF AMIFOSTINE ON THE LATE TOXICITIES DUE TO MULTIMODAL THERAPY IN HEAD AND NECK CANCER**

J. Büntzel, O. Micke, M. Glatzel

The late toxicities due to multimodal therapy of advanced head and neck cancers were analyzed. The impact of cytoprotection with amifostine was the specific objective.

**Materials and Methods:** 531 patients (442 men, 89 women) with head and neck cancer were included into this prospective study. 313/531 had received amifostine before radio(chemo)therapy, while 218 control subjects had not received any kind of cytoprotection before irradiation. Primary radiochemotherapy was performed in 143 patients, while adjuvant radiation was administered in 388 patients. The follow-up examination was done at our out-patient department 2 years (median) after the primary therapy.

**Results:** Late xerostomia was seen in 480/531 patients. Altered taste was reported by 183/531. Fibrotic tissue reactions were registered in 104/531 patients. These symptoms were significantly reduced by amifostine. No influence was seen in interstitial lymph edema (46.3%) or stenosis of the cervical esophagus (16.4%). Secondary symptoms, such as posttreatment pain (12.2%) or dysphagia (73.3%), were also significantly reduced.

**Conclusion:** The administration of amifostine offers a opportunity to reduce the important long-term toxicities for survivors of head and neck cancer.

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**MUTAGENICITY TESTING OF MOUTHWASH BRANDS**

R.E. Friedrich

Branded mouthwashes are under scrutiny for possibly causing oral cancer. Therefore, the mutagenicity of different mouthwash brands was tested in vitro, using Ames tests. They were also tested in the UDS assay on primary rat hepatocytes. Four brands were additionally tested in the M2-C3H mouse fibroblast malignant transformation assay. The different brands gave consistently negative results in the Ames test, the UDS assay and the transformation assay. These results indicate that the tested mouthwash brands are unlikely to present a mutagenic or carcinogetic hazard. However, these findings, derived from an in vitro study, can not imitate the plethora of pharmacologically or biochemically active agents which continuously pass through the oral cavity, thereby potentially reactive with each other and the oral mucosa. **Supported by Hamburger Stiftung zur Foerderung der Krebsbekämpfung, Germany.**

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**GROWTH TYPES OF PLEXIFORM NEUROFIBROMAS ON MAGNETIC RESONANCE IMAGES OF THE UPPER LIMB IN NEUROFIBROMATOSIS TYPE 1 PATIENTS**

R.E. Friedrich, C. Fünsterer, V.-F. Mautner

Neurofibromatosis type 1 (NF1) is an autosomal dominant inherited disease affecting about 1 in 3000 humans. Benign tumors of the peripheral nerve sheath, the neurofibromas, are the hallmark of the disease. Plexiform neurofibroma arise from peripheral nerves of any body region and are a frequent cause of disfigurement and impaired organ function. They are regarded as precancerous lesions. From recent studies of magnetic resonance images (MRI), we were able to define subtypes of the plexiform neurofibromas. These distinctions were suitable for surgical treatment planning. The classification was adapted to cases with plexiform neurofibroma of the upper limb in order to provide an image-based therapeutic approach.

**Materials and Methods:** The MRI of the upper limb of 12 patients with NF1 were investigated. All images were acquired with and without contrast enhancement. All patients fulfilled the updated diagnostic criteria for NF1 proposed by the US National Institute of Health. In all but one patient, the radiographic investigation was performed to identify the extension of a benign lesion. One patient with a history of MPNST was investigated to diagnose the extension of an axillary metastasis with a history of a distal primary tumor 10 years previously. In all patients, the diagnosis was histologically-confirmed on representative specimens of the resected tumor.
Results: Three types of growth were distinguishable: the superficial plexiform neurofibroma is a tumor forming a layer of thickened skin, sometimes folded and growing to a large extent. In these tumors the muscles are not affected. The displacing type of plexiform neurofibroma arises from subcutaneous nerves or nerve roots. The tumor might occur singularly or can grow along the entire length of a peripheral nerve, thereby causing crowding that can be associated with the palpation sign called the "bag of worms". Invasive plexiform neurofibromas are tumors that invade the surrounding organs, e.g. muscle or fascia. They usually extend from the epidermis and can grow through the entire limb and might be associated with skeletal deformity. MPNST is characterised by inhomogeneous contrast enhancement indicating areas of necrosis.

Conclusion: The distinctions of subtypes of plexiform neurofibroma made on MRI are a useful tool for surgical treatment planning. Superficial tumors can be resected without risk to neural function. However, the extent of the tumor might be crucial to gain complete resection. The resection of displacing tumors parallels the function of the nerve as tumor resection is identical to nerve resection. The invasive tumors can not completely be resected except possibly for curative therapy in an early growth stage, preferentially in children. The distinction published here might be helpful for treatment planning and medical advice to NF1 patients with plexiform neurofibroma of the upper limb. Supported by Deutsche Krebshilfe and Deutsche Forschungsgemeinschaft (Project FR 1035/6-1), Germany.

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RAPIDLY PROGRESSIVE AND METASTATIC MUCOEPIDERMOID CARCINOMA OF THE MINOR SALIVARY GLAND

R.E. Friedrich¹, R. Klapdor²

¹Maxillofacial Surgery Clinic, Eppendorf University Hospital; ²Praxis Rothenbaum, ZeTDT Hamburg, Germany

Mucocoeidermoid carcinoma (MEC) of the salivary gland is a rare entity. A distinction between 2 variants has been proposed: the low-grade tumor with a favorable prognosis and the high-grade tumor with a poor prognosis. Indeed, MEC is a cancer with a relatively favorable outcome, with more than 90% of patients surviving for more than 5 years after diagnosis, reduced to about 70% after 10 years. This excellent prognosis might contribute to the unacceptable retention of the term "mucocoeidermoid tumor" in the medical terminology of current textbooks. However, the distinction of MEC by grading is a guideline only and it is not appropriate to use the histological terms for prediction in individual cases. The rapid fatal outcome of a patient with MEC is described in order to emphasize the malignant characteristics of this tumor and the possible application of tumor markers for diagnosis in metastasizing MEC.

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CURRENT VALUE OF SERUM TUMOR MARKERS IN DIAGNOSIS AND FOLLOW-UP OF ORAL SQUAMOUS CELL CARCINOMA

R.E. Friedrich¹, R. Klapdor²

¹Maxillofacial Surgery, UKE; ²ZeTDT, Hamburg, Germany

Oral squamous cell carcinoma (OSCC) is one the most frequent malignant tumors worldwide. Ablative surgery is still the therapy of choice, if applicable. The 5-year overall survival of OSCC patients has not markedly improved over the past 40 years, despite many efforts in terms of extension of ablative surgery, combined radiotherapy and surgery, or the induction of tumor necrosis following variable protocols of chemotherapy. The limited therapeutic success of OSCC and the improvement in diagnostics led several working groups to study the expression of proteins that might be associated with the course of the disease. In contrast to the complex field of the immunohistochemical study of tumor samples or resection specimens, the quantitative determination of serum proteins allows a non-invasive and easy reproducible study of cellular products, possibly associated with the (malignant) disease. Further, the determination of these so-called serological tumor markers allows a follow-up control. Our personal experience with the evaluation of serological tumor markers in OSCC started about 15 years ago. In the focus of our studies were several proteins determined by well-established assays (e.g., TPA, SCC, Cyfra, CEA, p53). These proteins were not well suited for monitoring OSCC. Some reasons for the restricted value of serum tumor markers in OSCC might be the simple accessibility of the oral cavity to direct inspection (allowing direct measurement of tumor extension), the improvements of imaging techniques, e.g., ultrasound being currently the first-line diagnostic for determining the lymph nodes of the neck, and positron emission tomography (PET), e.g., being superior in identifying and localizing occult primaries in the carcinoma-unknown-primary (CUP) syndrome. These measures allow a topographic diagnosis (inspection), the determination of a strategy for surgery (palpation, ultrasound of the neck) and define limitations for surgery (PET: distant metastases), all convenient for the surgical decision process. On the other hand, it might be difficult to apply these investigation techniques in the follow-up control, first due to the severe alterations of the
sites following surgery (resulting in inconclusive diagnostic statements) and second, due to the disseminated growth of recurrent tumor that is hardly seen on any type of head and neck images in an early stage. Therefore, serological tumor markers could have a certain place in the follow-up control of OSCC. A second reason for the limited value of serum tumor markers in head and neck cancer is the lack of any association with tumor staging in this entity. Most "tumor marker studies" that tried to identify tumor-associated proteins and staging of OSCC have failed to produce reliable results. The low number of OSCC patients with serum levels above a "cut-off" level impairs the justification of routine determination of these proteins in cancer patients. Further, it was repeatedly shown that the serum levels of some of the current tumor-associated proteins, that were used for OSCC monitoring, might get altered following concomitant abuse of alcohol and cigarettes. These conditions are the basis of the conclusion that serological tumor markers are of use, if any, in those patients with pre-operative elevated serum levels. At present, this individualized use of tumor markers has only a few advocates. On the other hand, it is generally accepted that the determination of tumor-associated proteins would be a much appreciated, valuable and cost-effective tool for monitoring OSCC patients, in particular to determine therapy response and tumor relapse.

**Hematology**

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LAMDA- AND KAPPA-FREE LIGHT CHAINS IN HEMATOLOGICAL AND ONCOLOGICAL PATIENTS

P. Brück, H. Sauer-Eppel, G.M. Oremek

Zentrum der Inneren Medizin, Klinikum der Johann Wolfgang Goethe Universität, Frankfurt am Main, Germany

A new latex-enhanced assay, measuring free light chains (FLC) in serum and urine, has become available (Freelite™, The Binding Site). Evaluating the concentration of a soluble antigen by turbidimetry on the Hitachi-Analyzer 917 involves the addition of the test sample to a solution containing the appropriate antibody. From 51 hematological and oncological patients, serum samples were stored at –20°C. Determination of the FLC concentration was performed with the commercially available kit Freelite™ (The Binding Site Ltd., Birmingham, UK). This turbidimetry-based kit uses latex particle-bound polyclonal antibodies directed against lamda- and kappa-FLC. The detection limit of this method is 3.7 mg/l for kappa-FLC and 7.0 mg/l for lamda-FLC, respectively.

For statistical analysis, the patient's clinical history, specific MM markers, including, e.g., age at diagnosis, sex, current state of disease and further serum hematological laboratory parameters were determined.

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TUMOR MARKER MONITORING IN CHILDREN WITH ACUTE LEUKEMIA

O. Topolcan, T. Votava, Z. Cerna, L. Holubec Jr., L. Sasek, S. Svobodova, J. Finek

Charles University, Medical School, Faculty Hospital Pilsen, Czech Republic

Leukemia represents the majority of all childhood cancers. Significant progress in the therapy has been achieved during the past 20 years, improving the prognosis of these patients. An open question remains as to whether a reliable complex of biochemical screening methods exists for the early detection of disease relapse. Biochemical screening would provide a more cost-effective alternative to the well-established molecular biological methods.

**Patients and Methods:** A group of 37 children with acute leukemia was evaluated retrospectively from the period between April 1994 and April 2005 (33x ALL, 4x AML). The group consisted of 27 boys and 10 girls from 12 months to 15 years of age (median 9.2 years). The mean monitoring period was 49 months. Five parameters were monitored: thymidine kinase (TK), β2-microglobulin, lactate dehydrogenase (LD), ferritin and erythrocyte sedimentation rate (SR). Six disease relapses have occurred during the monitoring period (5x ALL, 1xAML).

**Results:** At the time of primary diagnosis, the most elevated parameter was TK, while the least elevated parameter was β2-microglobulin. At remission, elevated levels of ferritin were the most frequently elevated parameter. The largest variability was noticed with the erythrocyte sedimentation rate (SR). The TK serum levels remained, during the remission period, elevated slightly above the reference values and false-positive values were found only in patients with severe liver function impairment. At the time of disease relapse, a significant elevation of TK was found (83%), as well as elevation of ferritin (50%) and elevation of LD (25%). There were no significant changes in the SR and β2-microglobulin levels at the time of disease relapse. The levels of TK were significantly elevated 1 month prior to clinical signs of disease relapse in 3 cases.
Conclusion: Biochemical markers are convenient for acute leukemia monitoring in childhood. Monitoring of the TK serum levels seems to be the most suitable parameter.

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NUCLEOSOMES PREDICT EARLY THE RESPONSE TO INDUCTION THERAPY IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA

S. Mueller1, St. Holdenrieder1, P. Stieber1, T. Haferlach2, A. Schalhorn2, D. Nagel1, D. Seidel1

1Institute of Clinical Chemistry and 2Internal Medicine III, University Hospital Munich-Grosshadern, Germany

In patients with various solid tumours during systemic therapies, the initial changes of nucleosome concentrations in serum have shown to be predictive for the later outcome. Here, the courses of nucleosomes in sera of patients with acute myeloid leukaemia were investigated to evaluate their predictive potential for treatment outcome in systemic malignancies.

Materials and Methods: Nucleosomes were measured in the sera of 25 patients with de novo and relapse acute myeloid leukemia during the first cycle of induction chemotherapy. In addition, thymidine kinase, lactate dehydrogenase and leukocytes were analyzed in parallel. According to the criteria of the German AML Cooperative Group, 18 patients reached complete remission (<5% blasts and normal haematopoiesis in bone marrow after therapy), while 7 showed no remission.

Results: Nucleosome concentrations decreased in almost all patients during the first week, in some cases after initial peaks. In overall analysis, the nucleosome levels clearly distinguished between patients with complete remission and those with insufficient response (p=0.017). In detail, significantly higher concentrations were found on days 2 and 4 after the start of chemotherapy (p=0.014 and 0.022, respectively). A tendency to higher levels in patients with complete response was also found for thymidine kinase, lactate dehydrogenase and leukocytes, however the difference did not reach the level of significance (p=0.542, p=0.260 and p=0.144, respectively).

Conclusion: These results indicate that circulating nucleosomes are valuable markers for the prediction of therapeutic efficacy in patients with acute myeloid leukaemia, who are already at the very initial phase of the treatment.

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SCINTIGRAPHY FOR IMAGING OF MAXILLOFACIAL LYMPHOMAS AND DISTANT METASTASES OF CARCINOMAS

R.E. Friedrich
Department of Maxillofacial Surgery, Eppendorf University Hospital, Hamburg, Germany

The maxillofacial region is a rare site of distant metastases of malignancies of other body regions. They usually occur at a late stage of the disease. On the other hand, distant metastasis in the maxillofacial region can be the first finding of an already widespread malignoma.

Materials and Methods: The medical files of 92 patients with lymphomas or distant metastases of carcinomas to the maxillofacial region were evaluated (female: 45, male: 47; age 5 to 88 years, mean: 61.4 years). Lymphomas of the maxillofacial region were regarded as metastatic in cases with multilocular tumor development. All patients were treated at a single institution over a period of 29 years.

Results: In females, metastases of breast cancer constituted the largest group, followed by malignant lymphoma, malignant melanoma and renal cell carcinoma. In males, lymphomas constituted the largest group (25.5%), followed by bronchial carcinoma and those with a carcinoma of unknown primary syndrome (CUPD-syndrome; 17% each). Metastasis of renal cancer occurred in 4 patients (8.5%). Synchronous cancer was diagnosed in 4 patients. The latency period between the diagnosis of the primary cancer and the metastasis varied considerably. In 49 patients, the mean latency period was 48 months. In 35 patients, the maxillofacial findings led to the diagnoses of bronchial cancer: 4, hypernephroma: 1, lymphoma: 20, histiocytoma: 5, plasmacytoma: 5. Findings at the time of admission were non-specific (e.g.: swelling: 70.7%; paresthesia: 4.3%; impaired mouth opening: 5.5%; pathological fractures 4.3%; oral ulcers 4.3%). Scintigraphy for bone metastasis was performed in 34 patients. Scintigraphy was accurate in 32 patients, but failed to identify bone metastasis of the maxillofacial region in 2 patients with histologically-proven metastatic involvement of the bone.

Conclusion: Metastases to the maxillofacial region are rare, through some entities show a predilection for metastases to this region. During the extended period of time covered by this investigation, scintigraphy was the method of choice to define bone involvement of tumor spread. It was shown that scintigraphy might fail to correctly identify distant bone metastases in the maxillofacial region. Alternative imaging methods, such as positron emission tomography, might more precisely visualize the extent of tumor invasion in the maxillofacial region compared to scintigraphy.

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IMMUNOHISTOCHEMICAL ALGORITHM FOR THE DIAGNOSIS OF LYMPHORETICULAR DISEASES
Immunohistochemistry is used for the demonstration of antigens in tissues, cells and subcellular structures. However, the main focus of diagnostic immunohistochemistry is the identification of the cellular origin to classify normal and neoplastic lesions in order to validate the morphological diagnosis of the pathologist. Immunohistochemistry is, thus, an important tool in routine pathology and has important implications, not only for the diagnosis, but also for therapy and patient management. While currently immunohistochemistry is used in 10 to 20% of solid tumors, there is an essential need for immunohistochemical investigations of lymphoid and hematological neoplasms. We use immunohistochemical studies in almost all cases of lymphoid malignancies for accurate diagnosis and also to satisfy the upcoming interest in expression analysis (e.g. prognostic and therapeutical markers, like CD20, CD52, CD33). For this purpose, algorithms for lymphoma diagnosis are presented in the context of selected problems in the differential diagnosis of frequent problems in the diagnosis of malignant lymphomas and in the light of recent pioneering developments in immunohistochemistry and the molecular pathology of malignant lymphomas.

**Melanoma – Skin**

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S100, S100A1B AND S100BB AS CANCER MARKERS FOR PREDICTION OF SURVIVAL AND RELAPSE DURING LONG-TERM FOLLOW-UP OF MALIGNANT MELANOMA PATIENTS

A. Bolander¹, M. Bergqvist¹, D. Brattström¹, A. Larsson², G. Ullenhag¹, P. Hesselius¹, G. Wagenius¹, R. Einarsson³

¹Department of Oncology and ²Department of Medical Sciences, University Hospital, Uppsala; ³CanAg Diagnostics, Gothenburg, Sweden

Protein S100B belongs to the S100/calmodulin/troponin C superfamily of EF-hand calcium-binding proteins. S100 proteins (20 members) constitute a multigenic family of low molecular weight proteins (10-12 kDa). S100B and S100A1 form either homodimers (S100BB) or heterodimers (S100A1B). The combination of S100 markers may give additive information concerning the increased risk of relapse and death in malignant melanoma patients.

**Materials and Methods:** CanAg S100, S100A1B and S100BB are solid-phase, 2-step enzyme immunoassays, based on monoclonal antibodies specific for different epitopes expressed on S100B. S100 technical performance; high sensitivity (detection limit <10 ng/L, intra-assay CV 1.3-2.5%, inter-assay CV 1.5-2.5%) and measuring range 10-3500 ng/L. S100A1B and S100BB have a detection limit of <10 ng/L and <30 ng/L, respectively, and the analytical imprecision for S100A1B and S100BB is below 4%.

**Results:** An upper reference limit of 90 ng/L was obtained for S100 in 269 healthy individuals. 198 patients (UICC stage I and II) with cutaneous malignant melanoma were analysed after primary surgery and monitored for up 7.5 years or until TK activity levels in pathological samples than the old radioactive assay. Disturbances by factors in the serum and low substrate levels in the old technique cause underestimation of pathological TK activities. The novel assay uses saturating substrate and low serum and thus lacks disturbance from the clinical specimen. In summary, the novel technique should detect tumour growth earlier, making it more useful for therapy monitoring. Its sensitivity makes measurement in spinal fluid, pleural and ascitic fluids clinically relevant. Thus, the new technique will expand the use of TK activity measurement from mainly hematology to all kinds of tumors as they have a common denominator, since TK is associated with growth and cell death.
the time to death. Relapse was reocred in 28 patients and death in 16 patients. The S100 serum concentration was in the range 20-8330 ng/L, S100A1B 0-7267 ng/L and S100BB 0-2575 ng/L. Increased levels of S100, S100A1B and S100BB reflected worse overall survival (statistically significant) applying the decision levels of 150, 50 and 50 ng/L, respectively. Increased levels of S100 and S100BB correlated with the increased risk of relapse (p=0.02 and p=0.03), but this was not found for S100A1B (p=0.7).

Conclusion: S100A1B and S100BB might give further information for patients with malignant melanoma and also act as early indicators for progressive disease.

79 OSTEOPONTIN, S-100ß and MIA – MARKERS FOR METASTATIC UVEAL MELANOMA

J. Peter¹, R. Folberg³, K. S. Frenke¹, I. Kalickman², V. Barak²

¹Department of Ophthalmology and ²Immunology Laboratory for Tumor Diagnosis, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; ³Department of Pathology, University of Chicago, Illinois, U.S.A.

The aim was to test Osteopontin, S-100ß and MIA as potential markers for metastatic uveal melanoma.

Materials and Methods: Over 1000 blood samples of uveal melanoma patients (pts) were assessed. Fifteen pts were evaluated with proven metastatic uveal melanoma and 67 pts were under follow-up for more than 10 years without metastatic disease. For the 15 pts with metastases, kinetics before and after diagnosis of metastases were available. In addition, 50 healthy age- and sex-matched controls were included. All 3 markers were evaluated by ELISA assays. Tissue sections of metastatic melanoma were stained for Osteopontin and its RNA measured by RT-PCR.

