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Gastroenterology I

1
DELAY AND REFERRAL PATTERNS OF PATIENTS WITH ORAL AND MAXILLOFACIAL MALIGNANCIES

R.E. Friedrich

Oral and Maxillofacial Surgery, University Hospital, Hamburg, Germany

Therapy for malignant diseases in the oral and maxillofacial region in terms of overall survival has not improved over the last decades despite remarkable efforts in the field of reconstructive surgery which allow extensive ablation of advanced stage cancer. Many patients are admitted to hospital in an advanced stage of the disease, allowing only palliative treatment options. Efforts have been undertaken to improve diagnostics for head and neck cancer, including molecular-biology based screening techniques for malignant cells in smears of the mucosa of the upper aerodigestive tract, with modest results. Improvement of therapy mainly rests upon diagnosis at an early stage of the disease. Thorough knowledge of the cancerous lesions in the oral/maxillofacial region by physicians is an important factor to decrease the time of first symptoms and adequate treatment. This analysis was performed to detect the stations that cancer patients had taken until their arrival in the department and to disclose the referral patterns to a single institution, specialized in the field of treatment for oral and maxillofacial cancer.

Materials and Methods: The files of 646 patients with malignancies of the oral and maxillofacial region who were treated in a single institution during an interval of 19 years were retrospectively analysed.

Results: Mean age of patients was 59 years. The male/female ratio was 2.3:1. Localized swelling, pain and alterations of the mucosa were the predominant first signs and symptoms of the disease. Tumors were located in the oral cavity or oropharynx (75.1%), epipharynx (1.1%), larynx (0.3%), lips and facial skin (7.3%) and other regions (11.6%) (not exactly classified: 3.9%). Stage grouping according to the TNM-system (UICC, 1992) revealed advanced stages in the majority of cases (IV: 30%, III: 15%). In 22% of cases the stage grouping could not be found out due to initial treatment performed at other institutions. The majority of patients were referred by residents in oral and maxillofacial surgery (31.7%) and dentists (28.8%). The majority of patients were hospitalized within 4 months after notification of the first symptoms (66.9%). Less than one of two patients was correctly diagnosed within 2 weeks after first consultation of a doctor (46.3%).

Conclusion: Diagnosis of oral and maxillofacial cancer was performed at a late stage of the disease. Residents with a qualification in dentistry either as a dentist or as a maxillofacial surgeon were the prevailing subgroup of medical specialists who referred the patient to the institution. In addition to the ongoing outreach work of public health institutions, *e.g.* awareness of risk factors such as alcohol abuse and smoking as causative factors for oral cancer, continuous medical education during dentistry studies and in further education plays a key role in reducing diagnostic delay and in optimizing treatment options in oral and maxillofacial cancer.

2
DISTANT METASTASES AND MALIGNANT CELLULAR NEOPLASIA ENCOUNTERED IN THE ORAL AND MAXILLOFACIAL REGION

R.E. Friedrich

Maxillofacial Surgery, Medical Center University, Hamburg, Germany

Distant metastases to the oral cavity, the face or facial skeleton are rare. These locations are usually found at a late stage of the malignancy. In the majority of cases the malignancy is already known at the time of admission. However, a distant metastasis as the first sign of a cancer developing in other parts of the body may occasionally be found. Malignant cellular neoplasia, in particular those derived from the hematopoetic system, are also rarely diagnosed first in the oro-maxillofacial region. Therapy for these patients is difficult. Main parameters of therapy are type of tumor, general health condition and localization of the tumor. The aim of this study was to analyze the types of tumor, the treatment modalities and the outcome of patients who experienced a malignant disease in the oro-maxillofacial region under these conditions. A total of 92 patients were treated for distant metastases or cellular malignant neoplasia in the oro-maxillofacial region in a single institution (female: 45, male: 47, ratio 1:1.04; mean age: 61.4 years (ys); range: 5 to 88 ys).