Results: The serum levels of Osteopontin in pts with metastases were almost 3 times higher (28±2.6) than in NED (9.44±1.8) pts with long follow-up and the control group (6.71±1.2) (p<0.001). There was a significant rise in Osteopontin levels in the 15 pts who developed metastases during the study, which corresponded to liver metastasis. The levels of S-100ß in pts with metastases were 3 times higher (0.147±0.04) than those of pts with long follow-up (0.057±0.01) and the control group (0.073±0.03), but there was no statistical significance (p=0.07). There was no significant difference in MIA levels among the 3 groups: metastatic pts.-7.45±0.67, NED pts.-5.99±0.46 and controls 6.3±0.26. By RT-PCR highly-invasive primary and metastatic uveal melanoma expressed 6- to 250-fold excess Osteopontin RNA compared to poorly-invasive uveal melanoma cells.

Conclusion: Osteopontin and S-100ß serum levels can serve as markers of metastatic disease of uveal melanoma. Increasing levels of both markers are prognostic of metastatic disease development.

80 IMMUNOLUMINESCENT DETECTION OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) AND AMINOPEPTIDASE N (APN) ON HUMAN MELANOMA CELLS

F. Laube

Institute of Physiological Chemistry, Martin-Luther-University, Halle, Germany

During the complex process of melanoma cell detachment and subsequent metastasis, different cell surface proteins are involved in tumor cell interactions with extracellular matrix (ECM) components and surrounding cells. In different stages of tumor development, melanoma cells are able to vary their expression patterns of adhesion proteins and proteases/peptidases. In addition to the previous detection of CD44(v5) and uPA/uPAR in the melanoma cell line IGR-1, these cells were further characterized by ICAM-1 (CD54) and (aminopeptidase N) APN (CD13).

Materials and Methods: Both cell surface proteins were detected by immunoluminescence using two different antibodies (Abs) for each antigen. Horseradish peroxidase (HRP) conjugates were used for indirect labeling and to initiate the HRP-catalyzed enhanced luminescent reaction. Additionally, APN was detected on intact cells by an activity assay using alanine-p-nitroanilide (Ala-pNA) as substrate, including inhibition experiments.

Results: The immunological responses with Abs to ICAM-1 (15.2 and HAS8) were high and nearly equal (3.5 10⁵ RLU/s). Both Abs to APN (SJ1D1 and WM15) gave different signal intensities: 1.5 10⁵ RLU/s and 1.5 10⁴ RLU/s, respectively. APN activity was assayed using Ala-pNA. The peptidase activity was effectively inhibited by 1,10-phenanthroline and partly inhibited by EDTA. Both anti-APN Abs used for the immunophenotyping caused only a low inhibitory effect.

Conclusion: Both cell surface proteins have a strong impact on the tumor cell behavior and are typical cell markers, representing a high metastatic tumor stage in melanoma. ICAM-1 affects adhesion, migration and tumor cell interaction with lymphocytes. APN contributes to degradation of ECM components (e.g. collagen IV) and cleaves biologically active peptides, affecting tumor growth and angiogenesis. The clinical significance of APN in tumor therapy could be in the application of inhibitors such as bestatin/ ubiquinone. Soluble ICAM-1 (sICAM-1) was found
to be increased in the serum of patients with malignant melanoma who had a shorter survival time, but the results from another study did not show a difference in sICAM-1 between melanoma stages and the control group.

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NAVIGATION-ASSISTED RESECTION OF AN ATYPICAL PRIMARY EXTRAOCULAR MELANOMA
R. E. Friedrich, U. Grzyska, H. Schäfer, L. Li
Maxillofacial Surgery, Neuroradiology and the Institute of Pathology, Eppendorf University Hospital, Hamburg, Germany

A 21-year-old male presented with exophthalmos of the right eye and diplopia. On MRI, a well-delineated orbital tumor, medio-distal to the eye, was detected, respecting the orbital walls. The aim of surgery was to excise the progressive tumor while maintaining vision. Therefore, tumor resection via a lateral orbitotomy and intraoperative navigation (VectorVision, BrainLAB) was planned. For intraoperative referencing, a cortical fixed-reference system was used ("Latero Reference Star", z-touch, BrainLAB). The accuracy of this referencing method was checked by anatomical landmarks. A modified lateral orbitotomy, as revisited by Maroon and Kenderdell, was used. Microscopic analysis of the resection specimen revealed a melanoma. The patient's postoperative course was uneventful. The diplopia improved rapidly. Two further eye-saving revisions of the tumor site excluded melanoma and revealed melanophages in scar tissue. Intraoperative navigation was used during all the procedures. The tumor showed some interesting features concerning the histopathological and MRI appearance. The proliferation index in terms of Ki-67-positive tumor cells was low, not exceeding 10% (MIB1). The proliferation seemed to be higher in non-pigmented tumor cells. The tumor failed to stain for synaptophysin, tyrosin hydrolase, chromogranin, neurofilament, pan-cytokeratin (AE1/AE3), EGFR, GFAP or vimentin. The tumor cells stained for c-kit and HMB45. Some tumor cells were positive for S-100 and CD68. Electron microscopy excluded the presence of secret granula. The tumor cells failed to be surrounded by a basal membrane. The MIC2 antigen (CD99) was identified in tumor cells and strands of melanophages. The tentative differential diagnoses of pigmented paragranuloma, melanotic schwannoma or malignant melanocytoma were excluded on the basis of the afore-mentioned findings. Detailed histopathological investigations supported the decision for organ-saving surgery. Follow-up computed tomography after nine months showed no local tumor recurrence.

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DIAGNOSIS AND TREATMENT OF PATIENTS WITH BASAL CELL NEVUS SYNDROME [GORLIN-GOLTZ-SYNDROME (GGS)]
R. E. Friedrich
Maxillofacial Surgery Clinic, Eppendorf University Hospital, Hamburg, Germany

Basal cell nevus syndrome (GGS) is a rare and autosomal-inherited disease with syndromatic characteristics, first described by Gorlin and Goltz in 1960. In the head and neck region, the occurrence of basal cell carcinoma in atypical skin regions and multiple keratocysts of the jaws are the predominant findings. The aim of this study was to determine the diagnostic findings and therapy for patients with GGS.

Materials and Methods: The medical files of 17 patients with GGS, treated in a single institution, were evaluated (females: 9, males: 8).

Results: The age at the time of first surgical treatment related to the syndrome was 3 to 57 years (mean: 21.3 years). A family history of GGS was evident in 4 patients. The number of patients with characteristic head and neck findings in the spectrum of GGS varied: basal cell carcinoma (n=15), keratocysts of the jaws (n=13), dura calcification visible on plain radiographs (n=15), palmar pits (n=9). Facial dysmorphism (like hypertelorism) was evident in 4, and skeletal anomalies outside the skull occurred in 10 patients. The medical histories revealed a cleft lip and palate in 2, and a renal agenesis in a further 2 patients, emphasizing the variability of the syndrome. Treatment was exclusively surgical in all but 2 patients. One of these 2 underwent external irradiation for a basal cell carcinoma (BCC) of the frontotemporal region. Nine years later, another frontal BCC had to be treated. The second developed several other BCC inside and outside the irradiation field. Up to 50 BCC per patient had to be resected. The number of keratocysts of all patients was 66, with a predilection for the mandibular angle in 44%. On CT (n=9) a number of calcifications became evident: falx (8/9), tentorium (9/9), petrosellar ligament (2/9) and carotid siphon (1/9). Cerebral cysts occurred in 3/9. One patient underwent surgery for a medulloblastoma during childhood. In this series of CT of 9 patients, no cortical atrophy was found.

Conclusion: The GGS is a well-known syndrome with a variety of findings in the head and neck region. However, careful clinical investigation discloses many other findings. Interdisciplinary cooperation is mandatory in the diagnosis and follow-up of patients with GGS.
83 THE ULTRASONOGRAPHIC "TARGET SIGN" OF PLEXIFORM NEUROFIBROMAS IN NEUROFIBROMATOSIS TYPE 1 IS RESTRICTED TO THE NODULAR (DISPLACING) SUB-TYPE

R. E. Friedrich
Maxillofacial Surgery Clinic, UKE, Hamburg, Germany

Neurofibromatosis type 1 (NF1) is an autosomal dominant inherited disorder affecting about 1 in 3000 or 4000 newborns. Neurofibromas are the hallmark of the disease. Recent results of ultrasonographic investigations suggested a distinct ultrasonographic pattern indicative of a neurofibroma. These investigations were case reports only or based on small sample sizes and did not always differentiate between isolated neurofibromas and those developing in NF1 patients. Whereas cutaneous neurofibromas can easily be detected by inspection and palpation, the application of ultrasound might have some impact on detecting neurofibromas of deeper body layers, and those tumors that affect larger body sites and are currently termed neurofibromas of the plexiform type. We have recently proposed a subtyping of plexiform neurofibromas based on magnetic resonance images. This classification differentiates invasive, superficial and displacing plexiform neurofibromas. The aim of this study was to compare the ultrasonographic findings to those found and classified on magnetic resonance images.

Materials and Methods: Thirty patients with NF1 were investigated. All the patients fulfilled the current National Institute of Health (USA) diagnostic criteria for NF1. All were affected with cutaneous neurofibromas of variable size and numbers and 20 patients were also affected with a plexiform neurofibroma. Those patients who were surgically treated had a complete histological work-up of the resection specimens. Therefore, all ultrasonographical findings were compared with the microscopic diagnosis. B-scan ultrasound was applied, with the focus directed to the region of interest (Siemens, small-part applicator, 7.5 MHz emission frequency). Doppler imaging was used to estimate the vascularity of the tumor and the surrounding tissues.

Results: Neurofibromas of the skin are soft tumors of a few centimeters in diameter and can be felt below the skin surface. On ultrasonograms, they are of round to oval in shape, have well-defined borders and low echo-reflective internal structures. Clusters of cutaneous neurofibromas can be detected by ultrasound below the skin surface. In plexiform neurofibromas, the ultrasonogram showed an irregular tumor mass without any defined borders. Inside the tumor, isolated or few linear reflections were reminiscent of tension lines. These findings were exclusive to invasive plexiform neurofibromas. Superficial neurofibromas of the diffuse/plexiform type were indicated by a thin, homogeneous low echo-reflective region close to the applicator with distal enhancement of the signals. The linear reflections running in parallel, that were assigned to be the "target sign" of neurofibroma, were also seen. However, these tumors were all of the displacing type.

Discussion: This investigation showed that ultrasonography can visualize plexiform neurofibroma in NF1 patients. The so-called target sign for neurofibroma cannot be applied for this tumor type in general. Indeed, the target sign is restricted to a subtype of plexiform neurofibroma that constitutes nodules. Ultrasonography can be used to visualize this subtype of plexiform neurofibroma in NF1. Supported by Deutsche Krebshilfe/Mildred Scheel Stiftung and Deutsche Forschungsgemeinschaft: Project FR 1035/6-1, Germany.

Bone Metastasis

84 MEASUREMENT OF THE BONE TURNOVER PARAMETERS ICTP AND PINP IN PROSTATE CANCER PATIENTS – NEW SERUM MARKERS FOR DIAGNOSIS AND THERAPY MONITORING OF BONE METASTASES

G. Feil, C. Bock, S. Feyerabend, A. Anastasiadis, A. Stenzl
I. Department of Urology, Eberhard-Karls-University, Tübingen, Germany

The most sensitive method for detecting bone metastases in prostate cancer patients is bone scintigraphy, before clinical evaluation and bone radiographs. However, the imaging is not highly specific and reliable serum markers are currently not at hand. New laboratory parameters reflecting metastatic bone turnover are needed for both M-staging and monitoring of the therapeutic response to skeletal metastases. The aim of the study was to evaluate the new bone turnover markers, carboxyterminal cross-linked telopeptide of type I collagen (ICTP) and the aminoterminal propeptide of type I procollagen (PINP), for diagnosis and therapy monitoring of bone metastases in prostate cancer patients.

Materials and Methods: Eighty patients with histologically-proven prostate cancer, 41 of whom had bone metastases (PCaM1b) and 39 without bone metastases (PCaM0), and 51 controls with benign prostatic hyperplasia (BPH) were included in the study. The serum concentrations of ICTP and PINP were analyzed using specific radioimmunoassays.
The therapeutic response to bisphosphonate therapy in 7 out of the 41 M1b-patients was monitored 5 (3-7) and another 4 (3-9) weeks after the initialization of the therapy.

Results: The mean patient age was comparable between the BPH-, PCaM0- and the PCaM1b-cohorts at 68, 67 and 66 years, respectively. The mean (+/– SEM) values of the parameters measured in these groups were as follows: PSA 2.72 (+/–0.33), 42.82 (+/–19.17), 886.52 (+/–309.16) ng/ml; ICTP 4.74 (+/–0.41), 5.61 (+/–0.49), 15.18 (+/–3.48) ng/ml; and PINP 47.76 (+/–5.58), 46.02 (+/–4.90) and 211.76 (+/–52.30) ng/ml, respectively. Both serum markers of type I collagen metabolism were significantly elevated in the M1b-patients compared to the M0-patients (p<0.01, t-test). The areas under the ROC curve for PSA, ICTP and PINP were 0.682, 0.759 and 0.673, respectively. The most accurate sensitivity and specificity for PSA was 48.8% and 89.7%, for ICTP 73.2% and 74.4% and for PINP 46.3% and 87.2%, respectively. In the patients monitored for response to bisphosphonate therapy, the pre-treatment and the follow-up mean values (+/– SEM) detected were for PSA 364.59 (+/–183.32), 159.16 (+/–63.18) and 117.62 (+/–53.42) ng/ml; for ICTP 7.91 (+/–2.33), 5.97 (+/–1.25) and 5.46 (+/–1.17) ng/ml; and for PINP 178.24 (+/–79.71), 116.34 (+/–61.61) and 69.00 (+/–31.10) ng/ml, respectively.

Conclusion: Serum levels for both type I collagen degradation, ICTP and type I procollagen synthesis, PINP, were statistically significantly different between prostate cancer patients with and without bone metastases. ICTP and PINP may be important for pre-treatment staging and for the early detection of progression in patients with PSA failure and useful in monitoring the therapeutic response to bone metastases.

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P1NP EIN KNOCHENMARKER BEI GYNAKOLOGISCHEN KARZINOMEN

Ch. Hansen, H. Sauer-Eppel, G.M. Oremek

Zentrum Innere Medizin – Zentrallabor, Klinikum der J.W. Goethe Universität, Frankfurt am Main, Germany


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THE AMINO-TERMINAL PROPEPTIDE (PINP) OF TYPE I COLLAGEN IS A CLINICALLY VALID INDICATOR OF BONE TURNOVER AND THE EXTENT OF METASTATIC SPREAD IN OSSEOUS METASTATIC BREAST CANCER

D. Pollmann, S. Schildhauer, R. Geppert, K.-D. Wernecke, K. Possinger, D. Lüftner

Medizinische Klinik mit Schwerpunkt Onkologie und Hämatologie, Universitätsmedizin, Charité Campus Mitte, Humboldt-Universität zu Berlin, Germany

The efficacy control of any treatment of bone metastases is difficult and usually initiated later than restaging of visceral or soft tissue metastases. The amino-terminal propeptide (PINP) of type I collagen, as a biochemical indicator of bone turnover, might facilitate early and valid disease surveillance. The utility of PINP was investigated in metastatic breast cancer patients, with and without bone metastases, for monitoring of therapy. The results were compared to osteocalcin and β-carboxyterminal telopeptide (CTX) concentrations as historically used markers.

Materials and Methods: Baseline serum samples of 51 patients with metastatic breast cancer under chemotherapy were investigated. In total, 38 patients had been diagnosed with bone spread, while 13 had no evidence of osseous metastases. All the patients with bone spread received bisphosphonates in addition to systemic chemotherapy.
and/or antibody or hormone therapy. The Osteocalcin®, CTX and PINP levels were measured using the Elecsys values <41.3 pg/ml; CTX <1008 pg/ml; PINP <95 ng/ml.

**Results:** The baseline levels of PINP were significantly higher for patients with bone metastases (median: 92.8 ng/ml) than those without bone spread (median: 63.2 ng/ml, \( p=0.0044 \)). Patients with more than 7 bone lesions had a significantly higher P1NP level (median: 149.7 ng/ml, \( p=0.04 \)) than those who had less than 7 bone spread (median: 67.6 ng/ml). For Osteocalcin and CTX, these differences were significant, too, with \( p \)-values of \( p=0.02 \) and \( p=0.04 \), respectively. However, the median concentration remained below the cut-off of normal values (patients with more than 7 bone lesions: median of CTX 422 pg/ml; median of Osteocalcin 26.6 pg/ml). For patients with bone spread, the P1NP level decreased manifestly in responders (from 194.3 to 71.8 ng/ml) and increased in progressive disease (from 71 to 65.4 ng/ml). However, these courses did not show a similar biochemical pattern and must be considered as less sensitive for the surveillance of bone metastases.

**Conclusion:** PINP concentrations of patients with osseous metastatic breast cancer are elevated at baseline in comparison to patients without bone involvement; this PINP level correlates with the number of bone metastases and is independent of the menopausal status. The PINP changes under therapy seem to indicate the response to therapy. Osteocalcin and CTX did not show a similar biochemical pattern and must be considered as less sensitive for the surveillance of bone metastases.

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**DETECTION AND MOLECULAR CHARACTERIZATION OF MICROMETASTATIC CANCER CELLS**

K. Pantel

Institute of Tumor Biology, University Medical Center, Hamburg, Germany

The two major approaches to detect micrometastatic cells are immunocytochemical staining and polymerase chain reaction analysis. These assays are sensitive enough to detect a single metastatic cell in the background of millions of normal cells. For epithelial tumors, the cytokeratins (CK) have become the best markers for the immunocytochemical detection of micrometastatic cells. Bone marrow (BM), which can easily be collected from the iliac crest, is the most important site for detecting micrometastatic cells, which are present in BM samples of 20–40% of patients with carcinomas at various primary sites; even in the absence of
lymph node metastases (stage N0), or clinical signs of overt distant metastases (stage M0). The prognostic value of CK+ cells in BM at primary surgery has been recently demonstrated by an international study on more than 4700 breast cancer patients (Braun et al: NEJM, 2005). BM samples can also be monitored for the presence of micrometastatic cells after primary surgical treatment, to detect tumor recurrence.

Interestingly, the presence of micrometastatic cells in BM is not only useful in predicting the development of skeletal metastases, but also in predicting the development of metastases in other distant organs, such as the lung or liver. This is even true for tumors that rarely show skeletal metastases, such as colon cancer. In fact disseminated cancer cells have also been found in the BM of patients with head and neck cancer who did not have lymph node metastases, although the clinical significance of these cells is not clear. So BM might be an important reservoir that allows for micrometastatic cells to adapt and disseminate into other organs. An alternative explanation is that the presence of occult cancer cells in the BM might only reflect the general propensity of these cells to disseminate and to survive in organs, rather than just in the BM. Several studies have documented that adjuvant systemic treatment may be insufficient to eliminate micrometastatic cells and that at least some of the surviving micrometastatic cells can escape dormancy. It will be now a major challenge to unravel the mechanisms underlying this capacity.

One approach to achieve this task might be the further characterization of micrometastatic cells. Anti-CK antibodies, which are used to identify micrometastatic cells in BM, can be used in combination with antibodies against other tumor-associated antigens to profile these cells. Using such immunocytochemical procedures, it has been possible to identify a number of tumor-associated characteristics of the CK+ cells found in BM. Phenotyping of disseminated tumor cells in BM of early-stage patients has also yielded additional prognostic information. Recent technical developments have made it possible to examine the genome of disseminated tumor cells. A combination of immunocytochemistry and fluorescence in situ hybridization (FISH) has shown that BM contains proliferating micrometastatic cells with various numeric chromosomal aberrations found of malignant origin. The viability and proliferative capacity of these cells has also been monitored in these models and correlated with clinical outcome. By developing a new procedure for whole genome amplification and subsequent comparative genomic hybridization (CGH) of single immunostained cells, it was found that CK+ cells in the BM of epithelial breast cancer patients, without clinical signs of overt metastases (stage M0), are genetically heterogeneous.

In conclusion, the detection and characterization of micrometastatic cells will open new insights into metastatic progression in cancer patients with potential implications for targeted therapy.

**ECONOMIC ASPECTS OF RADIONUCLIDE THERAPY OF PAINFUL BONE METASTASES**

M. Fischer¹, F.-U. Fricke²

¹Kassel and ²Nürnberg, Germany

Besides conservative treatment with radiotherapy, radionuclide therapy is an effective treatment in patients with painful bone metastases. ⁸⁹Strontium, ¹⁸⁶Rhenium and ¹⁵³Samarium are routinely used in patients with osteoblastic metastases from different primary tumors, who develop severe pain syndrome in about 75% of cases. In about 70% of these patients, pain reduction can be observed after one single intravenous administration of a radionuclide.

**Materials and Methods:** Data from the literature about radionuclide therapy with ¹⁵³Samarium were the basis for developing an economic model for the evaluation of the cost-effectiveness of this therapeutic option. The results were compared to standard pain therapy from the perspective of the German Statutory Health Insurance.

**Results:** Whereas the average cost per responding patient in the standard therapy group was about € 22,000, the average cost in the radionuclide therapy group per responder was only about € 11,000.

**Conclusion:** Treatment of painful osteoblastic bone metastases from different primary tumors with radionuclides is improving the pain syndrome in the majority of patients. The treatment modality is also pharmaco-economically reasonable compared to standard therapy.

**CLINICAL VALUE OF BISPHOSPHONATES IN CANCER THERAPY**

D. Lüftner

Medizinische Klinik II, Charité Campus Mitte, Berlin, Germany

More than 50 % of patients with breast cancer will develop bone metastases in the long run. It is still unclear whether the course of the disease would be different if the detection of small, clinically asymptomatic bone metastases were possible at an earlier stage than is now feasible. The sensitivity of the currently available methods is too low to allow for the
detection of clinically still occult bone lesions. The use of specific biochemical markers of bone metabolism might improve the detection of bone metastases, leading to early therapeutic intervention. However, due to limited sensitivity and specificity, the main use of the parameters of bone metabolism is monitoring of the treatment outcome of advanced disease with osseous involvement. More than 90% of organic bone matrix consists of type I collagen, which is preferentially synthesized in bone. During normal bone catabolism, mature type I collagen is degraded and small fragments pass into the bloodstream and are excreted via the kidneys. In physiologically or pathologically elevated bone resorption, type I collagen is degraded to an increased extent.

On the other hand, reparative mechanisms try to antagonize bone degradation to keep the net bone mass in a steady-state condition. During this continuous bone remodeling process, mature type I collagen must be formed from precursor molecules by splicing off propeptides at the C-terminal and N-terminal ends of the procollagen molecules. The differential value of different markers of bone turnover in relation to the clinical use of bisphosphonate therapy is discussed.

Quality Control and Standardization

91 QUALITY CONTROL AND TUMOUR MARKERS – A UK NEQAS PERSPECTIVE
C. Sturgeon

UK NEQAS, Department of Clinical Biochemistry, Edinburgh Royal Infirmary, U.K.

The primary function of external quality assessment (EQA) schemes must always be to provide each participating laboratory with an objective assessment of its own performance, enabling comparison with that of other laboratories. Data from EQA schemes influence laboratory decisions when considering changes of method, particularly for complex analytes such as the tumour markers. Suppliers of diagnostic kits also find EQA information of interest as it provides unique and up-to-date information about how methods are performing in routine practice. Increasingly, data generated by EQA schemes inform the strategic decisions of clinical Pathology Accreditation (UK) Ltd., those working to improve analytical standardisation and comparability of clinical results for specific analytes [e.g. the International Federation of Clinical Chemistry] and those specifying quality requirements for national screening programmes [e.g. the National Heath Service Prostate Cancer Risk Management Programme in the UK]. Such reliance on EQA data places a major responsibility on EQA providers to ensure that the specimens distributed are appropriate, i.e. as similar as possible to patient specimens and of clinically relevant concentrations. The validity of target values (usually trimmed consensus means) should also be regularly confirmed, by assessing recovery of International Standards where available, by assessing linearity on dilution and by determining their reproducibility on repeat distribution of the same pool. Assessment of long-term assay stability is particularly important for tumour markers. Experience suggests that major factors contributing to between-method differences include errors in calibration, differences in antibody specificity and method design. Between-method agreement has improved for some tumour markers, including PSA, for which between-method coefficients of variation in the UK NEQAS for PSA have fallen from 21.9% to 9.5% following the widespread adoption of International Standard 96/670 for PSA and highly commendable efforts by diagnostic companies to calibrate their PSA methods accurately in terms of this standard. However, there are still significant method-related differences in the results for all tumour markers, particularly CA125 and the other CA antigens. The lack of International Standards for these markers continues to limit progress in improving between-method comparability and should be addressed urgently by the international scientific community. Attention to method design is also required, e.g. to achieve equimolarity of recognition of different isoforms where relevant (e.g. complexed and free PSA) and to minimize the risk of clinically-relevant interferences (e.g. heterophilic antibodies). Much effort is already being expended, both nationally and internationally, to improve the quality of tumour marker measurements. EQA provides a powerful tool with which to assess the success of these initiatives, as well as to identify targets for further improvement.

92 QUALITÄTSSICHERUNG IN DER TUMORMARKER-ANALYSE: CUT-OFF-UNABHÄNGIGE EINZELMARKERBEWERTUNG FÜR DIE VERALLGE-MEINERUNGSFÄHIGE PROFILAUSWERTUNG
N. Bitterlich1, J. Schneider2

1Medizin and Service GmbH Chemnitz, 2Institut und Poliklinik für Arbeits- und Sozialmedizin der Universitätskliniken Gießen/Marburg, Germany

93 BIOLOGICAL VARIABILITY OF 10 TUMOUR MARKERS

P. Heiss, K. Jaensch, R. Sedlmaier-Prasselsperger, B. Eckert

Roche Diagnostics GmbH, Penzberg, Germany

The intra-individual biological variability of 10 tumour markers was compared. Six samples (taken monthly for 6 months) were measured in 36 apparently healthy subjects (16 women, 18 men; age 22-57 years) recruited from our laboratory staff (Penzberg, Germany). The sera, stored at −80 °C, were measured with the following Elecsys® assays: AFP, CEA, CA 125, CA 15-3, CA 19-9, CA 72-4, NSE, CYFRA 21-1, total PSA and Ferritin. For each individual, the coefficient of variation (CV [%]) over the 6 samples was calculated.

The results demonstrate that most tumour markers have a minor biological variability. Therefore, these markers are suitable for follow-up measurements in monitoring patients. However, in the case of CYFRA 21-1 and CA 72-4 a higher biological variation was found. The clinical interpretation of kinetic measurements for these parameters must be done carefully and requires more control measurements.

94 EXTERNAL QUALITY CONTROL OF TUMOR MARKERS

The assays AFP, CA 125, CA 15-3, CA 19-9, CEA, NSE and PSA showed intra-individual CV's generally below 25%, with the exception of 1 or 2 individuals. With Ferritin 4 individuals had intra-individual CV's higher than 25%. However, 3 of them had a mean concentration of Ferritin lower than 16 ng/ml. The intra-individual CV's of CYFRA 21-1 and CA 72-4 were higher than 25% in 44% and 47% of the tested subjects, respectively.

The results demonstrate that most tumour markers have a minor biological variability. Therefore, these markers are suitable for follow-up measurements in monitoring patients. However, in the case of CYFRA 21-1 and CA 72-4 a higher biological variation was found. The clinical interpretation of kinetic measurements for these parameters must be done carefully and requires more control measurements.
LACK OF BETWEEN-TEST COMPARABILITY IN TUMOR MARKER TESTS: PROBABLE CAUSES AND SOLUTIONS

M. Zwirner¹, K. Korte¹, N. Bewarder²

¹Department of Obstetrics and Gynecology, University of Tübingen; ²BIOREF GmbH, Mömbris, Germany

The goals of the quality control system in diagnostic laboratories are to assess accuracy, precision and comparability of test results. One of the most confusing aspects for untrained clinicians, and a still unsolved problem, is the between-test comparability of results when the tumor markers are measured using different test kits. In several proficiency studies using BIOREF tumor marker monoanalyte controls, differences in method means ranging from 5% to 300% were found, depending on the concentration range. Generally, the quantification of proteins or glycoproteins by immunological tests is based on a calibration curve with a defined concentration of an antigen derived from a master calibration, using a standard reference preparation. Unfortunately, valid international reference preparations are not available for most of the tumor marker tests on the market to date. Therefore, the test manufacturers are free to choose standard preparations for the calibration of their test systems. Since there is no reference method available for tumor marker immunoassays, the true value is unknown. Information about the reference materials used for calibration of most tests is difficult to obtain. Of the four test systems used in the CA 125-proficiency study in 2002, two calibrators were of "commercial origin", one was described as being of "internal origin" and in one test description no information about the origin of the calibrator was available. In consequence, the heterogeneity of "unknown" calibrators could be one reason for the incomparable test results. On the other hand, some of the tests exhibited reagent batch-dependent drifts of about 10% to 30%, indicating problems in test design. Further problems in immunoassay testing are also the antibodies used, as well as the antigen itself, which has to be detected. The monoclonal antibodies are sequence-specific and their binding depends on the sequence repeatability in the antigen. If the antigen in a reference preparation is treated inadequately one would find artefacts or dissociated molecule fragments which, in consequence, are immunoreactive but do not correspond to the native antigen in a patient serum. This phenomenon can be observed in several long-established protein assays, e.g. in the proteohormone field. In conclusion, a first step to increase the comparability of test results is to establish an adequate internal reference preparation. This reference preparation should fulfill all the requirements of international reference preparations. Based on the experiences gained with BIOREF reference materials, it could be feasible to use the patented production procedure applied for these materials to produce a large amount of a reference preparation, which could be offered by international federations, like IFCC or EGTM, to test manufacturers. The reference materials manufactured by BIOREF contain tumor antigens produced from a defined human tumor propagated in tissue culture. Because of this special production method, the antigens are genetically uniform and are present in their native forms in a physiological human serum matrix similar to the analyte in
a patient serum. The high titer antigens produced in this way must not be (bio)chemically extracted, only having to be diluted using a standardized human serum matrix. A further requirement is that the reference materials should be offered in large quantities of identical quality and should be of high long-term stability. All requirements are fulfilled by BIOREF’s reference materials. A further attempt to place an international reference preparation and, thus, probably increase the comparability of test results could also be the use of recombinant material which, to date, is only available for the tumor marker CA 125.

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LOT-TO-LOT CONSISTENCY OF ABBOTT
ARCHITECT® TUMOR MARKER ASSAYS

K. Kula¹, P. Roth¹, K. White¹, Y. Kobayashi², S. Kochler³, S. O’Morchoe¹, T. Kettlety⁴, G. Dickson⁴, M. Schulten³

¹Abbott Laboratories, Abbott Park, IL, U.S.A.; ²Abbott Laboratories, Tokyo, Japan; ³Abbott Laboratories, Wiesbaden, Germany; ⁴Fujirebio Diagnostics Inc., Malvern, PA, U.S.A.

The objective of this study was to evaluate reagent lot-to-lot performance over time of Abbott ARCHITECT tumor marker assays: Total PSA, Free PSA, CEA, AFP, CA 125 IIITM, CA 15-3®, CA 19-9XR, SCC, Pepsinogen I and Pepsinogen II using chemiluminescent microparticle immunoassay technology. Serial testing of patients for specific antigen levels in the serum or plasma aids in determining the presence of residual tumor, tumor recurrence, progressive malignant disease or therapeutic response. Consistency among manufactured reagent lots is essential for cancer patient management.

Materials and Methods: Quality control testing procedures report control and serum panel values at various concentration levels throughout the assay ranges on each manufactured reagent lot to monitor assay performance over time. Data were collected from all reagent lots manufactured for each assay with a market availability of ten months to six years. The mean values and % coefficient of variation were determined for each level of controls and serum panels.

Results: Control samples, prepared from spiked analyte in a human serum matrix used to mimic the expected values of patient specimens in clinical laboratories exhibited the following %CV: Total PSA, <4.9%; Free PSA, <7.6%; CEA, <5.5%; AFP, <10.0%; CA 125 II, <7.0%; CA 15-3, <5.3%; CA 19-9XR, <3.8%; SCC, <1.9%; Pepsinogen I, <5.0%; and Pepsinogen II, <6.9%.

Conclusion: Consistent results among manufactured reagent lots were demonstrated for all tumor marker assays on the ARCHITECT family of instruments with a market availability of ten months to six years. Consistent performance allows for laboratories to implement quality control programs with high levels of measurement resolution, resulting in the ability to statistically discriminate significant differences in sample values. These consistent results over time allow the laboratories to have greater confidence in the reliability and reproducibility of the results they provide to their physicians, resulting in more timely and more accurate clinical decisions and management of patients. Physicians and patients can be confident that changes in the concentrations of tumor markers in the serum or plasma are indicative of changes in the patients’ disease status and not a result of variability in assay manufacturing.

New Assays

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EVALUATION NEUER AUTOMATISIERTER
IMMUNOASSAYS ZUR MESSUNG VON GESAMT- UND FREIEM PSA

K. Fischer¹, A. Jurczok¹, C. Heße¹, T. Erden², H. Loertzer¹, P. Fornara¹

¹Universitätsklinik und Poliklinik für Urologie der Martin-Luther-Universität Halle-Wittenberg; ²Tosoh Bioscience Deutschland, Germany

Vor dem Hintergrund zahlreicher kommerziell erhältlicher Testkits zur Bestimmung des prostataspezifischen Antigens (PSA) und seiner molekularen Formen mit entsprechenden diskreptanten Ergebnissen gehört zur Evaluierung eines neuen Immunoassays neben der Prüfung seiner analytischen Zuverlässigkeit auch der Vergleich mit einer schon etablierten Methode. In dieser Arbeit wurden entsprechende Untersuchungen zur Messung des Gesamt- und freien PSA auf dem automatischen Immunoanalyzer AIA-600 II der Firma Tosoh Bioscience durchgeführt.

Methodik: Zur Prüfung der analytischen Zuverlässigkeit wurden Präzision, Richtigkeit, analytische Nachweisgrenze, Verdünnungsliniearität und Präzisionsprofil der immunoenzymetrischen Assays ST AIA-PACK PA (Gesamt-PSA)

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**Ergebnisse:** Sowohl der Immunooassay ST AIA-PACK PA für die Messung des Gesamt-PSA als auch der AIA-PACK ucPA für die Messung des freien PSA zeigten gute analytische Validitätskriterien. Der Methodenvergleich zwischen den AIA-PACK- und Immulite®-Assays auf der Grundlage einer linearen Regressionsanalyse ergab Korrelationskoeffizienten von 0,998 für das Gesamt-PSA und von 0,909 für das freie PSA. Die maximale mögliche Abweichung eines AIA-PACK – Wertes konnte für Gesamt-PSA bis zu 35% über bzw. bis zu 32% unter dem Immulite-Wert betragen. Für freies PSA war diese maximale mögliche Abweichung eines AIA-PACK – Wertes mit 61% über bzw. 16% unter dem Immulite-Wert deutlich größer.

**Zusammenfassung:** Wir können anhand unserer vorliegenden Untersuchung feststellen, daß die Bestimmungsmethoden ST AIA-PACK PA für Gesamt-PSA und AIA-PACK ucPA für freies PSA bei einfacher Handhabbarkeit analytisch zuverlässige Ergebnisse liefern. Der Methodenvergleich zwischen den beiden Immunooassays für Gesamt-PSA (ST AIA-PACK PA und PSA-Immulite®) zeigte, dass bei hoher Korrelation eine relativ gute Übereinstimmung besteht, allerdings sind die Einzelwerte nicht austauschbar. Erhebliche Unterschiede dagegen bestehen zwischen den Ergebnissen der beiden Bestimmungsmethoden für freies PSA (AIA-PACK ucPA und Freies PSA-Immulite®) und – daraus folgend – für die entsprechenden Quotienten aus freiem und Gesamt-PSA (% PSA). Die Ergebnisse des Methodenvergleichs mit einem anderen quantitativen Verfahren zur PSA-Bestimmung unterstreichen, daß ein Methodenwechsel in der Verlaufs kontrolle eines Patienten nicht ungeprüft vollzogen werden darf und daß sowohl Referenzbereiche als auch Entscheidungsgrenzen, die nicht für die betreffende Methode ermittelt wurden, keineswegs aus der Literatur übernommen werden können.

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**IDENTIFICATION AND VALIDATION OF NOVEL CANCER BIOMARKERS BY PROTEOMICS**


1Roche Diagnostics GmbH, Centralized Diagnostics, Penzberg, Germany; 2Hoffmann-La Roche Ltd., Roche Center of Medical Genomics, Basel, Switzerland

In many diagnostic areas there is a great medical need for novel biomarkers with improved sensitivity and specificity. Proteomics technologies are now offering unique chances to identify new candidate markers. We have applied proteomics technologies to establish new biomarkers in the field of oncology. Here, the first results on the identification of markers for the early diagnosis of colorectal cancer (CRC) are presented. In the marker discovery phase, a variety of proteomics technologies was applied to compare tumor and matched control tissue from CRC patients in order to identify tumor-specific proteins. Particularly, a novel proteomics approach was employed, applying conventional 2D-gel electrophoresis for protein separation, but substituting the generally applied image analysis for identification of regulated proteins by a pure mass spectrometry-based approach. Proteins found to be elevated in cancer tissue were further validated by generating antibodies which were used for immunoblotting of tissue samples to confirm and prioritize the proteomics findings. In the validation phase, highly sensitive immunooassays to selected candidate markers have been developed, to assess their presence in the sera of CRC patients. Elevated levels of one candidate marker, nicotinamide N-methyltransferase (NNMT), were found in the sera of CRC patients. ROC analysis based on a serum panel limited to 109 CRC samples, 87 benign colon diseases and 317 healthy donors resulted in an area under the curve of 0.80 for NNMT and of 0.75 for carcinoembryonic antigen (CEA). Validation of additional candidate markers is currently ongoing.

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**SITE-SPECIFIC IMMUNIZATION WITH PHAGE DISPLAYING DEFINED ANTIGEN EPITOPES**

Ch. Fermér, M. Karlsson, K. Majnesjö, E. Röijer, I. Persson, O. Nilsson

CanAg Diagnostics, Gothenburg, Sweden

The aim was to establish antibodies with pre-defined specificities, by using phage particles constructed to display multiple copies of defined antigen segments as immunogens. In the present study, two antigens were chosen as the test system: the Progastrin gastrin-releasing peptide (ProGRP), which, in our hands, has proven to be a poor immunogen, and the E7 oncoprotein of human papillomavirus 16 (HPV 16), a protein that contains both conserved motifs as well as unique segments.
Materials and Methods: Rabbits and mice were immunized with phage particles displaying defined parts of the chosen antigens. Serum samples were analyzed in ELISA assays for reactivity against the corresponding native antigen. Antibody transcripts from B-cells of ProGRP-positive mice were sequenced. The results of the present study are especially encouraging, indicating that "site-specific immunization" may be useful for antigens that otherwise are difficult to raise antibodies against. Furthermore, the establishment of specific anti-HPV 16 E7 antibodies demonstrated that it is possible to direct the immune response to specific parts of the antigen, by avoiding motifs that are shared by other proteins. Thus, "site-specific immunization" has proven to be an efficient way to establish highly-specific antibodies.

Results: Both ProGRP and E7 phage clones triggered strong immune responses in mice and rabbits against the corresponding native antigen. Anti-ProGRP scFv antibodies from the constructed library were selected and the epitopes were roughly mapped to at least three parts of the antigen. Hybridomas producing anti-HPV 16 E7 antibodies were established and the preliminary results indicate a specific reactivity against the target antigen.

Conclusion: Earlier immunization schemes with recombinant ProGRP, with or without carrier molecules (KLH, Ovalbumin), as well as with peptides, have shown poor results. Therefore, the results of the present study are especially encouraging, indicating that "site-specific immunization" may be useful for antigens that otherwise are difficult to raise antibodies against. Furthermore, the establishment of specific anti-HPV 16 E7 antibodies demonstrated that it is possible to direct the immune response to specific parts of the antigen, by avoiding motifs that are shared by other proteins. Thus, "site-specific immunization" has proven to be an efficient way to establish highly-specific antibodies.

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NEW ELISAS FOR THE DIRECT QUANTITATION OF CA 72-4, CYFRA 21-1 AND SCC

H. Brahms1, A. Janetzko1, S. Chari1, C. Geacintov2

1DRG-Instruments GmbH, Marburg, Germany; 2DRG International, Mountainside, NJ, U.S.A.

Tumor markers are important diagnostic tools for the early detection of tumor relapses or formation of metastasis in the aftercare of cancer patients. CYFRA 21-1 (used in differential diagnosis and monitoring of lung cancer as well as bladder cancer), CA 72-4 (used in monitoring of gastrointestinal cancers and ovarian cancer) and SCC (lung cancer) are well-characterized tumor markers. Despite their clinical importance, these three tumor markers are available on only few automates. We therefore developed sensitive and precise Enzyme-linked Immunosorbent assays (ELISA) for the routinely-used tumor markers CYFRA 21-1 and CA 72-4. Both ELISAs are based on the sandwich principle using the Gold Standard Fujirebio (formerly Centocor) monoclonal antibodies B72-3 and CC49 for CA 72-4 and BSM19.21 and KS19.1 for CYFRA 21-1. For the DRG CA 72-4 ELISA (standard range 0-100 U/ml) the following specifications were found: analytical sensitivity 0.311 U/ml; intra-assay precision 3.34%-4.54%; inter-assay precision 5.32%-7.81%; linearity of dilution 80.1%-112.8%; recoveries of spiked sera 86.6%-106.2%. The ELISA results showed a good correlation (r>0.95) with two commercially available radioimmunoassays when pathological and healthy sera were assayed in a method comparison study. For the DRG CYFRA 21-1 ELISA (standard range 0-50 ng/ml) the following specifications were found: analytical sensitivity 0.266 ng/ml; intra-assay precision 1.92%-2.33%; inter-assay precision 4.76%-7.60%; linearity of dilution 89.2%-101.9%; recoveries of spiked sera 88.5%-108.8%. The ELISA results showed a good correlation (r>0.95) with a commercially available radioimmunoassay when pathological and healthy sera were assayed in a method comparison study. For the DRG SCC ELISA (standard range 0-50 µg/l) the following specifications were found: analytical sensitivity 0.3 µg/ml; intra-assay precision 1.9%-2.4%; inter-assay precision 1.1%-1.9%; linearity of dilution 90%-110%; recoveries of spiked sera 90%-110%. The ELISA results showed a good correlation (r=0.98) with Immunoassay MEIA. Because of the accurate detection of tumor marker values below and above the cut-off values, DRG ELISA kits for CYFRA 21-1, CA 72-4 and SCC are suitable for routine diagnostic monitoring in clinical oncology.