Results: In females the most frequent primary tumor was breast cancer (40%), followed by malignant lymphoma (17.8%), malignant melanoma and hypernephroma (8.9% each). In males the most frequent primaries were lymphomas (25.5%), followed by bronchial carcinoma and carcinoma of unknown primary site (CUPD-syndrome; 17% each). Hypernephroma was the site of origin in 8.5%. Mean survival of patients with solid tumors was 1.28 years and 4.85 years in patients with cellular neoplasia. Survival rates differed significantly in both diagnostic groups (p=0.001). The 5-ys

overall survival rate of patients with distant metastases of solid tumors was 0 years, independent from gender. In patients with malignant cellular neoplasia significant differences of survival rates were identified. Male survival was calculated to be 90% at 5 years follow-up control, but was poor for females (0%).

Conclusion: Prognosis is poor in patients with distant metastases of solid tumors of other body parts to the oro-maxillofacial region. Patients with malignant cellular neoplasia becoming symptomatic in this region share their fates depending on their gender, with males having a better prognosis.

3

BASALOID LESIONS OF ORAL SQUAMOUS CELL EPITHELIA AND HUMAN PAPILLOMA VIRUS (HPV) INFECTION

R.E. Friedrich, C. Sperber, K. Roeser, T. Loening

Oral and Maxillofacial Surgery, Eppendorf University Hospital, Institute of Pathology, Albertinen-Krankenhaus, Hamburg, Germany

Basaloid squamous cell carcinomas are a variant of squamous cell carcinoma preferentially arising in the head and neck region. Current reports point to an association of basaloid squamous cell carcinoma and human papilloma virus (HPV)-infection. This virus is supposed to be an aetiologic factor in this entity. The aim of this study was to analyse the HPV-infection status in different entities of oral neoplasms or dysplasias.

Materials and Methods: This study comprises data from 34 oral lesions of squamous epithelia origin: 17 basaloid squamous cell carcinoma, 10 papilloma and 7 hyperplasia/dysplasia of oral epithelia. HPV-DNA was detected by means of hybrid capture technique. HPV-types were identified by direct sequencing. Immunohistochemical investigation of the specimens with anti-p16 antibody was performed in order to elucidate the putative role of p16 as a surrogate marker of HPV infection.

Results: The rate of HPV-infected basaloid squamous cell carcinoma was extraordinarily high. About 2 out of 3 cases (61%, 11/17) were infected with HPV-high-risk types, predominantly with HPV genotype 16 (>90%, 10/11). The infection status differed significantly between basaloid squamous cell carcinoma and other oral lesions in terms of frequency of HPV infection and HPV genotypes. p16 expression was proved unsuitable as a surrogate marker of HPV-high-risk infection in oral lesions, particularly in basaloid squamous cell carcinoma. This is an essential difference of this collective compared to genital carcinoma with HPV-high-risk infection.

Conclusion: This study reveals a tremendously high association between basaloid squamous cell carcinoma and HPV type 16. This close phenotype-genotype-correlation can be of diagnostic value. Type-specific analysis of HPV infection in head and neck cancer might be important in the differential diagnosis of malignancies in the head and neck region with a "basaloid" growth pattern. However, the investigation is technically demanding, including hybridization and sequencing techniques. A simplified test of HPV in basaloid squamus cell carcinoma of the oral cavity using the immunohistochemical proof of p16 expression as a surrogate marker is non-effective. *Supported in part by Deutsche Krebshilfe*.

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TUMOUR VOLUME, TRACE ELEMENT STATUS AND TUMOUR MARKERS IN HEAD AND NECK CANCER PATIENTS

Jens Büntzel[1], Ute Knolle[2], Oliver Micke[3]

[1]Department of Otolaryngology, [2]Department of Anesthesiology, Südharzkrankenhaus, Nordhausen, Germany, [3]AKTE

Objective: To establish whether there is a relation between tumour volume and SCC/CEA/CYFRA 21-1 and/or trace element status (selenium (Se), zinc (Zn)) in patients with advanced head and neck cancer.