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SENSITIVE IMMUNOASSAY FOR DETERMINATION OF PRO-GRP31-98

A. Öhrvik, U. Dahlén, I. Persson, C. Fermér, O. Nilsson

CanAg Diagnostics AB, 414 55 Göteborg, Sweden

ProGRP (Pro gastrin-releasing peptide) is a stable precursor of the gut hormone gastrin-releasing peptide (GRP). This marker of small cell lung cancer (SCLC) is more organ- and tumor-specific than other markers of relevance for lung cancer. The development of a sensitive assay for serum proGRP is here described.

Materials and Methods: Monoclonal antibodies were developed from mice immunized with recombinant proGRP peptide 31-98. Mab E146, reactive against proGRP aa 48-53, was fragmented to F(ab’)2 and biotinylated. Mab E168, reactive against proGRP aa 83-88, was conjugated to horseradish peroxidase. The assay is a one-step sandwich ELISA performed in microtiter wells coated with streptavidin using recombinant proGRP as the calibrator.
**Results:** The analytical detection limit was below 2.3 ng/L and the measuring range covered 2-2000 ng/L. Inter- and intra-assay % cv was <5%. Linearity on dilutions ranged from 90-107%. No high-dose hook was noticed up to 200,000 ng/L. Heterophilic antibodies did not interfere in the assay. The estimated upper 95th percentile in healthy individuals was 28 ng/L. The results of the new assay CanAg proGRP EIA (ng/L) = 0.96 x proGRP ELISA (Advanced Life Sciences Inc.) –16.5 demonstrated a high correlation between the methods (r=0.98).

**Conclusion:** CanAg proGRP EIA is a precise, sensitive and reproducible method for measuring proGRP in human sera. It may provide a practical tool in the management of lung cancer.

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**LIAISON® TK – A FULLY AUTOMATED NON-RADIOACTIVE TEST FOR THYMIDINE KINASE**

A.-Ch. Aronsson¹, K. Gouze¹, S. Eriksson², G. Olson¹

¹DiaSorin Inc, Stillwater, U.S.A.; ²Department of Molecular BioSciences, Swedish University of Agricultural Sciences, BMC, Uppsala, Sweden

Thymidine kinase 1 (TK1) is a cytoplasmatic enzyme, produced in the S-phase of proliferating cells. Since this enzyme is found only in proliferating cells, the enzymatic activity of TK1 has been shown to be a reliable marker of cell proliferation. Serum TK1 has been determined by use of a commercially available radioenzyme assay (DiaSorin TK REA). This assay provides clinically valuable information in the monitoring of haematological malignancies. However, the cumbersome handling procedure of the present method has hampered its general use in cancer management. A new, fully-automated, non-radioactive assay for the determination of TK1 activity is described. The assay principle is based on phosphorylation of a specific substrate (AZT) to the corresponding 5’-monophosphate. The produced AZT-MP then competes with an AZT-MP-analogue conjugated to N-(4-Aminobutyl)-N-ethylisoluminol (ABEI) for an AZT-MP-specific antibody. The antibody has negligible cross reactivity with the substrate (AZT) and naturally occurring nucleosides or nucleotides. Free AZTMP-ABEI is washed away and the amount of AZTMP-ABEI bound to magnetic particles is determined by chemiluminescence. AZT is a very efficient substrate for TK1, but not for the mitochondrial iso-enzyme, TK2, which makes the assay specific for TK1. The new assay has been compared with the TK REA test in a group of patients with haematological malignancies and an excellent correlation coefficient of 0.98 was found. The analytical sensitivity was determined to be 1 U/L and within-run CVs of <20% were seen down to 1.99 U/L.

**Conclusion:** The new LIAISON TK is a fully-automated test measuring the enzymatic activity of TK1. The assay is based on magnetic particle technology and chemiluminescence with an isoluminol derivative as label. It uses a stable substrate, shows a good precision and an excellent correlation to TK REA. The new TK test may, therefore, provide a practical tool in the management of haematological malignancies.

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**A NOVEL SERUM THYMIDINE KINASE ASSAY USING 96-WELL PLATE FORMAT AND COLORO-OR FLUOROMETRIC ELISA FOR PRODUCT DETECTION**

L. Nirell, J. Siljasson, S. Gronowitz

Biovica AB, Uppsala, Sweden

The sensitivity, specificity and reproducibility of serum TK activity determinations using a novel non-radioactive assay are presented. The assay, using 96-well plate format with pre-added reference material and measurement of the TK activity product by ELISA using coloro- or fluoro-metry, is easy to perform and automate. Age-related reference TK activity levels of a large cohort of blood donors and a range of pathological levels found in patients with different tumor diseases are presented. Comparisons of data obtained with the novel technology to those obtained with the traditional radioactive method shows a correlation >0.95 (Spearman). However, after calibrating the reference levels for the two assay procedures, it was found that the novel assay detects higher TK activity levels in pathological samples than the old radioactive assay. Disturbances by factors in the serum and low substrate levels in the old technique cause it to underestimate pathological TK activities. The novel assay uses saturating substrate and low serum and, thus, lacks disturbance from the clinical specimen. In summary, the novel technique should detect tumor growth earlier, making it more useful for therapy monitoring. Its sensitivity makes measurement in the spinal fluid, pleural and ascites fluids clinically relevant. This new technique will spread the use of TK activity measurement from hematology to all kinds of tumors, since TK is associated with growth and cell death.

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**EVALUATION OF THE ANALYTICAL AND CLINICAL PERFORMANCE OF THE ACCESS® BR MONITOR (CA15-3 ANTIGEN) ASSAY ON BECKMAN COULTER’S UNICEL® DXI 800 IMMUNOASSAY SYSTEM: A EUROPEAN MULTICENTER STUDY**
The Beckman Coulter Access® BR-Monitor assay detects the CA15-3 antigen and is a potential new test for the management of breast cancer patients.

**Materials and Methods:** The Beckman Coulter Access® BR-Monitor assay uses Ma552 as the tracer and Ma695 as the capture antibody. It was assessed on the Beckman Coulter’s UniCel® DxI 800 Immunoassay System in 5 European centers on its analytical and in 2 centers (Munich, Barcelona) on its clinical performance. In total, the sera from 1812 patients were tested, among them 267 healthy individuals, 550 patients with benign diseases (breast, gastrointestinal, lung, gynecological, urological and other benign diseases), and 995 with malignant diseases (breast, gastrointestinal, lung, gynecological, breast, urological and other malignant diseases).

**Results:** The imprecision of the assay was good (intra-assay: 4.0%-10.0%; inter-assay: 3.9%-8.6%; inter-laboratory reproducibility: 3.4%-5.1%). In all centers, linearity upon dilution was very good. Endogeneous interference of, e.g., hemoglobin, bilirubin and triglycerides had no or only negligible effects on the marker concentrations. The results in serum and heparin-plasma samples were comparable. In healthy individuals, the median level of the BR-Monitor was 11.9 U/mL and the 95th percentile was 23.5 U/mL. In benign diseases, the medians ranged from 11.3 U/mL (breast) to 15.6 U/mL (others) and the 95th percentiles from 21.6 U/mL (gynecological) to 54.6 U/mL (urological) to 54.4 U/mL (gastrointestinal, lung, and renal insufficiencies). In malignant diseases, the medians ranged from 11.2 U/mL (gastric) and 16.9 U/mL (breast) and the 95th percentiles from 30.0 U/mL (urological) to 429.7 U/mL (breast). Discrimination between the malignant and respective benign diseases was best for ovarian cancer (AUC 0.77; 53.1% sensitivity at 95% specificity) and breast cancer (AUC 0.71; 38.7% sensitivity at 95% specificity).

**Conclusion:** The Beckman Coulter Access® BR-Monitor assay shows a good analytical performance and reveals the known profile of specificity and sensitivity for the measurement of the MUC1 protein in ovarian and breast cancer.
U/mL), pancreatic cancer (95th percentile at 623.8 U/mL) and lung cancer (95th percentile at 538.8 U/mL). Discrimination between the malignant and respective benign diseases was best for ovarian cancer (AUC 0.90; 74.1% sensitivity at 95% specificity).

Conclusion: The Beckman Coulter Access® OV Monitor assay showed good analytical performance and is a reliable tool for the detection and differential diagnosis of ovarian cancer.

EVALUATION OF THE ANALYTICAL AND CLINICAL PERFORMANCE OF THE ACCESS® GI MONITOR (CA19-9 ANTIGEN) ASSAY ON BECKMAN COULTER’S UNICEL® DXI 800 IMMUNOASSAY SYSTEM: A EUROPEAN MULTICENTER STUDY


The Beckman Access® GI-Monitor assay detects the CA19-9 (GICA) antigen and is a potential new test for the differential diagnosis of pancreatic tumors as well as other gastrointestinal tumors, such as stomach cancer or colorectal cancer.

Materials and Methods: The Access® GI-Monitor assay uses C192 as the tracer and capture antibody. It was assessed on the Beckman Coulter’s UniCel® DxI 800 Immunoassay System in 5 European centers on its analytical and in 2 centers (Munich, Barcelona) on its clinical performance. In total, the sera from 1812 patients were tested, among them 267 healthy individuals, 550 patients with benign diseases (gastrointestinal, lung, breast, gynecological, urological and other benign diseases), and 995 with malignant diseases (pancreatic, gastric, hepatic, colorectal, lung, breast, gynecological and urological cancers).

Results: The imprecision was very good (intra-assay: 2.2%-4.8%; inter-assay: 2.9%-7.0%; inter-laboratory reproducibility: 3.6%-4.0%). In all the centers, the linearity upon dilution was very good. Endogeneous interference of, e.g., hemoglobin, bilirubin and triglycerides had no or only negligible effects on the marker concentrations. The results in serum and heparin-plasma samples were comparable. In healthy individuals, the median level was 6.0 U/mL and the 95th percentile was 23.8 U/mL. In benign diseases, medians ranged from 5.8 U/mL (breast) to 13.8 U/mL (urological) and 95th percentiles from 30.1 U/mL (breast) to 221.1 U/mL (gastrointestinal, particularly cholestatic diseases). In malignant diseases, medians ranged from 8.4 U/mL (prostate) to 233.8 U/mL (pancreatic) and 95th percentiles from 9.5 U/mL (prostate) to 13902 U/mL (pancreatic). Very high levels were also observed in colorectal cancer (95th percentile at 11014 U/mL), gynecological cancer (95th percentile at 3174 U/mL) and ovarian cancer (95th percentile at 1298 U/mL). Discrimination between the malignant and respective benign diseases was best for pancreatic cancer (AUC 0.82; 51.6% sensitivity at 95% specificity).

Conclusion: The Beckman Coulter Access® GI-Monitor assay shows a good analytical performance and is a reliable tool for the detection and differential diagnosis of pancreatic tumors.

Imaging Methods in Tumor Diagnosis

NUCLEAR MEDICINE IN ONCOLOGY: CONVENTIONAL SCINTIGRAPHY AND POSITRON EMISSION TOMOGRAPHY (PET)

W. Brenner

Department of Nuclear Medicine, University Medical Center, Hamburg, Germany

Nuclear medicine radionuclide procedures have a long-standing tradition in oncology to help localize both primary tumors and metastases. Bone scintigraphy for the detection of bone metastases or radiiodine scanning in thyroid cancer, for example, are well-established and thus longstanding routinely applied procedures which are part of guideline-recommended diagnostic work-ups in these patients. Besides standard radionuclide techniques, two major developments during the last 10-15 years have shifted nuclear medicine towards new areas of so-called functional imaging in oncology: receptor imaging (and therapy) in neuroendocrine tumors, and positron emission tomography (PET).

Radionuclide receptor imaging with gamma-tracers, like Indium-111-octreotide, In-111-gastrin, Iodine-123-MIBG, or, recently, with PET tracers such as Gallium-68-DOTATOC, has become increasingly important in the diagnostic work-up of neuroendocrine tumors, e.g. gastroenteropancreatic tumors, medullary thyroid carcinoma, pheochromocytoma, or...
neuroblastoma. Tumor diagnosis in these patients is usually based on clinical symptoms and elevated tumor markers or metabolites, whereas tumor localization is often hampered by the small size of these tumors. Functional imaging, such as whole-body somatostatin receptor scintigraphy, can help detect these tumors independently of their size and localization in patients in whom standard radiological procedures failed to show the predicted tumors. Furthermore, the combination of these highly tumor-specific radiopharmaceuticals with ß-emitters such as Yttrium-90 or Iodine-131 offers therapeutic options in metastatic disease.

Besides conventional radionuclide imaging with gamma-emitters, PET has become the most widely used nuclear medicine tool in oncology. PET has a significantly better image resolution and allows the quantification of tracer uptake in the tumor, which is mandatory for therapy monitoring and follow-up investigations. F-18-fluorodeoxyglucose (FDG) is the standard tracer in oncology to date since almost all tumor cells depend on increased glucose metabolism. New tracers for measuring proliferation, hypoxia, apoptosis, tumor vascularization, hormone receptor status, multidrug resistance and other cell-specific functions, however, are under clinical investigation. PET and its ability to measure cellular function and characterize tissue on a molecular basis is of increasing interest, especially for therapy regimens with specific new substances primarily aiming at tumor control rather than tumor killing. Monitoring these new therapies, therefore, requires functional information in addition to morphological imaging. Combined PET/CT, as a new imaging tool, offers both functional and morphological data to define tumors and their response to therapy.

**IMAGING METHODS IN TUMOR DIAGNOSIS**

Gerrit Krupski-Berdien

Center for Diagnostic Imaging and Interventions, University Hospital, Hamburg, Germany

Two major questions are asked by oncologists: what kind of tumor is it and how big is it or where does it lie, respectively. Improvements in diagnostic imaging have been tremendous during the last decade. With the introduction of multislice CT scanners and advanced MRI coil and breath-hold sequence technology, both modalities now offer single session whole-body imaging. Particularly, high resolution multiplanar reconstruction in CT improves oncological staging. Apart from the increased imageable volume, spatial resolution can be broken down to sub-millimeter dimensions. Although these features have resulted in better primary and secondary tumor staging and follow-up, as well as depicting of resectability, there has been no substantial benefit in terms of tumor typing and differential diagnosis. MRI-based (spectroscopy) documentation of the therapeutic effects of both systemic and locoregional therapy is possible but not in routine use. In terms of oncological imaging, today we can witness the dawning of a new age: molecular imaging. The combination of PET and multislice CT within one single scanner facilitates image fusion of glucose-uptake studies and morphological background. Particularly 3 Tesla high-field MRI enables the detection of small amounts of Fe-labeled antibodies for specific cell line detection, which has already been shown to work for stem cell tracing etc. The possibilities and limitations in 2005 compared to 2000 and 1995 are illustrated and perspectives for 2010 elucidated.

**Immunohistochemistry**

S. Abarzua1, M. Szewczyk1, S. Gailus1, D.-U. Richter3, W. Ruth2, V. Briese3, P. Piechulla1

1Universität Rostock, Institut für Biowissenschaften, Abtl. Biochemie; 2Institut für Chemie, Abtl. Technische Chemie; 3Südstadtklinik, Forschungslabor der Frauenklinik, Rostock, Germany


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**STIMULATION OF PROGESTERONE, ESTROGEN AND CORTISOL IN TROPHOBLAST TUMOUR CELLS BEWO BY GLYCODELIN A N-GLYCANS**

U. Jeschke¹, D.-U. Richter², B.-M. Möbius², V. Briese², I. Mylonas¹, K. Friese¹

¹Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University of Munich, Munich; ²Department of Obstetrics and Gynaecology, University of Rostock, Germany

The immunosuppressive protein glycodelin A (GdA) is secreted by a large number of gynaecological tumours. GdA has a unique glycosylation including fucoylated LacdiNAc structures. Trophoblast tumour cells produce a variety of steroid hormones and hCG. The purpose of this study was to investigate the effect of GdA N-glycans on hCG, cortisol, estrogen and progesterone release by the chorion carcinoma cell line BeWo in vitro.

**Materials and Methods:** Chorionic carcinoma cells of the cell line BeWo (1x10⁶ cells/ml) were cultivated in the presence of 25 mmol/ml glycodelin A and 25 mmol/ml glycodelin A N-glycans RH-3, RH-4 and RH-5 for up to 72 hours. Unstimulated BeWo cells were used as controls. After 24 h, 48 h and 72 h, 1 ml cell culture supernatant was removed, stored at –25°C and replaced by fresh medium. All experiments were carried out at least in triplicate. Collected cell culture supernatants were analysed for hCG, progesterone, estradiol (E2) and cortisol concentration.

**Results:** The production of estrogen, cortisol and progesterone are increased in GdA N-glycan-treated cell cultures as compared to untreated BeWo cell cultures. GdA N-glycans did not stimulate the hCG production in BeWo cells. A significant increase in E2 secretion was observed in BeWo cells incubated with glycodelin A and glycodelin A N-glycans (RH-3: 2.6% E2 increase compared to control, p=0.401; RH-4: 8.9% increase, p=0.012; RH-5: 4% increase, p=0.017; glycodelin: 7.7% increase, p=0.017). Although an increase in progesterone secretion was observed in BeWo cells incubated with glycodelin A and glycodelin A N-glycans (RH-3: 16.2%, RH-4: 2.8%, RH-5: 8.7%) compared to controls, these differences were not statistically significant (RH-3 to control p=0.051, RH-4 to control p=0.4, RH-5 to control p=0.139, glycodelin A to control p=0.237).

**Conclusion:** Estrogen, cortisol and progesterone are hormone markers for the trophoblast tumour cells BeWo. The results suggest that GdA, with its distinct glycosylation, modulates the hormone production of trophoblast tumours.

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**IMMUNOHISTOCHEMICAL VISUALISATION OF CATHEPSIN-D EXPRESSION IN BREAST CANCER**

E. Barthell, I. Mylonas, S. Kunze, C Kuhn, U. Jeschke, K. Friese

Universitätsfrauenklinik Innenstadt der Ludwig-Maximilians-Universität, München, Germany

Cathepsin D (Cath D), a lysosomal protease secreted by normal and malignant cells, is considered to be involved in the breakdown of the extracellular matrix during the process of metastases of tumors. The aim of this study was to investigate if the degree of immunohistochemical staining of Cath D correlates with grading, nodal status, hormone receptors or histological type in human ductal and lobular breast cancer.

**Materials and Methods:** Paraffin-fixed tissue of 120 pure (without carcinoma in situ or hyperplasia) breast cancer tissues (81 invasive ductal carcinomas and 39 invasive lobular carcinomas) from postmenopausal women were analyzed immunohistochemically with a monoclonal
anti-body. The grade and distribution of staining was evaluated with the immunoreactive score (IRS). Statistical evaluation was done by the Mann-Whitney test. \( P<0.05 \) was considered to be statistically significant.

**Results:** For nodal-positive tumors, a statistically significant increase of Cath D immunohistochemical reaction was found. In histological subgroups this could only be demonstrated for the number of positive cells. There was also a significant difference in expression depending on the histological type. Ductal carcinomas showed a significantly higher immunohistochemical reaction compared to lobular carcinomas. With respect to grading, a non-significant increase from G1 to G2 and G1 to G3 was observed in ductal carcinomas. In addition, an increase of Cath D expression from all receptor-positive to all receptor-negative tumors was identified.

**Conclusion:** In this immunohistochemical study to investigate the staining of Cath D in human breast cancer significant correlations with known prognostic values (especially nodal status) were found. Interestingly, a difference depending on the histological type. Ductal carcinomas expressed higher amounts of Cath D than lobular carcinomas. Therefore, Cath D might be a useful marker to discriminate between these two subtypes of breast cancer. However, the clinical relevance of this observation needs further investigation.

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**EXPRESSION OF INHIBIN/ACTIVIN SUBUNITS (ALPHA, BETA A AND BETA B) IN NORMAL AND CARCINOGENIC ENDOMETRIAL TISSUE: POSSIBLE IMMUNOHISTOCHEMICAL DIFFERENTIATION MARKERS**

S. Worbs, N. Shabania, D. Mayr, C. Kuhna, U. Jeschke, K. Friese, I. Mylonas

1st Department of Obstetrics and Gynaecology, Institute of Pathology, Ludwig-Maximilians-University, Munich, Germany

Inhibins (INH) are dimeric glycoproteins, composed of an alpha-subunit (INH-\( \alpha \)) and one of two possible beta-subunits (INH-\( \beta A \) or \( \beta B \)), with substantial roles in human reproduction and in endocrine-responsive tumours. The aims of this study were to determine the frequency and tissue distribution of INH-\( \alpha \), -\( \beta A \) and -\( \beta B \) in normal and malignant endometrium. Samples were obtained from normal (\( n=46 \)), atrophic (\( n=8 \)) and endometrioid carcinoma tissue (EC; G1 = 93; G2 = 32; G3 = 14). INH-\( \alpha \) was significantly higher in normal compared to malignant endometrial tissue, showing a cyclical variation throughout the menstrual cycle. EC G3 did not express this subunit. INH-\( \beta A \) and -\( \beta B \) showed specific staining reactions within the tumour cells. The highest intensity of INH-\( \beta A \) was observed in the normal secretory phase compared to adenocarcinomas (\( p<0.05 \)). For INH-\( \beta B \), the significantly highest expression was noted for EC G3 compared to EC G2 (\( p<0.05 \)) and atrophic endometrial tissue.

**Conclusion:** INH-\( \alpha \), -\( \beta A \) and -\( \beta B \) were immunolabelled in normal and malignant endometrium. INH-\( \alpha \) was expressed in a declining relationship, suggesting a tumour-suppressive function in EC. Interestingly, a high expression of INH-\( \beta B \) was observed in EC G3 compared to G2, suggesting an important role in endometrial carcinogenesis. However, the utilisation of these subunits as specific tumour markers still remains unclear.

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**THE IMMUNOHISTOCHEMICAL EXPRESSION OF THE INHIBIN/ACTIVIN SUBUNITS ARE UP-REGULATED BY INTERFERON-\( \beta 1A \) IN THE ISHIKAWA CELL LINE**

A. Höing, C. Kuhn, S. Schulze, U. Jeschke, K. Friese, I. Mylonas

Frauenklinik der LMU, München, Germany

Inhibins are dimeric glycoproteins, belonging to the TGF-\( \beta \), that are composed of an \( \alpha \)-subunit (INH-\( \alpha \)) and one of two possible \( \beta \)-subunits (\( \beta A \) or \( \beta B \)) INH-\( \beta A \) and INH-\( \beta B \). They have a substantial function in human reproduction and also seem to play an important role in endocrine-responsive tumors. Interestingly, there is an association between interferon and TGF-\( \beta \) expression. However, this relationship has not been assessed in endometrial tissue regarding the inhibin/activin expression. Therefore, the aim of this study was to determine the expression changes of inhibin/activin subunits in the endometrial Ishikawa carcinoma cell line after stimulation with interferon-\( \beta 1A \).

**Materials and Methods:** The Ishikawa cell line was cultured until confluence was observed (after 2 days). After adding interferon-\( \beta 1A \) (1000IE/ml), the Ishikawa cells were immunohistochemically analyzed for INH-\( \alpha \), INH-\( \beta A \) and INH-\( \beta B \) subunits. All experiments were performed in triplicate. The immunohistochemical expression was analyzed with a semi-quantitative score (IRS) and statistical analysis was performed.

**Results:** No immunohistochemical reaction with INH-\( \alpha \) could be demonstrated in unstimulated cells, while it was significantly up-regulated in interferon-stimulated cells (\( p<0.02 \)). INH-\( \beta A \) and INH-\( \beta B \) were primarily observed during the mitotic phases of unstimulated cells. After
stimulation, their expression was significantly higher ($p < 0.05$ each) compared to the controls and could be observed, not only during the mitotic phases, but also in non-mitotic cells.

**Discussion:** A functional relationship between interferon and the inhibin/activin subunits was demonstrated for the first time. The expression of INH-α, INH-βA and INH-βB were immunohistochemically significantly up-regulated in the Ishikawa endometrial cell line after stimulation with interferon-β1a. Since INH-α is thought to be tumor suppressive in the mouse model, interferon-β1a might activate this gene. Whether this effect can be used as a therapeutic option in endometrial carcinomas still remains to be clarified.

**114 OVEREXPRESSION OF P16INK4A IS INDICATIVE FOR THE ASSOCIATION OF NASOPHARYNGEAL CARCINOMA WITH HUMAN PAPILLOMA VIRUS (HPV)**

R.E. Friedrich¹, L. Riethdorf², S. Bartel-Friedrich³, C. Hagel²

¹Maxillofacial Surgery Department, UKE; ²Institute of Pathology, Hamburg; ³ENT Department, Martin-Luther-University, Halle/S.; ⁴Institute of Tumorbiology, UKE, Hamburg, Germany

Nasopharyngeal carcinomas (NPC) are rare tumors in Western Europe and have a poor prognosis. Irradiation of the primary and efferent lymphatics is the therapy of choice. The undifferentiated type of nasopharyngeal carcinoma is a well-described entity closely associated with the Epstein-Barr virus (EBV), a human herpes virus, in the nucleus of carcinoma cells. EBV-associated NPC are endemic in some areas, e.g. Southeast China. In recent studies, it was shown that the integration of certain human viruses into the genome of tumor cells might be favorable for the response to radiotherapy in terms of better survival rates. This was shown for EBV in NPC and for human papilloma virus (HPV) and subtypes of tonsillar carcinoma (Wentzensen and von Knebel Doeberitz: Pathologie 25: 21-30, 2004; Wittekindt et al: Adv Otorhinolaringol 62: 72-80, 2005). Both papilloma viruses (HPV 16/18) and EBV infect squamous epithelia of the skin and mucosa and are involved in a number of infectious diseases, benign and malignant lesions. P16 overexpression is indicative of HPV-infected tumor cells of oropharyngeal carcinomas (Kluesmann et al: Am J Pathol 162: 747-753, 2003; Weinberger et al: Clin Cancer Res 10: 5684-5691, 2004). Since both EBV and HPV are able to infect cells of epithelial origin and are closely associated with carcinomas, it was interesting to determine the presence of HPV infection by means of p16 overexpression in samples of undifferentiated carcinomas that were simultaneously investigated for the presence of EBV in carcinoma cells (Tyam et al: J Clin Microbiol 31: 53-56, 1993).

**Materials and Methods:** Twenty-six specimens of undifferentiated carcinomas (primaries and regional lymph node metastases) from 12 patients were investigated for the presence of HPV-dependent E6/E7 expression, the expression of p16, the proliferation rate (Ki-67, MIB-1), presence of EBV DNA (in situ hybridization) and CMV infection. The patients (mean age: 61 years) were predominantly males (n=12).

**Results:** The proliferation rate was 0 - 5% in 15 specimens, 5 - 20% in 4 specimens and >10% in 4 specimens (no staining: 3). Immunostaining revealed a diffuse nuclear and/or cytoplasmatic reaction for p16 in 18 specimens. EBV was identified in lymphoepithelial NPC. The simultaneous presence of EBV and p16 overexpression was found in 1 case (3 specimens).

**Discussion:** This study revealed that the simultaneous appearance of oncogenic HPV and EBV occurs in undifferentiated carcinomas of the nasopharynx. These findings support the current hypothesis of a synergistic effect or interaction of both viruses in the pathogenesis of such tumors. Further studies, with a larger sample size, are needed to confirm these results. Supported by Deutsche Krebshilfe, Germany.

**115 EXPRESSION OF INSULIN-LIKE GROWTH-FACTOR-RECEPTOR (IGF-IR) IN PERIPHERAL NERVE SHEATH TUMORS IN NEUROFIBROMATOSIS TYPE 1**

R.E. Friedrich¹, D. Keiner², V. F. Mautner¹, C. Hagem²

¹Maxillofacial Surgery Clinic and ²Neuropathology, Eppendorf University Hospital, Hamburg, Germany

Neurofibromatosis type 1 (NF1) is an autosomal-dominant inherited disease, characterized by the development of nerve sheath tumors. NF1 is the most frequent inherited disease associated with a predisposition for cancer (in particular malignant peripheral nerve sheath tumors, MPNST). NF1 is a progressive disease with phase-like growth spurts of dermal or plexiform neurofibroma (PNF). These tumors can cause severe disfigurement of patients. The growth control of these tumors is poorly understood. The aim of this study was to identify the expression of insulin-like growth factor receptor (IGF-IR) in peripheral nerve sheath tumors. Factor and receptor are involved in the growth control of numerous physiological and pathological processes, including Schwann cell development.
Materials and Methods: The investigation included 210 tumors of 68 NF1-patients (neurofibroma, MPNST). Sections of the specimens were immunohistochemically typed for several antigens (target antigens: IGF-IR, S-100, EMA, CD34, MIB-1), both in single- and double-staining. Double-staining allowed the sub-typing of the IGF-IR expression pattern in the mixed nerve sheath tumors. The expression was also measured in Schwann cell cultures and co-cultures with fibroblasts.

Results: Following staining of S-100 and IGF-IR, PNF were more intensely marked than MPNST \((r=-0.439, p<0.002, n=49)\). The proliferation index was tumor type-dependent: MPNST > neurofibroma. The IGF-IR expression correlated positively with the MIB-1-index in neurofibroma \((r=0.372, p=0.021, n=38)\). The receptor expression was higher in PNF than in dermal neurofibroma \((r=0.335, p=0.040, n=38)\). IGF-IR was detected in perineural fibroblasts (EMA-positive) of all nerve sheath tumors. However, the receptor was identified in CD34-marked endothelia of neurofibromas, but not in the endothelia of MPNST. In cell cultures, a strong receptor expression became evident. This expression was independent from the co-cultivation of tumor cells with fibroblasts. The statistical calculations excluded the impact of gender on the receptor expression.

Conclusion: This investigation provides evidence for the expression of IGF-IR in nerve sheath tumors in NF1. The expression pattern varied between the tumor types, the cell types and between tumors of the same type. IGF and IGF-IR are a prerequisite to maintain Schwann cell stability in the postnatal period and to prevent Schwann cells from apoptosis. This first evidence for IGF-IR expression in mutated Schwann cells points to a tumor-type-associated receptor expression in NF1. Supported in part by Deutsche Krebshilfe, Germany.

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**QUANTITATIVE ESTIMATION OF MATRIX METALLOPROTEINASES 2 AND 7 (MMP-2, MMP-7) AND TISSUE INHIBITORS OF MATRIX METALLOPROTEINASES 1 AND 2 (TIMP-1, TIMP-2) IN COLORECTAL CARCINOMA TISSUE SAMPLES**

M. Pesta, O. Topolcan, L. Holubec Jr., K. Rupert, M. Cerna, L. Holubec Sen, V. Treska, J. Finek, R. Cerny

2nd Internal Clinic, Department of Surgery, Department of Oncology and Radiotherapy and Department of Biochemistry, Medical Faculty, Charles University, Pilsen, Czech Republic

Matrix metalloproteinases play an important role in the process of extracellular matrix (ECM) degradation. The aim of our study was to assess the levels of MMP-2, MMP-7, TIMP-2 and TIMP-1 mRNA expression in colorectal carcinoma tissue samples with the real-time RT-PCR method, and to correlate the values obtained with the clinical status of the patients.

Materials and Methods: The tumor tissues were obtained from 38 colorectal carcinoma patients. From 19 of these patients, normal colon tissue was obtained and from 11 patients with benign disease of the colon, benign tissue was obtained. The expression levels of mRNA MMPs, TIMPs and GAPDH as housekeeping gene were quantified using the method of real-time RT-PCR.

Results: It was found that the level of mRNA expression of MMP-2, MMP-7 and TIMP-1 was significantly higher in tumor tissue samples that in the control tissue \((p<0.0005, resp.<0.0007 and resp.<0.0004)\). It was observed that the levels of mRNA expression of MMP-2, MMP-7, TIMP-2 and TIMP-1 did not correlate with stage of disease \((p<0.7129, resp.<0.5334, resp.<0.3648 and resp.<0.6068)\), with disease-free survival \((5\text{-year analysis}; p<0.5196, resp.<0.9197, resp.<0.6997 and resp.<0.6525)\), or the metastatic process \((p<0.4893, resp.<0.6721, resp.<0.9752 and resp.<0.4599)\).

Conclusion: It was proved that there are significant differences in the levels of mRNA MMP-2, MMP-7 and TIMP-1 expressions between tumor colorectal tissue and control tissue.

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**LOSS OF HETEROCYOGOSITY IN TUMOR CELLS OF A RECURRENT MANDIBULAR GIANT CELL GRANULOMA IN NEUROFIBROMATOSIS TYPE 1**

R.E. Friedrich, V.-F. Mautner

Maxillofacial Surgery Clinic, Eppendorf University Hospital, Hamburg, Germany

Neurofibromatosis type 1 (NF1) is an autosomal inherited disease affecting about 1 in 3,000 humans at birth. Neurofibromas are benign soft tissue tumors, which are the hallmark of the disease. Neurofibromas are composed of different cells types, e.g. Schwann cells, fibroblasts and mast cells. The neurofibromatosis 1 gene, \(NF1\), is located on chromosome 17q11.2. Loss of heterocogyosity (LOH) of the \(NF1\) gene in NF1 patients was identified in Schwann cells from cutaneous and plexiform neurofibroma. One striking finding in NF1 is numerous skeletal lesions, e.g. scoliosis or bone cysts. Indeed, some alterations of the skeleton are pathognomonic for NF1. They are included in the NIH (USA) diagnostic criteria on NF1. Giant cell granuloma (GCG), first described by Jaffé, is a benign tumor-like lesion that is preferentially located in the jaws. The origin of GCG is unknown. One skeletal finding associated with NF1 is GCG in bones. Recently, it was shown that a new mutation of the \(NF1\) gene
was found in leukocytes of a NF1 patient with GCG. We were able to investigate a patient with NF1 who developed a GCG and to compare the genetic alterations identified from different sources of the same patient. Case Report: A 7-year-old female NF1 patient was submitted to our out-patient clinic for diagnosis and treatment of a recurrent GCG of the right premolar region of the mandible. The serum levels of calcium and phosphate were in the normal range. Removal of the tumor was followed by uneventful wound healing and proved to be successful in terms of rapid eruption of the depressed premolar. Histological investigation confirmed the diagnosis of a GCG. Genetic analysis of the tumor sample and blood by 7 microsatellite markers revealed the same LOH on the NF1 gene in both sources.

Conclusion: This study revealed a genetic alteration of the NF1 gene in a GCG of a NF1 patient. Krammer et al. (2003) have also investigated a NF1 patient, an 11-year-old girl, who had a mandibular GCG. However, in that study the identification of a novel NF1 mutation was restricted to the blood sample. This investigation is, to the best of our knowledge, the first report on a genetic link between GCG and NF1, based on the investigation of different sources. Supported by Deutsche Krebshilfe and Deutsche Forschungsgemeinschaft, Germany.

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OPTICAL SECTIONING BY STRUCTURED ILLUMINATION. BACKGROUND AND APPLICATION IN FLUORESCENCE IMAGING
H. Bauch
Carl Zeiss, Göttingen, Germany

In conventional (widefield) fluorescence microscopy, the focal plane is always blurred with information from above and below the actual focus, decreasing the contrast and possible axial resolution. This problem is especially pronounced if the thickness of the used specimen is considerably extended compared to the depth of field of the objective used. This effect prevents 3-D reconstruction and 3-D visualisation of the specimen. The blurring can be prevented or reversed by techniques, which allow the detection of only the fluorescence signals, which originate from the focal plane resulting in a "optical section" of the specimen. Several techniques, like confocal laser-scanning microscopy, 3-D deconvolution, etc., are well known to generate optical sections. In this presentation, "structured illumination" is introduced as an additional possibility to create optical sections in fluorescence applications. With structured illumination, a regular grid pattern is projected in the focal plane. The grid pattern is moved in 3 defined positions. At each position of the grid, a digital image is acquired and cleared to an optical section using image analysis. This approach was integrated in the ApoTome system from Carl Zeiss.

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PLASDIC – A USEFUL MODIFICATION OF THE DIFFERENTIAL INTERFERENCE CONTRAST METHOD DESIGNED BY SMITH/NOMARSKI
R. Danz, H. Bauch
Carl Zeiss, Göttingen, Germany

Living cells and tissues are invisible in a normal transmitted light microscope, because these structures are phase objects that, unlike amplitude objects (fixed, stained specimen), affect not the amplitude but the phase of the light with which they interact. So, in order to create high-contrast images of these invisible structures without actually interfering with them (e.g. using staining), phase-optic aids have to be used. Much has been written over the past century about the possibilities of high-contrast depiction of phase objects in light microscopes, and the differential interference contrast method according to Smith/Nomarski certainly ranks among the best of them today. In this presentation, PlasDIC is introduced as a new polarization-optical transmitted light differential interference contrast method where, unlike conventional differential interference contrast (DIC) according to Smith or Nomarski, linearly polarized light is only generated after the objective. Consequently, the differential interference contrast is invariant in relation to the unwanted optical anisotropies that may stem from the condenser, slide, object and objective. Cells grown in plastic petri dishes, for example, can be studied with DIC.

EOSTT (EOSPC-EOSMMT)

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SEQUENTIAL CHEMOTHERAPY IN NON-SMALL CELL LUNG CANCER (NSCLC)
M. Reck
Hospital Grosshansdorf, Department of Thoracic Oncology, Germany

After the introduction of the new third-generation regimens for the treatment of advanced non-small cell lung cancer
Efficacy-oriented sequential therapy of exocrine pancreatic cancer

R. Klapdor
ZeTDT GmbH, Hamburg, Germany

During recent years, clinicians and companies have mainly been interested in overall survival after first-line therapies with new drugs or new combinations. Consequently, for many years no representative studies were done concerning the effects of a sequence of 2 or 3 more or less active drugs or drug combinations on the course of a tumor disease and survival, respectively. Recently, however, a few studies have been published indicating that sequences of drugs or drug combinations with more or less comparable antitumoral activities may increase survival compared to trials with only first-line treatments, e.g. a sequence of 5-FU/Oxaliplatin followed by 5-FU/Irinotecan in colorectal cancer or of gemcitabine followed by 5-FU/Oxaliplatin in pancreatic cancer. Our own experience since 1997 seems to confirm this concept.

For more than 7 years we have been trying to significantly improve survival of exocrine pancreatic cancer patients by an efficacy-oriented sequential polychemotherapy with at least 2 or 3 sequences. Looking for survival of patients with no effective treatment, 1 or more than 1 effective treatments, respectively, we found a relevant increase in survival in localized and metastasised pancreatic cancer patients in relation to the number of effective treatment sequences (Anticancer Res 19: 2459-2469, 1999; Anticancer Res 20: 5201-5208, 2000; Anticancer Res 23: 841-844, 2003; Anticancer Res 25: 1687-1692, 2005). A precondition for this kind of sequential chemotherapy in pancreatic cancer is a short-term follow-up in order to detect further or new progression of the disease early enough to start a second- or third-line treatment before the death of these patients. For this purpose, monthly determinations of the relevant tumor markers and bi-monthly imaging (CT/MR) are proposed. The combination of both diagnostic procedures allows the diagnosis of a progressive disease or a new progression within 6-8 weeks in the majority of patients. This procedure also allows cost reductions, since early detection of new tumor progression generally allows shortening of ineffective treatment by some weeks at least. Further, gemcitabine treatment costs more than 400-500 € per week, exceeding an annual monthly determination of CA19-9. There is some evidence that, even in the case of colorectal and gastric cancer, respectively, sequential cytostatic treatment strategies may induce longer survival times compared to first-line treatments.

In conclusion, these data should stimulate companies, as the main sponsors of clinical treatment trials: a) to test the drugs or drug combinations not only in first-line strategies, but also with regard to their potential value as second- or third-line treatments; and b) to switch from first-line treatment protocols to efficacy-oriented sequential trials with at least 2 or 3 different regimens.

Sequential treatment of ovarian cancer

P. Schmidt-Rhode
Department of Gynecology, Gynecol. Senolog, Hamburg, Germany

The treatment of ovarian carcinoma is based on extensive tumor-debulking surgery. In cases of complete intra-abdominal tumor resection, retroperitoneal lymphadenectomy is recommended for patients with tumor stages of FIGO Ic and higher. Subsequent adjuvant combination chemotherapy improves the prognosis and increases the survival time. In patients with primarily inoperable findings, secondary tumor debulking operations after induction chemotherapy can improve prognosis in cases of remission under this treatment. The therapy of recurrent ovarian disease follows the same principles as in primary ovarian cancer. Secondary or third surgical interventions make sense if complete tumor removal
is possible again, or at least a significant tumor reduction can be achieved. Additional re-induction chemotherapy of primarily used substances or application of newly-developed and highly effective drugs have a positive impact on patients' quality of life and survival time. Follow-up examinations after effective therapy include tumor marker determination of CA 125 and, in special cases, CA 72.4. Both markers are highly sensitive in the detection of recurrence. However, the decision about the timing and extent of a reoperation should be planned, not depending upon the tumor marker level, but on clinical criteria in relation to the benefit to the patient.

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C-PSA/PSA FOR OPTIMIZING TREATMENT FOR PROSTATE CANCER (PCA) PATIENTS IN A MULTIMODAL CONCEPT

P. Hammerer
Klinik für Urologie, Braunschweig, Germany

The high incidence of latent and slow-growing prostate cancers (PCa) has resulted in ongoing debates concerning the most effective therapies for different patient groups. In order to manage PCa patients effectively, it is critical that early detection systems, appropriate risk assessment tools and effective treatment strategies are available, such that the management plans can be tailored to patient requirements.

Materials and Methods: Based on recent publications on early detection trials, PSA and c-PSA, a new statistical approach (Clin Chem 51(3): 532-539, 2005), recommendations are given for utilizing PSA to assess risk and predict outcomes in the management of prostate cancer.

Results: PSA is used as a part of the formal staging of tumors, to evaluate the likelihood of metastatic disease and to determine the probability of cure or long-term control of the disease. Serum PSA values also form an integral component of nomograms for the development of prognoses and prediction of treatment efficacy. Data from different European Prostate Cancer Detection Studies suggest that, in patients with elevated PSA levels (4-10 ng/mL), complexed PSA is more accurate than total PSA in differentiating between patients with benign and malignant disease, especially around the PSA cut-off. PSA doubling-time (PSADT) may be used as an indicator of hormone-refractory PC (HRPC) and as a surrogate end-point for determining survival in patients receiving surgery or radiotherapy.

Conclusion: By combining PSA measurements with additional key variables, patients can be assigned to different levels of risk of progression, which may allow an optimized multimodal management of patients with prostate cancer.

Immunotherapy – State and Trends

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21% FIVE-YEAR SURVIVAL IN HIGH-RISK METASTATIC RENAL CELL CARCINOMA PATIENTS ON INHALED INTERLEUKIN-2: A GERMAN OUTCOME STUDY

E. Huland, H. Heinzer, H. Huland
Department of Urology, University of Hamburg, Germany

Israel's oncologists recently reported a unique progression-free survival of 8.7 months (m) in multimorbid metastatic renal cell carcinoma (mRCC) patients unfit for systemic immunotherapy using well-tolerated interleukin-2 (IL-2) inhalation therapy (Merimsky, 2004). High-risk patients barely achieve a median survival of 6 m and a maximum survival of 36 m. Here, overall survival using inhaled IL-2 was evaluated in multimorbid high-risk patients in 9 centers in Germany. Ninety-seven high-risk patients on inhaled IL-2 were collected. Twenty-eight % had ECOG 2, 87% had diagnostic treatment interval (DTI) of ≤24 m, 97% had metastases to the lung and other sites, 31% had no prior nephrectomy, 11% had prior chemo- and 17% had prior immuno-therapy. Survival was compared to 103 high-risk patients on systemic immunotherapy from approval studies. All patients met the European exclusion criteria for subcutaneous IL-2 therapy (e.g. ECOG >1 or ECOG=1 and >1 metastatic site and ≤24 m DTI). Inhalation was well-tolerated and doubled the median survival compared to systemic therapy. The 5-year survival for inhalation patients was 21% and for systemic patients it was 0.

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MOLECULAR TARGETED THERAPY IN ADVANCED CANCER

U. Vanhoefer
Zentrum für Innere Medizin, Marienkrankenhaus, Hamburg, Germany

Currently, anticancer strategies implicate molecular targets such as the vascular endothelial growth factor (VEGF) or epidermal growth factor (EGF) receptor. Tumor growth and the development of metastasis are dependent on angiogenesis. The VEGF has been demonstrated to play a crucial role in the angiogenesis of various solid malignancies, including colorectal cancer and lung cancer. Thus, preventing neovascularisation by
inhibiting the VEGF-dependent signaling pathway appears promising as a new strategy in cancer treatment. The combination of chemotherapy with the anti-VEGF mAb bevacizumab, compared to chemotherapy alone, showed superiority in terms of survival in advanced colorectal cancer and lung cancer. Furthermore, several compounds, addressing different targets of the signaling pathway, are currently being evaluated in clinical trials. The EGF receptor is also being investigated as a target for anticancer therapy in a variety of malignancies. Recent trials have shown that the chimeric anti-EGFR mAb cetuximab reverses the clinical resistance to irinotecan in advanced colorectal cancer. The EGFR tyrosine kinase inhibitor tarceva showed antitumor activity in advanced lung cancer. With increasing in treatment options, more individualized treatments for patients become possible. The role of molecular targeted therapies in advanced solid tumors will be discussed.

126 APPLICATION OF MONOCLONAL ANTIBODIES TO THE TREATMENT OF MALIGNANT LYMPHOMAS

J. Dierlamm

Clinic of Oncology and Hematology (with the sections Bone Marrow Transplantation and Pneumology), UKE, Hamburg, Germany

The potential for antibodies to target cancer cells specifically has long been recognized. With the advent of monoclonal antibodies, it became possible to identify and target specific molecules on the surface of tumor cells. For B-cell malignancies, target antigens have included the unique immunoglobulin idiosyncrasy of the B-cell clone (anti-idiotypic antibodies), as well as lineage-specific markers such as CD20 and CD52. The development of genetic engineering techniques has allowed the creation of antibodies incorporating selected human and murine characteristics. Rituximab (MabThera) is a genetically-engineered monoclonal antibody that targets CD20 to kill B-cells by multiple mechanisms, including mobilization of host immune effector mechanisms, induction of apoptosis and synergy with chemotherapy. Rituximab plus chemotherapy represents a new standard first-line treatment in patients with indolent and aggressive non-Hodgkin’s lymphomas. The addition of Rituximab to chemotherapy similarly improves the outcome in patients with refractory or relapsed disease. In patients with indolent lymphomas who can not tolerate chemotherapy, Rituximab monotherapy remains an effective treatment option. Patients can also be retreated with Rituximab, with no loss of efficacy. Another example of the efficacy and applicability of monoclonal antibodies is the anti-CD52 antibody, alemtuzumab (MabCampath), which is currently being applied in the treatment of CLL and T-cell lymphomas. Clinical trials are in progress to evaluate the role of other antibodies in the treatment of Hodgkin’s and non-Hodgkin’s lymphomas.

127 RADIOIMMUNOTHERAPY: FROM LABORATORY BENCH TO TREATMENT GUIDELINES

B. M. Dohmen

Section of Nuclear Medicine, Department of Radiooncology and Nuclear Medicine, Diakonie Hospital, Rotenburg/Wümme, Germany

One may wonder why an antibody-radionuclide conjugate, which – from a strictly scientific point of view – is neither particularly sophisticated nor innovative compared to other contemporary developments, has become the only approved radioimmunotherapy in Europe. However, some unique aspects of this type of treatment might explain why it was particularly difficult for this modality to progress from the laboratory bench into the treatment guidelines.

To be effective, it is mandatory that both the antibody and radionuclide exhibit a homogeneous accumulation and distribution in the tumor tissue, and that they remain there. Accumulation as well as distribution, however, may vary from antibody to antibody and from radionuclide to radionuclide. Factors such as the affinity, internalization and biological half-life of the antibodies need to be considered. Equally important are considerations of radionuclide half-life, the use of whole antibodies vs. fragments and of radiometals vs. radioiodines – to mention just a few relevant issues. The combination of antibody and radionuclide may result in deteriorated immunoreactivity, radiolysis and aggregation. These problems need to be addressed. Pretargeting concepts add, among other factors, the need to optimize sequential timing.

To turn what amounted to probably thousands of "promising" radioimmunotherapy results into true medical progress, two conclusions had to be drawn: i) a moderate response rate might be considered as a good result, if currently available treatments provide poorer results; ii) pharmaceutical agents need to be practicable, or, in the case of radioimmunotherapy, even simple. After more than a decade of development, a new therapy has finally emerged that meets both criteria: Zevalin™
Therapy

128 TUMOR CELLS XENOTRANSPLANTATION – NOVEL IMMUNOTHERAPEUTIC APPROACH FOR CANCER

S.E. Severin, V.K. Sologub, I.A. Koromislova, A.G. Kotelevits
Moscow Research Institute of Medical Ecology, Moscow, Russia

Biocompatible gels are widely used in plastic surgery. Soft polyacrylamide gel (PAAG), after subcutaneous injection, is encapsulated by connective tissue within three weeks. Such a capsule creates a media suitable for xenogenic cell growth and multiplication for months at least. In our experiments, the growth of solid mouse and human melanoma tumors in PAAG capsules in rats and human melanoma and adenocarcinoma in mice were shown. Rejection of xenogenic tumors was finally observed, but only after two or three months. In case of allogenic mouse - mouse tumor cells transplantation using the PAAG capsule, there was no rejection of allogenic B-16 melanoma and CA-755 adenocarcinoma tumors in Balb/c mice. As a consequence of the xenogenic tumor rejection, strong humoral and cellular immune responses of the experimental animals to tumor cells were observed. The vaccinating effect of xenogenic human tumors for syngeneic mouse melanoma and adenocarcinoma were shown. Human melanoma SK-Mel-1 and human breast carcinomma MCF-7 protected C57Black/6 mice from their syngeneic melanoma B-16 and adenocarcinoma CA-755 after rejection of human xenotransplants growing in the PAAG capsule. Antitumor vaccination of humans with xenogenic mouse tumor cells for immunotherapeutic purposes would be a very promising approach, if we could use the adjuvant effect of the PAAG capsule and find the most matched mouse tumors. Mouse tumor cells seem to be safer and easier to control for human pathogens than allogeneic human tumors at least. The preliminary results of clinical trials will be discussed.

129 ALLELE-SPECIFIC PEPTIDE PRESENTATION BY HUMAN LEUKOCYTE ANTIGENS: IMPLICATIONS FOR TUMOR IMMUNOTHERAPY

H.-A. Elsner1,2, R. Blasczyk2, C. Bade-Doeding2

1Laboratory Dres. Fenner and Partners, Hamburg; 2Institute of Transfusion Medicine, Hannover Medical School, Germany

HLA (human leukocyte antigens) are characterized by a large polymorphism, with, e.g., almost 700 HLA-B and 500 HLA-DRB alleles currently recognized. The single alleles of HLA genes can be grouped into families, mostly reflecting their serological and phylogenetic relationships. HLA plays a central role in the immune defense against viruses, parasites and tumors by presenting peptides to T-cells, resulting in the destruction of infected or malignant cells. The sequence comparison of peptides eluted from HLA molecules often reveals a distinct "peptide motif", i.e., a preference for peptides that have distinct amino acid residues at the so-called anchor positions of the peptides. For a variety of alleles, peptide motifs have been described, but few studies have addressed the question as to which extent variants of the same allelic HLA group differ in their peptide motifs. To answer this question for the HLA-A*66 family, the peptide repertoire of A*6602 and A*6603 was analyzed and compared with the data previously reported for A*6601.

Two HLA class I-deficient lymphoblastoid cell lines were transfected with plasmid-constructs leading to the expression of soluble, truncated HLA-A*6602 and A*6603 molecules. The recombinant cells were cultured in hollow fiber bioreactors; the harvests were purified by affinity chromatography, and the peptides were eluted from the recombinant HLA molecules. The peptides were sequenced and subjected to tandem mass spectrometry and Edman degradation after RP-HPLC.

HLA-A*6601 differs from A*6602 by two amino acid (AA) exchanges at positions 90 (outer loop) and 163 (pocket A). A*6603 differs from both alleles by an additional exchange at position 70 (pockets A and B). There were no significant differences between the peptide motifs of A*6602 and A*6603, suggesting a minor importance for the exchange Glu703>His, with both AA having neutral side-chains. However, a striking difference at the auxiliary anchor P1 of peptides bound by A*6601 (polar/acidic AA: Asp, Glu) and A*6602/6603 (polar/neutral AA: Ser), which interacts with pocket A, was observed. This finding may be best explained by the exchange Arg1633>Glu resulting in a shift towards higher acidity in pocket A of A*6602/6603, and apparently leading to the loss of preference for acidic auxiliary anchors.

This comparative study on the peptide-binding properties of closely-related HLA-A molecules demonstrates that, in some cases, minor amino acid differences between HLA molecules may already have a large impact on peptide presentation. This finding has important implications for tumor immunotherapy: for vaccination studies or T-cell-based approaches, it is crucial that the tumor-specific peptide is presented by the patient’s HLA molecules, i.e., that it fits into their peptide motif, because otherwise therapy will be inefficient. For this reason, immunotherapy based on tumor-related peptides requires the patient to be
typed not only at the group-level, e.g., A*66, but also at high-resolution (allelic) level by molecular methods. Moreover, the systematic analysis of allele-specific peptide motifs will help to define permissive HLA mismatches in allogeneic blood stem cell transplantation.

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MONITORING OF THE SIDE-EFFECTS OF ANTICANCER THERAPY USING LABORATORY METHODS

L. Holubec Jr., O. Topolcan, J. Finek, S. Svobodova, J. Salvet
University Hospital and Medical Faculty, Charles University Pilsen, Czech Republic

With improving anticancer therapy and the associated prolongation of life, there has been an important emphasis put on the quality of life of the patient during and after completing therapy. Quality of life is significantly influenced by the effects of anticancer therapy. The influence of radio- and chemotherapy on the thyroid and myocardial function was studied.

Materials and Methods: The study group involved 60 patients after radio- or chemotherapy. Myocardial function was evaluated using cardiac-specific enzymes in these patients: BNP, pro BNP and Seristra. Sixty patients were also examined for thyroid function using TSH, FT3, FT4 and antithyroid antibodies. The laboratory value results correlated with the clinical status of the patients using imaging methods.

Results: Thirty % of patients showed pathological values of BNP. Heart failure was evident during the anticancer therapy. Fifteen % of patients showed subclinical thyroid disease (hyper- or hypothyreosis) or clinical aggravation of the pre-existing disease.

Conclusion: Based on our results, it can be concluded that it is very important to monitor the side-effects of the anticancer therapy using laboratory methods. This is very important when conventional imaging methods are not available or their prompt interpretation is missing.

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LOCAL CHEMOTHERAPY WITH MAGNETIC NANOPARTICLES

C. Alexiou1, R. Jurgons1, C. Seliger1, S. Kolb1, K. Amman2, S. Odenbach3, H. Iro1

1Department of Otorhinolaryngology, Head and Neck Surgery, 2Department of Institute of Pathology and Anatomy, University Erlangen-Nürnberg; 3Department of Physics, Technical University of Dresden, Germany

Magnetic nanoparticles are used in medicine in vitro and in vivo. Magnetic cell separation is meanwhile performed with biocompatible nanoparticles in clinical routine settings and these particles are also used as contrast agents in vivo. A new approach to local cancer therapy is Magnetic Drug Targeting (MDT). Starch-coated magnetic nanoparticles, ionically-bound to mitoxantrone, were given intraarterially into the tumor-supplying artery and focused in the tumor region with an external magnetic field (1.7 Tesla) in rabbits. With this delivery, total tumor remissions without negative side-effects, with the use of only 20% and 50% of the regular systemic chemotherapeutic dosage, could be achieved. The aim of the present study was to investigate the biodistribution of magnetic nanoparticles after MDT at cellular and sub-cellular levels and to compare this with common imaging techniques. The tumor tissues were examined after MDT with light- and electromicroscopy. The same tumors were also investigated with a high resolution 3-dimensional X-ray tomography (CCD camera, 1024x1024 pixels). Light microscopy showed the particles in the endothelium of tumor vessels and in macrophages and in the intracellular space (electromicroscopy). X-ray tomography pictures revealed that the whole vascular system of these tumors can be reached by MDT. Thus, magnetic nanoparticles, bound to a chemotherapeutic agent (Mitoxantrone) can be delivered to a tumor region by MDT. High-resolution X-ray tomography offers the opportunity to achieve information about the biodistribution and this could be very important to non-invasively control cancer therapy.

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DEFINITIVE EXTERNAL BEAM RADIOTHERAPY FOR LOCALIZED HIGH-RISK PROSTATE CANCER: A LONG-TERM FOLLOW-UP STUDY WITH PSA COURSE

F. Bruns3, C. Franzki1, G. Wegener2, J.H. Karstens1

1Department of Radiotherapy, Hannover Medical School; 2Clinical Cancer Registry, Tumorzentrum, Hannover, Germany

Prostate-specific antigen (PSA) has affected the management of prostate cancer by allowing better case selection. The comparison between the two definitive treatment modalities, radiotherapy (RT) and radical prostatectomy (RP), can now be made accurately with respect to case selection and treatment outcome. The treatment results of definitive conformal RT for localized high-risk prostate cancer patients are reported.
Patients and Methods: The clinical courses of 91 patients with localized high-risk (T stage >T2: 66%; Gleason score ≥7: 49%; initial PSA >20: 49%) prostate cancer, who were treated with conformal RT alone or in combination with temporary androgen deprivation (AD), between 1996 and 2000, were reviewed. The median pre-treatment PSA level was 20.2 ng/ml (range: 2.0 to 95.6). The median radiation dose was 70.2 Gy (range: 64.8 to 75.6). Seventy-six patients (84%) received a temporary AD; 51% of these for ≤3 months and 49% for >3 months. The median follow-up time was 4.8 years (range: 0.4 to 9.6).

Results: During the follow-up period, 26 patients (29%) experienced biochemical failure, including 3 with hematogenous metastases. Sixteen patients (18%) have died to date. The 5-year overall survival was 83.6%±4.4%; the 5-year biochemical relapse-free survival (bRFS) was 74.4%±5.4%, with biochemical relapse being defined as 3 consecutive rising PSA levels ≥0.4 ng/ml after RT. The median PSA nadir after RT was 0.1 ng/ml (range: <0.04 to 2.89). The side-effects of RT were mild and tolerable.

Conclusion: For localized high-risk prostate cancer patients, definitive high-dose conformal RT with temporary AD is an effective treatment with an acceptable incidence of severe late complications in a median follow-up period of 4.8 years.

133 SHORT-TERM EFFECTS OF FRACTIONATED IRRADIATION OF THE FACIAL NERVE. AN EXPERIMENTAL STUDY IN RATS

R.E. Friedrich1, K. Lasson1, R. Laas2, C. Hagel2, S. Bartel-Friedrich3

1Maxillofacial Surgery Clinic and 2Department of Neuropathology, Eppendorf University Hospital, Hamburg; 3Department of Otorhinolaryngology, Martin-Luther-University, Halle-Wittenberg, Germany

The aim of this study was to analyse the effect of external irradiation on the facial nerve.

Materials and Methods: X-rays were applied in daily fractions of 2 Gy to the left side of the neck and cheek region of female Wistar rats (5 days/week, total: 60 Gy) in an experimental model with half-side exposure of the neck to the radiation source (n=8). After 3 to 4 months follow-up, the animals were sacrificed and the biopsies of the masseter muscle with adherent facial nerve segments were immersion-fixed in glutaraldehyde. Semi-thin sections were stained with toluidine blue and morphometrically investigated, using an integrated imaging system and standard computer software. The facial nerve of the shielded right side served as an internal control for determining the scattering effects of irradiation. Further, facial nerves of non-irradiated rats were investigated (n=37). The study was based on the evaluation of 11,193 measurements. The Scheffé-test was used to determine statistically significant differences (p<0.05).

Results: The microns area (i.e. area of single fibre) and average feret (mean diameter of a single fibre) and hole area (axon area) were not altered compared to the nerves of untreated animals, but the ratio axon to total area of single fibres increased. This result indicates an axon swelling as the short-term effect of direct roentgen-ray exposure. On the other hand, nerve fibres of the shielded right side demonstrated a reduction of the axon/total area of single fibres ratio. All findings were statistically significant.

Conclusion: This study revealed short-term irradiation effects on peripheral nerves. These experimental morphological findings could have some importance for human conditions concerning neurological deficiencies in the follow-up control and microsurgery of the facial nerve in patients with a history of radiotherapy for head and neck cancer. This study was supported in part by Hamburger Stiftung zur Förderung der Krebsbekämpfung (project No. 149) and Deutsche Forschungsgemeinschaft (proj. FR 1035/1-2), Germany.

134 RADIATION-ASSOCIATED SARCOMA AROUND BREAST IMPLANTS IN A RAT MODEL

E. Eltze1, O. Micke2, U. Schäfer2, O. Bettendorf1, A. Roda1, F. Herchenröder4, T. Chiwritsch1, C. Jackisch3, B. Pfleiderer4

1Institute of Pathology, 2Department of Radiotherapy, 3Department of Obstetrics and Gynaecology and 4Department of Clinical Radiology, University of Muenster, Germany

A known but poorly-characterized phenomenon is the occurrence of radiation sarcoma after breast implants in cancer patients. Tumor induction and other complications (e.g. capsule formation) around various filled breast implants were assessed after 12 months of implantation in a rat radiation model.

Materials and Methods: Model implants, one per rat (saline n=40, controls without implants n=10), were implanted subcutaneously in the lower back of rats. After one month, high voltage radiation (3 x 7.75 Gy) followed. Two groups with (n=27) and without postoperative radiation (n=13) were formed. The tumors were characterized histo- and immunohistologically (2=6.927; p=0.008). Radiation was also an important cofactor – none of the control animals developed tumors.
Results: Development of malignant tumors in the implantation site was two times higher than in the radiated animals group. The malignant tumors in the radiated animals had a much higher rate of high-grade sarcomas than in unirradiated animals (Mann-Whitney, \( p=0.008 \)). The presence of an implant is a factor for tumor development in our rat model (Chi-squared test).

Conclusion: Risk for radiation-induced sarcoma might be higher in younger patients who probably have a longer implantation time, since the presence of an implant may be a cofactor for tumor development. Thus, a complete capsulectomy seems advisable when an implant must be changed.

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EXPRESSION AND DISTRIBUTION OF CYTOKERATINS AND VIMENTIN IN RAT LARYNX AND TRACHEA FOLLOWING IRRADIATION

S. Bartel-Friedrich, C. Lautenschläger, H.-J. Holzhausen, R.E. Friedrich

1Department of Otorhinolaryngology, Section: Phoniatrics and Pedaudiology; 2Institute of Medical Biometry and Informatics and 3Institute of Pathology, Martin-Luther University Halle-Wittenberg; 4Department of Maxillofacial Surgery, UKE, Hamburg, Germany

Cytokeratins (CK), which are among the principal components of the intermediate filament proteins (IF; cytoskeleton) of epithelial cells, and vimentin, a marker of mesenchymal cells, provide intracellular mechanical support and play an important role in the structural stability of both the single cell and the tissue unit. While CK alterations in lung tissues have been reported in association with the morphological changes in pulmonary alveolar remodeling processes, during lung development and in cultured type II pneumocytes, as well as, albeit rarely, in radiation-induced lung injury (alveolar and bronchial epithelia), reports on radiogenic CK/IF alterations in laryngeal-tracheal tissues are rare. External irradiation (IRR) of advanced head and neck tumors often includes "normal tissues" (tissues unaffected by cancer) of the larynx and trachea. In these normal tissues, both single cell damage (necrosis, apoptosis, functional cell death) and interstitial damage (edema, fibrosis, vascular alterations, cellular infiltrations) resulting in tissue remodeling can occur, depending on various IRR parameters. The aim of this study was to add to our knowledge of the phenotypic characterization of the normally integrity-supporting CK/IF following a clinically relevant IRR protocol in laryngeal-tracheal tissues.

Materials and Methods: In 61 laryngotracheal specimens from Wistar rats, the expression profiles and distribution patterns of CK (CK13, CK17/19, CK18) and vimentin, depending on the IRR dose (fractionated IRR, 2 Gy per day, total dose of 20, 40 or 60 Gy), time since IRR (6 months vs. 12 months) and animal age (1 year vs. 1.5 years), were investigated using immunohistochemical methods, semi-quantitative assessment and multivariate analysis.

Results: In irradiated specimens, the expressions of both CK and vimentin showed slight to moderate dose-dependent alterations. The expressions differed in frequency and level among the various tissue structures and showed remarkable heterogeneity, with increases, decreases and fluctuations in staining. In the glottic mucosal layer (non-keratinizing squamous epithelium), the CK13 expression decreased with increasing dose. The CK17/19 expression of supra- and subglottic respiratory epithelia following 20- and 60-Gy exposure was significantly lower than in controls. The respiratory epithelia and, in part, the cuboidal epithelia of the indifferent type at the inner side of the aryepiglottic fold, revealed increasing CK17/19 immunoreactions up to 40 Gy IRR, but a distinct decrease in expression at 60 Gy. In subglottic gland structures, CK18 was detected at significantly higher levels than in controls. There was increasing expression with increasing dose. The CK18 reactions of the supra- and subglottic respiratory mucosal layers, supraglottic gland structures and thryocytes tended towards increasing expression. Tracheal mucosal epithelia, tracheal glands and respiratory epithelia of the inner side of the aryepiglottic fold showed a tendency towards decreasing expression of CK18. In addition, these tissues showed dose-dependent fluctuations. Furthermore, the vimentin reactions showed dose-dependent, heterogeneous patterns, with increases, decreases and fluctuations in staining. Additionally, there were differences in the frequency and intensity of expression among the various tissue structures. Age and time since IRR had no significant effect on immunoreaction.

Conclusion: The staining of CK and vimentin predominantly showed a notable dose-dependent heterogeneity, with increases, decreases and fluctuations in expression. The expression pattern persisted for up to 1 year after completion of irradiation. Thus, these findings must reflect late radiation effects. The altered expressions of CK and vimentin may play at least a partial role in the structural (e.g., edema) and functional (e.g., voice disorders) changes associated with irradiation of the head and neck. This study was generously supported by the Hamburger Stiftung zur Förderung der Krebsbekämpfung (project No. 149), Germany.
Complementary Medicine –
Hyperthermia

136 COMPLEMENTARY AND ALTERNATIVE MEDICINE IN ONCOLOGY
M. Azemar, M. Rostock, U. Mengs
Tumorbiology Center, University Freiburg, Madaus AG Cologne, Germany

Standard therapies in oncology, like surgery, radiotherapy and chemotherapy, have made tremendous progress during recent decades, so that nowadays 40% to 50% of newly-diagnosed cancers can be put on remission, with some of them even cured. However, these remissions are reached at the price of very cumbersome therapies. Most patients complain about the stressful side-effects of the therapies and their disappointment at being confronted with relapse or ineffective therapies and death. At the point when a majority of patients seek alternative, complementary and integrative therapeutic approaches, they are frequently confronted with a lack of reasonable and objective advice from their physicians, highlighting the cardinal role of the physician-patient communication to explain the role of different aspects of therapeutics in the treatment of cancer, encompassing standard, experimental, phythotherapeutics, cultural, social and psychological, as well as different unconventional methods. In an attempt to guide the patient in this particularly heterogeneous field, recently it has become clear that the treatment of cancer can not be restricted to the objective remission criteria, but has to include the important aspects of quality of life (QoL) in unconventional, integrative therapies. Only recently have clinical studies been published assessing the role of integrative therapies in maintaining QoL. Mistletoe is popular in German cultural areas and recent studies point toward its useful role in maintaining QoL in tumor patients.

137 COMPLEMENTARY IMMUNOTHERAPY AND HYPERThERMIA
H. Wehner
Gisunt-Klinik for Integrative Medicine, 1 Nordwestd, Hyperthermiezentrum Zetel, Germany

Background: Many cancer diseases take a problematic course. In order to try to control the disease and to improve the prognosis, theses have to be considered that suggest an impact of the immune system on the course of the disease. This aspect is interesting because all therapeutic measures necessary (operation, radiation, chemotherapy) can develop further immunosuppressive effects. Therefore, the consideration of the subpopulations of lymphocytes with immunological significance and their ability to be influenced are of interest. The use of hyperthermia in cancer treatments should thus be inspected and any possible synergism investigated. The effect of hyperthermia on different immunological aspects could be important, especially in matters of cancer treatment.

Materials and Methods: The damage of certain subpopulations of lymphocytes, due to certain diseases or therapeutic measures in a standardized laboratory, provides an insight into the side-effects of hyperthermia. The influences of extreme whole-body-hyperthermia (before, during and at different time-intervals after the therapy) were evaluated. The current knowledge emphasises that there still is a need for further research.

Results: The analysis of publications confirms our own perceptions. Hyperthermia has – beside the effects on the clinical picture – an influence on the subpopulations of lymphocytes. The distance to the application of hyperthermia shows that, even after a 4-week interval, the result is better than without hyperthermia, even under the known immunosuppressive aspects of chemotherapy.

Conclusion: Application of hyperthermia in most cases of cancer would be desirable. Especially reducing the side-effects on the immune system, in addition to possibly increasing the cytoreductive effects, is worthy of examination.

138 DENDRITIC CELL THERAPY IN ADVANCED SOLID TUMORS – AN INNOVATIVE NEW THERAPEUTIC APPROACH
Th. Neßelhut1, C. Matthes1, J. Neßelhut1, D. Lorenzen1, D. Marx1, J.H. Peters2
1Institute for Tumor Therapy and 2University of Göttingen, Germany

Several groups have shown that immunotherapy with monocyte-derived dendritic cells (MoDC) can induce a clinical antitumour response through induction of tumour-specific cytotoxic T-lymphocytes. On treating cancer patients since 1999, we found that this new treatment approach is successful in patients with various types of tumours, stabilising the disease or leading to remission even
in advanced stages. After isolating monocytes from the peripheral blood of cancer patients, dendritic cells were generated ex vivo in the presence of recombinant cytokines (IL-4, GMC-SF) and autologous serum. Flow cytometric analysis of the surface markers showed that the phenotype of the MoDC of the patients’ group was similar to the phenotype of healthy individuals. Using a phagocytosis assay, it was demonstrated, by flow cytometry and electron microscopy, that immature dendritic cells are able to phagocyte different-sized particles. The generated MoDC can induce an antigen-specific lymphocyte proliferation in vitro. Tumour escape mechanisms as, for example, down-regulation of immunological relevant molecules could have an influence on the clinical response. To investigate this hypothesis the expressions of MHC-I and -II, ICAM-1 and LFA-3 on the tumour cells of treated cancer patients were analysed. None of the analysed cases showed a complete down-regulation either of MHC-I/II or of ICAM-1 and LFA-3. However, the percentages of positive cells differed from patient to patient. After 7 days of culture, the generated autologous MoDC loaded with tumour cell lysate, known peptides, as well as with tumour proteins from the autologous serum, were administered to the patients intradermally, intravenously or intranodally. In inoperable tumours, as well as in local tumour lesions, unloaded immature MoDC were administered intratumorally leading to the in vivo uptake of tumour proteins, which can be internalised and presented by the MoDC. In order to enhance the efficiency of the dendritic cell vaccines, a combination with fever-range hyperthermia (physiological thermal stress) was performed. Summarising, all the above-mentioned application routes can induce a clinical antitumour response in cancer patients accompanied by only minor side-effects.

Glutoxim is a drug belonging to this new class of thiopoietsins, that possess a unique biological effect in that they have a modulating impact on intracellular thiol metabolic processes significant for genetic and metabolic regulation in cells and tissues. A specific drug feature is responsible for a differential impact on normal and transformed (cancer) cells. Glutoxim has a stimulating effect on the proliferation and differentiation of normal cells, while it activates mechanisms of genetically-programmed cell death (apoptosis) in transformed cells. Owing to its unique capability of inducing p53-dependent and p53-independent apoptosis pathways in the transformed cells only, Glutoxim basically increases cancer cell sensitivity to cytostatic agents. By activating normal cell metabolism, proliferation and differentiation, Glutoxim increases the resistance of intact cells to the impact of chemotherapy, thus preventing hepato-, nephro- and cardiotoxicity. Its protective effect against neurotoxicity may be additionally enhanced by the simultaneous administration of Liglutin. Considering its mechanism of action on a molecular level (endogenous production of a wide range of cytokines, reproduction of p53, p21 and ras protein effects), Glutoxim provides new levels of immune support and may thus be indicated for antibacterial, antiviral and antitumor chemo- and radiation therapy. In conclusion, this novel methodology and innovative approach to antitumor and antiviral chemotherapy may provide new treatment efficacy and grant patients a better quality of life.

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CHEMOTHERAPY SUPPORTIVE TREATMENT – NEW IDEOLOGY

L.A. Kozhemyakin

Sodarm Pharma, Saint-Petersburg, Russia

Innovative conceptual developments by the Russian pharmaceutical company Sodarm Pharma have resulted in the introduction of the following: i) a theory and methodology for designing drugs with predefined properties, analogs of key peptide metabolites, to regulate MDR gene systems, p53, p21 and cyclin-dependent protein kinases; ii) a drug that represents a new class of substances – the thiopoietsins – acting as systemic cell protectors, immunomodulators and hemopoietic factors.

Glutoxim is a drug belonging to this new class of thiopoietsins, that possess a unique biological effect in that they have a modulating impact on intracellular thiol metabolic processes significant for genetic and metabolic regulation in cells and tissues. A specific drug feature is responsible for a differential impact on normal and transformed (cancer) cells. Glutoxim has a stimulating effect on the proliferation and differentiation of normal cells, while it activates mechanisms of genetically-programmed cell death (apoptosis) in transformed cells. Owing to its unique capability of inducing p53-dependent and p53-independent apoptosis pathways in the transformed cells only, Glutoxim basically increases cancer cell sensitivity to cytostatic agents. By activating normal cell metabolism, proliferation and differentiation, Glutoxim increases the resistance of intact cells to the impact of chemotherapy, thus preventing hepato-, nephro- and cardiotoxicity. Its protective effect against neurotoxicity may be additionally enhanced by the simultaneous administration of Liglutin. Considering its mechanism of action on a molecular level (endogenous production of a wide range of cytokines, reproduction of p53, p21 and ras protein effects), Glutoxim provides new levels of immune support and may thus be indicated for antibacterial, antiviral and antitumor chemo- and radiation therapy. In conclusion, this novel methodology and innovative approach to antitumor and antiviral chemotherapy may provide new treatment efficacy and grant patients a better quality of life.

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INDIVIDUALISIERTE CHEMOTHERAPIE – OPTIMIERT FÜR DEN PATIENTEN, ZUSÄTZLICHE OPTIONEN FÜR DEN BEHANDELNDEN ARZT

Ch. Sartori

DCS Innovative Diagnostik-Systeme GmbH & Co. KG, Hamburg, Germany


New Aspects in Systemic Treatment of Malignant Nerve Sheath Tumors

141 SPORADIC AND NF1-ASSOCIATED MALIGNANT PERIPHERAL NERVE SHEATH TUMOURS DIFFER IN THE CLINICAL COURSE AND IN HISTOPATHOLOGY

Ch. Hagel

Institut für Neuropathologie, UKE, Hamburg, Germany

One of the most promising approaches to the biology of malignant peripheral nerve sheath tumours (MPNST) seems to be the comparative study of Neurofibromatosis Type 1 (NF1)-associated and sporadic tumours because of the high frequency of MPNST in NF1, and because of the differences in the clinical course in these two groups of patients. In a retrospective study, the clinical course and histopathology of 14 sporadic and 37 NF1-associated MPNST were compared. The NF1 patients were found to be significantly younger at diagnosis ($p<0.001$) and had a significantly shorter survival time as compared to the sporadic cases (median survival 17 months vs. 42 months, Breslow $p<0.05$). Time to local recurrence and metastatic spread were shorter in NF1 patients (interval to local recurrence 9.4 vs. 30.0 months, $p<0.01$; interval to metastatic spread: mean 9.1 months vs. 33.2 months, $p<0.001$). In a subgroup of patients in whom the original histopathological data was available (22 NF1 patients, 14 sporadic cases), NF1-associated MPNST showed a significantly higher cellularity compared to sporadic tumours ($p<0.001$), whereas sporadic MPNST were characterised by a significantly higher pleomorphism ($p<0.01$). Histopathological variables correlated with FNCLCC grading only in sporadic cases, but not in NF1-associated tumours. The clinical and histopathological findings indicate that NF1 gene mutations not only lead to an increased prevalence of MPNST, but also result in more aggressive tumours.

142 PROGNOSTIC RELEVANCE OF FDG PET IN PATIENTS WITH MALIGNANT PERIPHERAL NERVE SHEATH TUMORS (MPNST)

W. Brenner

Department of Nuclear Medicine, University Medical Center, Hamburg, Germany
In patients with malignant peripheral nerve sheath tumors (MPNST) and neurofibromatosis type-1 (NF1), overall survival rates are low and time to death is often less than 2 years. However, there are patients who develop metastases quite late or not at all and, thus, have a much more favorable prognosis. Histopathology and tumor grading are not well correlated with prognosis in these patients, which is similar to the situation in many types of sarcoma. In sarcoma patients, however, the standardized uptake value (SUV), determined by pre-therapeutic FDG PET, has been shown to be an independent and significant predictor of survival and disease progression. More recently, tumor heterogeneity in FDG uptake has been suggested as predictive for patient outcome and survival. The aim of this presentation was to show our data on FDG PET for risk assessment in NF1 patients with MPNST and to demonstrate the role of SUV as a parameter for prediction of patient outcome. SUVs were calculated for each tumor in pre-therapy PET scans and correlated to tumor grade, according to the FNCLCC system, and patient outcome in terms of survival or death. Three patients with tumor grade II had SUV <3. None of these patients developed metastases or passed away during a follow-up of 41-62 months. Thirteen patients with tumors grade II and III had an SUV >3. Only 1 of these patients is still alive after 20 months, while 12 patients passed away within 4-33 months. The SUV predicted long-time survival with an accuracy of 94% compared to 69% for tumor grade. In Kaplan-Meier survival analysis, patients with SUV >3 had a significantly shorter mean survival time of 13 months compared to 52 months in patients with SUV <3 (p=0.007). Tumor grading did not reveal differences in survival time (15 vs. 12 months; p=0.141). Pretherapeutic tumor SUV, obtained by FDG PET imaging, is a significant parameter for risk assessment and prediction of survival in NF1 patients with MPNST. A SUV of less than 3 has not resulted in metastatic disease to date, but indicated a significantly increased survival time, allowing the identification of MPNST patients with a more favorable outcome. FDG PET, therefore, might help to define low-risk patients and improve patient management.

144 WHICH NF1 PATIENTS DEVELOP MALIGNANT NERVE SHEATH TUMORS?

J. M. Friedman

Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Malignant peripheral nerve sheath tumours (MPNSTs) occur in about 10% of people with neurofibromatosis 1 (NF1) and are one of the most frequent causes of premature death in this population. Most MPNSTs develop from pre-existing plexiform neurofibromas. Some NF1 patients appear to be at higher risk of developing a MPNST than others. Some factors that have been associated with an increased risk of MPNST include: deletion of the entire NF1 locus and adjacent genomic region as the constitutional mutation; previous occurrence of an MPNST; previous therapeutic radiation; occurrence of NF1 neuropathy; occurrence of MPNST associated with NF1.

In collaboration with Dr. Pierre Wolkenstein and his associates in Paris, we recently conducted a study to determine whether the burden of benign neurofibromas in people with NF1 could be used to predict their risk of developing a MPNST. Clinical information on 476 NF1
proband in the Henri Mondor Database was analysed by logistic regression to look for associations between the occurrence of MPNSTs and various kinds of benign neurofibromas. Individuals found to have internal plexiform neurofibromas on imaging studies were 18 times (95% confidence interval, 5 to 73 times) more likely to develop MPNSTs than individuals without internal plexiform neurofibromas, after adjustment for age and the presence of other kinds of benign neurofibroma. This observation suggests that NF1 patients who have internal plexiform neurofibromas warrant increased surveillance for malignancy.

LIMITATIONS AND PERSPECTIVES OF RADICAL SURGERY FOR NF1 PATIENTS WITH MPNST

R. E. Friedrich, K. A. Gawad
Maxillofacial Surgery and General Surgery, UKE, Hamburg, Germany

Neurofibromatosis type 1 (NF1) is the most frequent hereditary disease with a predisposition for the development of cancer. Malignancies in the course of the disease are the main reason for the reduced life expectancy of NF1 patients. These tumors occur sporadically and are associated with NF1. Malignant peripheral nerve sheath tumors (MPNST) in NF1 have a very poor prognosis. Ablative surgery with wide safety margins is the therapy of choice. However, the resectability of MPNST depends heavily on the location of the primary and the tumor size. In tumors arising in the extremities, ablative surgery often includes amputation of the limb. Tumors arising from the trunk are very difficult to treat due to the often widespread tumor growth at the time of admission. In these patients, surgical tumor reduction is a limited therapeutic option. In the head and neck region, MPNST are predominantly derived from nerves running through the neck (or are metastases). In these patients, tumor-free resection margins are rarely achieved due to the tumor spread to the mediastinum and skull base. From our individual experience, the prognosis is better for patients with tumors arising in the extremities who allowed limb amputation. Currently, neither chemotherapeutics nor advanced radiotherapy strategies offer longer-lasting inhibitory effects on tumor growth.

Conclusion: Ablative surgery is the therapy of choice for the treatment of MPNST in NF1. Additional therapeutics are warranted. These new therapeutic strategies have to be proved in clinical studies, emphasizing the need for medical centers experienced in the treatment of the NF1.

PDGFRα AND KIT DYSREGULATION AND MUTATION IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

Charité – Universitätsmedizin Berlin, Germany

Platelet-derived growth factor receptor alpha (PDGFRα) and c-Kit are receptor tyrosine kinases targeted by the kinase inhibitor imatinib mesylate. Mutations in these receptors have been found in gastrointestinal stromal tumors (GIST) and gliomas. Since the expressions of PDGFRα and c-Kit have been found in malignant peripheral nerve sheath tumors (MPNST), we aimed to assess, in more detail, the molecular rationale for imatinib treatment of MPNST patients.

Materials and Methods: Applying single-strand conformation polymorphism analysis, 34 MPNST, 6 corresponding plexiform neurofibromas and 1 MPNST cell culture were investigated for sequence aberrations in PDGFRα (exon 2-21) and KIT (exon 9, 11,13, 17). In addition, the gene amplifications and protein expressions of PDGFRα and KIT were determined using real-time PCR, immunohistochemistry and Western blot. The expressions of the PDGFRα ligands, PDGF-A and PDGF-B, were examined by PCR and immunohistochemistry. A cell culture was utilized to determine the effect of imatinib on MPNST cells.

Results: Two MPNST carried somatic PDGFRα mutations in exons 4 and 10 leading to amino acid exchanges. Several polymorphisms detected in PDGFRα. PDGFRα and KIT were amplified in tumors from 6 and 4 NF1 patients, respectively. Both genes were amplified in the MPNST cell culture. The expression of PDGFRα was present in 21 out of 28 (75%) MPNST patients. Focal c-Kit expression was detected in 2 out of 29 (7%) MPNST patients. PDGF-A and PDGF-B were detected in MPNST and neurofibromas indicating an autocrine loop. Imatinib treatment of the MPNST cell culture exerted a growth inhibitory effect. PDGF-AA and PDGF-BB were potent mitogens for 2 human MPNST cell cultures. PDGF-AA-induced phosphorylation was prevented by imatinib.

Conclusion: The results show that dysregulation of PDGFRα and KIT occur in MPNST and suggest that MPNST patients may benefit from imatinib treatment.

CIRCULATING GROWTH FACTORS IN SYSTEMIC TUMORIGENESIS AND FOR DIAGNOSIS IN NF1 PATIENTS
Neurofibromatosis type 1 (NF1) is caused by mutations in the NF1 gene and is characterized by the systemic development of neurofibromas. Dysregulation of a number of growth factors has been suggested to be a mechanism of the pathogenesis. The secretion of some of these factors has been shown, and their identification in the circulation may provide a specific diagnostic profile for NF1. In addition, the temporal composition of this profile may influence the systemic pattern of tumor growth observed in NF1. Our data show that circulating growth factors contribute to diffuse tumorigenesis in NF1 and support the diagnosis. Furthermore, we showed that serum from NF1 patients enhanced the proliferation of human neurofibroma-derived primary Schwann cells and endothelial cells, significantly better than serum from healthy subjects. By analyzing serum growth factor levels in a cohort of 39 NF1 patients between the ages of 7 and 65 years, the concentrations of midkine (MK) and stem cell factor (SCF), but not epidermal growth factor (EGF), were shown to be significantly increased in the serum of NF1 patients when compared to healthy controls. Within the NF1 group, MK levels increased dramatically at puberty, from an average of 0.79 ng/ml in patients younger than 18 years to 1.18 ng/ml in patients over 18 years old. If the serum concentrations for SCF and MK together were applied for diagnosis, 96% of patients in the cohort tested were assigned correctly. MK and SCF both stimulate neurofibroma cell growth in vitro, while the omega-3 fatty acid docosahexaenoic acid (DHA) inhibits neurofibroma cell growth in vitro and in vivo. This effect can be modulated by growth factors in vitro, suggesting that a balance of stimulating and inhibitory factors in the patient’s circulation may mediate the effects on tumor growth rates. Indeed, it was shown by others that DHA blood concentrations decrease during pregnancy, at a time when women with NF1 frequently complain about increased tumorigenesis. The data showed that analyzing the molecular composition of the circulation in NF1 may provide valuable information for diagnosis, prognosis and tumor pathology.

1Medical Department II, Oncology and Hematology with the sections Bone Marrow Transplantation and Pneumology and 2Department of Neurology, Institute of Neuropathology, University Hospital, Hamburg, Germany

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive malignancies with poor prognosis. Although rare in the general population (incidence of 0.001), MPNSTs arise in 8-13% of patients with neurofibromatosis type 1 (NF1). In adult NF1 patients MPNST are the main cause for mortality. Therapeutic options are unsatisfying. Studies regarding the effectiveness of chemotherapy have been carried out mainly for soft tissue sarcoma. For MPNST, mainly single case reports regarding the effectiveness of chemotherapy exist. The molecular events underlying malignant progression are largely unknown. Recently, we demonstrated, by immunohistochemistry, that PDGFRalpha is highly expressed in the majority of MPNST. Western blotting confirmed PDGFRalpha expression in MPNST. Additionally, we now provide evidence for PDGFRα gene amplification and PDGFRα mutation in a subgroup of MPNST. MPNST was also examined for c-Kit expression. Only a few cases showed focal c-Kit expression by immunohistochemistry. Interestingly, MPNST with PDGFRα amplification generally also showed KIT amplification. PDGFRalpha and c-Kit are among the tyrosine kinases targeted by imatinib (imatinib). Imatinib treatment (10 μM) of a PDGFRalpha-expressing MPNST cell culture resulted in more than 50% reduced cell proliferation after 48 hours. Our observations will soon be submitted. Taken together, our results suggest that treatment of MPNST with imatinib may be indicated. This was an open-label phase II study to determine the safety and efficacy of imatinib in patients (planned: 14 evaluable patients) with unresectable MPNST. The primary objective was the response rate during the imatinib therapy of patients with inoperable or metastatic MPNST. The secondary objective was the time to progression and overall survival. The treatment consisted of imatinib, an oral drug, once daily with a starting dose of 400 mg. After 6 weeks, a first evaluation of the tumour is necessary to be sure that it is not growing. This is performed using MRI as well as FDG-PET. In the case of progressive disease, the therapy is changed and the patient receives a chemotherapy containing, e.g., anthracycline and ifosfamide. If the tumor volume does not change during therapy with imatinib accompanied by an otherwise good clinical condition, dose escalation is possible to 400 mg imatinib bid. If it is possible to progress to surgery in locally-advanced tumour following regression, it is performed; otherwise treatment is continued until progression. This study will start recruitment beginning in 2006.
MPNST IN NF1-AFFECTED CHILDREN

M. Hartmann

Department of Maxillofacial Surgery, Section of Phacomatosis, UKE, Hamburg, Germany

Malignant peripheral nerve sheath tumors (MPNST) have currently been described as tumors occurring mainly between the age 20 to 40 years, leading to a significant decrease of lifespan in patients with NF1. In the time-period from 1985 to 2005, 52 NF1 patients with MPNST were followed in our out-patient department. Out of this cohort, 8 patients were aged 1-17 years (mean age 12 years; 5 girls and 3 boys). The following characteristics were observed: MPNST arose from plexiform neurofibromas with invasive or displacing growth characteristics. They mainly presented with pain and neurological deficits. The diagnosis of MPNST in this age group took longer than for adults. This cohort did not show longer survival periods than adults with MPNST, even taking into account treatment by chemotherapy or radiation. The overall survival time of this small cohort was 30.5 months, but those children who died showed a median survival time after diagnosis of 20 months. The longest survival of 112 month was achieved by a girl who presented with a MPNST of the distal upper arm and underwent amputation of the distal extremity. The NF1 mutation analysis in the pediatric age group revealed the same mutational spectrum compared to the adult group. Our data indicated that MPNST also occurs in children with NF1. As children are not as able as adults to report pain, tumor growth or neurological deficit, each complaint should be investigated thoroughly, because it might be a hint of malignant transformation of PNF.

CULTURING AND GENETIC VERIFICATION OF MPNST CELLS

L. Kluwe, K. Grimm

Department of Maxillofacial Surgery, UKE, Hamburg, Germany

Malignant peripheral nerve sheath tumor (MPNST) cell cultures are essential for studying the biology and pathogenesis of this malignancy. They are also important for in vitro pharmacological studies for therapy development. However, for most previous MPNST cultures, the identity of the cells was unclear. Recently, we have cultured cells from 16 MPNSTs from NF1 patients with well-documented clinical and pathological findings. These cells were examined for loss of heterozygosity (LOH) of the NF1 gene (17q), p53 region (17p) and p16 region (9p). Constitutional NF1 mutations found in the patients were also used for verification of the identity of the cells. It is generally been believed that cells derived from a tumor represent the tumor cells. However, according to our finding using genotyping, this is often not the case. More than half of our cell cultures derived from MPNSTs did not exhibit the genetic alterations such as NF-LOH, or only at very low levels. Such alterations were clearly visible in corresponding tumors. These results demonstrate that cells derived even from a malignant tumor are often not the tumor cells and that genotyping is effective and essential for verification of tumor cells in culture. Without genetic verification, the risk of studying totally inappropriate cells is high. So far, one stable MPNST culture (#462) has been established, which has grown for more than 45 passages. This culture has NF1-, p53- and p16-LOH. The constitutional NF1 mutation in the patient, a nonsense mutation in exon 37, can be found homozygously in this culture. The genotype of the culture is checked regularly and before every important experiment. Other MPNST cultures either stopped growing gradually or lost, over passages, the genetic alterations which they exhibited at the beginning.

EVALUATION OF 18FLUOREODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY AS A DIAGNOSTIC TOOL FOR MALIGNANT PERIPHERAL NERVE SHEATH TUMOURS (MPNST) IN NEUROFIBROMATOSIS 1 (NF1)

R.E. Ferner¹, M. O’Doherty¹, J.F. Golding², I. Chaudhry¹, E. Calonje¹, A. Robson¹, M.A. Smith¹

¹Guy’s and St. Thomas’ Hospitals, London; ²University of Westminster, London, U.K.

Individuals with neurofibromatosis 1 (NF1) have an increased risk of developing malignant nerve sheath tumours (MPNST). These NF1-associated MPNST are difficult to diagnose, metastasise widely and often herald a poor prognosis. The clinical symptoms of malignancy are also found in patients with benign plexiform neurofibromas. Magnetic resonance imaging (MRI) does not always differentiate between benign and malignant tumours and blind biopsy may miss the site of malignancy in a heterogeneous lesion.

Materials and Methods: ¹⁸FDG PET was evaluated as a diagnostic tool for NF1-associated MPNST. One hundred
and five NF1 patients, were assessed with plexiform neurofibromas and one or more symptoms including persistent pain, change in texture, rapid growth and impaired neurological function associated with the neurofibroma. MRI determined the location and extent of the tumour. $^{18}$FDG PET evaluated malignant transformation of the lesion.

**Results:** There were 51 males and 54 females with an age range between 5-71 years (mean age 30.78, SD 13.49). Eighty benign plexiform neurofibromas were detected and 5 patients had atypical neurofibromas. The diagnosis was confirmed on biopsy in 26 patients. Thirty-five MPNST were identified including 16 low-grade, 4 intermediate-grade and 15 high-grade tumours. MPNSTs were found primarily in the proximal lower limb, whereas benign plexiform neurofibromas were located predominantly in the pelvis, head and neck, as well as in the proximal lower limb. Twenty-one patients had simultaneous multiple tumours and 15 individuals had successive MPNST in different sites. $^{18}$FDG PET revealed 4 false-positive and 3 false-negative scans (Chi-square=76.5 df=1 $p<0.001$). The 3 patients with false-negative scans had atypical or low-grade MPNST. The sensitivity of $^{18}$FDG PET in diagnosing NF1-associated MPNST was 0.83-0.90 and the specificity was 0.95-0.97. One hundred and five patients were followed clinically for 0-110 months (mean 40.6 months, SD 29.55). Fifty-two patients had benign plexiform neurofibromas that were not confirmed on biopsy; none developed the clinical features of MPNST and the majority were asymptomatic on follow-up. One individual with intermediate-grade MPNST had recurrent disease. 2 patients with high-grade MPNST developed metastases and 5 patients with high-grade tumours have died.

**Conclusion:** $^{18}$FDG PET is a highly sensitive and specific diagnostic tool for distinguishing benign plexiform neurofibromas from MPNST.

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**Addendum**

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**GROWTH PATTERN OF NEUROFIBROMAS IN NF1 PATIENTS**

U. Zils, C. Hagel, R. Friedrich, V. Mautner, A. von Deimling

Institute for Neuropathology, Charité-Universitätsmedizin, Berlin; Department of Neuropathology and Department of Oral and Maxillofacial Surgery, Hamburg, Germany

The classification of plexiform neurofibromas is in need of refinement, since current systems do not allow for simple interaction between clinicians and pathologists. Therefore, a series of 155 plexiform neurofibromas from 79 patients were examined by histological staining and immuno-cytochemistry. These data were compared with clinical and magnetic resonance imaging (MRI) findings. The striking difference in morphology was a more diffuse or a more lobulated and encapsulated growth pattern. The diffuse-type exhibited a higher grade of nuclear pleomorphism, higher cellularity ($p=0.005$), lower grade of differentiation and a higher proliferation rate than the lobulated / encapsulated type.

**Results:** Older patients suffered from plexiform neurofibromas of more peripheral localization ($p=0.04$) and with deeper localization ($p<0.001$). Further, elderly patients carried histologically more diffuse tumors ($p=0.001$) with fewer collagenous fibers ($p=0.025$). Male patients had larger tumors than women ($p=0.012$) and they were more deeply localized ($p=0.02$). The facial plexiform neurofibroma were more superficial than those of other localizations ($p<0.001$). Tumor depth, determined by MRI, matched the pathological findings.

**Conclusion:** Long-term follow-up of these tumors will be required to determine any potentially differing growth pattern.

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**EINFLUSS EINER CHEMOTHERAPIE AUF DIE KNOCHENDICHTE VON FRAUEN MIT PRIMÄREM MAMMAKARZINOM - ERGEBNISSE EINER PILOTSTUDIE**

T. Bauer, C. Maskow, M. Gottschalk, U. Wagner, P. Hadji

Klinik für Gynäkologie, Gynäkologische Endokrinologie und Onkologie, Universitätsklinikum Gießen und Marburg, Standort Marburg, Germany

Ziel dieser prospektiven, fall-kontrollierten Pilotstudie war es, den Einfluss einer Chemotherapie (Adriamycin/ Cyclophosphamid) auf die Knochendichte von prämenopausalen Frauen mit Mammakarzinom zu untersuchen.

**Material und Methoden:** Untersucht wurden 53 Patientinnen mit einem Durchschnittsalter von 43.5±9.4 Jahren, bei denen auf Grund eines primären Mammakarzinoms unter Ausschluss ossärer sowie viszeraler Metastasen eine Chemotherapie mit 4 Zyklen AC durchgeführt wurde. Frauen mit einer den Knochenstoffwechsel beeinflussenden Erkrankung oder Therapie wurden von der Studie ausgeschlossen. Die Knochendichtemessungen an der LWS und am Oberschenkelhals erfolgten mittels DXA-Methode.

Ergebnisse: Die Ergebnisse der DXA-Messung an der LWS und am Oberschenkelhals zeigten einen signifikanten Abfall der T- und Z-Score-Werte bei den Frauen mit Mammakarzinom im Vergleich zur Kontrollgruppe ($p<0.001$). In Übereinstimmung mit den Ultraschallresultaten zeigten sowohl die Messungen am Os Calcaneus als auch an den Phalangen ähnliche Signifikanzen und einen linearen Abfall der T- und Z-Score-Werte ($p<0.001$), mit dem größten Unterschied zwischen der Baseline- und der 12-Monatsmessung des T-score (AD-SOS; $p<0.001$).


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MUCIN 1, THOMSEN-FRIEDENREICH AND GALECTIN EXPRESSION IN ENDOMETROID ADENOCARCINOMAS: AN IMMUNOHISTOCHEMICAL ANALYSIS

I. Mylonas¹, N. Shabani¹, D. Mayr², D. Dian¹, C. Kuhn¹, S. Kunze¹, U. Jeschke¹, K. Friese¹

¹First Department of Obstetrics and Gynecology and ²Institute of Pathology, Ludwig-Maximilians-University, Munich, Germany

Altered mucin 1 (muc1) secretions patterns have been implicated in several cancerous conditions including gastric, colorectal and breast carcinomas. Additionally, an association between the expression of muc1, Thompson-Friedenreich (TF) and galectin has been proposed. Therefore, the aims of this study were to determine the frequencies and the tissue distributions of muc1, TF and galectin in endometroid adenocarcinomas.

Materials and Methods: Endometrial carcinomas, diagnosed with only one histological tumor form (endometroid adenocarcinomas), were obtained from 70 patients and classified according to the WHO grading system (G1=50; G2=12; G3=8). An immunohistochemical analysis was performed with specific antibodies against muc1, TF and galectin, followed by a semi-quantitative evaluation.

Results: The muc1 and galectin immunoreactions increased from G1 to G3, while TF demonstrated a lower intensity in G3 compared to G1, although with no statistical significance. However, TF showed a significant correlation with muc1 ($p=0.019$) within endometroid adenocarcinomas G1 and G2, but with no observed correlation in G3 tumors. Muc1 and TF demonstrated significant ($p=0.006$ and $p=0.046$, respectively) down-regulations in surgical stage FIGO III/IV compared to FIGO I/II. Galectin was up-regulated in FIGO III/IV, although with no statistical significance. Interestingly, there was an association between galectin and lymphangiosis ($p=0.008$).

Conclusion: The immunohistochemical expressions of muc1, TF and galectin were demonstrated in human endometrioid adenocarcinomas. Although no significant expression patterns could be demonstrated within different nuclear grading, TF and muc1 showed a significant correlation in G1/G2 tumors. Therefore, muc1 and TF might be associated in endometrial malignant transformation. Additionally, muc1 and TF were down-regulated in stage III/IV tumors, while a higher expression of galectin could be observed in stage III/IV tumors, suggesting a substantial role of this antigen in endometrial carcinogenesis. Interestingly, galectin was associated with lymphangiosis, which is thought to be a poor prognostic marker in endometrial adenocarcinomas. Therefore, muc1, TF and galectin might have important roles in endometrial pathogenesis and malignant transformation. However, the utilization of these antigens as specific tumor markers still remains unclear and further studies are warranted.

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INVASIVE MOLE: EXPRESSIONS OF INHIBIN/ACTIVIN SUBUNITS, GLYCODELIN A, MUCIN 1, THOMSEN-FRIEDENREICH, SIALYL-LEWIS A (CA 19-9, sLEA) AND SIALYL-LEWIS X (sLeX) CARBOHYDRATE ANTIGENS IN A RARE CASE

I. Mylonas¹, A. Rhode², N. Shabani¹, K. Friese¹, B. Gerber¹, J. Makovitzky²

¹First Department of Obstetrics and Gynecology, Ludwig-Maximilians University, Munich; ²Department of Obstetrics and Gynecology, University of Rostock, Rostock, Germany

Invasive trophoblastic mole is an extremely rare condition. Its early recognition is essential since, in some cases, it can transform into invasive tumours.
Materials and Methods: The biopsies had been routinely processed by paraffin-embedding and stained with Hematoxylin and Eosin. Immunohistochemical staining reactions were performed with monoclonal antibodies against the inhibin-α, inhibin-βA and inhibin-βB subunits. Additional immunohistochemical reactions were performed with mucin 1 (muc1), Thomsen-Friedenreich (TF) antigen, Sialyl Lewis-A (sLeA), Sialyl Lewis-X (sLeX) and glycodelin A.

Results: Intervillous invasive trophoblasts showed cell- and nuclear polymorphy with a wall invasion of the myometrium. The immunohistochemistry exhibited strong positivity for the inhibin-α, inhibin-βA and inhibin-βB subunits in trophoblastic and syncytiotrophoblastic tissue, while the invasive mole demonstrated no immunoreaction with these antibodies. Glycodelin A, TF, sLeA and sLeX also showed no or only minimal focal immunohistochemical reactions. Muc1 demonstrated minimal immunoreaction.

Conclusion: There were no expressions of the inhibin/activin-α, -βA and -βB subunits, glycodelin A, TF, sLeA and sLeX in the invasive mole. Since trophoblastic diseases, like hydatidiform moles, demonstrate strong immunohistochemical reactivity with antibodies against inhibin/activin subunits, these subunits might be useful in uncertain or difficult pathological diagnosis of invasive moles. Additionally, inhibin-α might also act as a tumor suppressor in placental tissue, as observed in gonadal, gonadotropin-responsive tissue. Muc1 showed minimal immunostaining and might, therefore, also be involved in carcinogenesis of placental tissues. Early recognition of gestational trophoblastic diseases is of extreme importance for the course of disease and the prognosis. Therefore, since the pathological diagnosis of an invasive mole is difficult, the immunohistochemical expressions of the inhibin/activin subunits, glycodelin A, muc1, TF, sLeA and sLeX might be useful additional tumour markers.

MESOMARK: A NEW MARKER FOR MESOTHELIOMA
D. Pons-Anicet, V. Cartalas, C. Mombrial, N. Sardasai
Cisbiointernational, Bagnols sur ceze, France; Fujirebio Diagnostics, Malvern-MA, U.S.A.

Until now, malignant mesothelioma (MM) was a rare aggressive tumour frequently arising from mesothelial cells of the pleura. It has recently been described as an epidemic disease related to asbestos exposure in industrialised countries. The symptoms are most commonly insidious, thus, the diagnosis of pleural mesothelioma is difficult and often delayed by 6 to 8 months after the initial symptoms present. Mesothelin is a phosphatidylinositol-linked glycoprotein that is present only on normal mesothelial cells and on tumours derived from them.

Soluble mesothelin-related proteins (SMRP) belong to the Mesothelin family of proteins and are also expressed by mesothelial cells. It has recently been observed that elevated levels of SMRP can be measured in ovarian cancers and mesothelioma. An ELISA assay, allowing the measurement of such fragments, has been developed using two specific monoclonal antibodies, OV569 and 4H3. This test is a two-step manual assay using 100 µL of pre-diluted serum. The precision is less than 6% for mid- and high values and <10% for low values; the analytical limit of detection is below 0.3 nM and there is no hook effect up to 10,000 nM. In a first clinical study, Mesomark has been measured in 409 healthy individuals which were all below 1.5 nM. ROC curves between those individuals and 88 mesothelioma patients showed an area under the curve at 0.87, demonstrating a high sensitivity for mesothelioma. Serum samples collected during affected patients’ follow-ups showed an excellent relationship between the level of Mesomark and progression of the disease, suggesting that SMRP levels in the sera may be of value in managing this disease.
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