Materials and Methods: Magnetic resonance imaging (MRI) based tumour volumetry in was performed, pre-treatment, on 21 patients (2 female, 19 male) suffering from advanced head and neck cancer. The following tumour localizations were included - oropharynx 6 patients (pts), larynx 2 pts, tongue 5 pts, hypopharynx 6 pts and others 2 pts. At the time of diagnosis classical tumour markers SCC, CEA and CYFRA 21-1 were measured as well as the serum-concentrations of selenium and zinc (atomic absorption spectrometry). Relations between tumour volume and laboratory data were calculated with MS Excel.

Results: The median tumour volume of the primary tumour was 16.45 cm^3 (range 1.14 to 209.87 cm^3). Reduced Zn and Se concentrations were observed in 6/21 patients (28.6%) and 16/21 patients (76.1%), respectively. Increased values were observed in 14/21 cases for SCC (66.7%), 1/21 cases for CEA (4.8%) and 4/21 cases for CYFRA 21-1 (19%). These sensitivities were increased to 50% for Zn, 83.3% for Se, 75% for SCC, 8.3% for CEA and 16.7% for CYFRA 21-1 if the tumour volume was >10 cm^3 (n=12).

Conclusion: SCC and serum Se levels seem to be effective tumour markers in head and neck cancer patients with primary tumour volumes >10 cm^3.

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EXPRESSION OF THE CARBOHYDRATE TUMOR MARKER SIALYL LEWIS A (CA19-9) IN SQUAMOUS CELL CARCINOMA OF THE LARYNX

I. Wiest[1], D. Mayr[2], C. Alexiou[3], S. Schulze[1], C. Kuhn[1], S. Kunze[1], M. Büttner[4], P. Betz[5], U. Jeschke[1]

[1]Department of Obstetrics and Gynecology and [2]Department of Pathology, LMU Munich, [3]Department of Oto-rhino-laryngology, Head and Neck Surgery, [4]Department of Pathology and [5]Department of Legal Medicine, FAU Erlangen, Germany

The clinical relevance of the carbohydrate antigen Sialyl Lewis a (SLe a) as a serum tumor marker in diagnosis and follow-up treatment is unquestioned in a broad spectra of human carcinomas. Over-expression of this antigen is combined with poor prognosis and malignant relapse. The aim of this study was the systematic investigation of SLea expression in squamous cell carcinoma of the larynx *versus* normal and phlogistic tissue.

Methods: Paraffin-embedded sections of normal, phlogistic and squamous cell carcinoma tissue were incubated with a monoclonal antibody against SLe a. Staining reaction was done with ABC-Peroxidase and DAB. Breast cancer tissue was used as a positive control and a negative control was performed with unspecific mouse IgM. Double-blind semi-quantitative evaluation was carried out by two independent investigators, including a pathologist.

Results: A total of 15 different tissue slides were analysed. A very faint expression of SLe a (Ca 19-9) was found in normal laryngeal tissue (IRS 4), a moderate up-regulation was observed in phlogistic tissue (IRS 4.66) and a dramatic up-regulation in some types of squamous cell carcinoma of the larynx (IRS 6). Due to the small number of cases the results did not reach statistical significance (p=0.066).

Conclusion: The results of this study indicate that Sialyl Lewis a is a potential tumor marker in carcinoma of the larynx.

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SQUAMOUS CELL CARCINOMA ANTIGEN ISOFORMS IN THE SERA FROM PATIENTS WITH BENIGN AND MALIGNANT OESOPHAGEAL DISORDES

B. Slesak[1], A. Harlozinska[1], M. Strutynska[2], R. Einarsson[3], P. Sedlaczek[1], J. Adamiak[1]

[1]Department of Clinical Immunology and [2]Department of Gastrointestinal and General Surgery, Wroclaw Medical University, Wroclaw, Poland and [3]Fujirebio Diagnostics AB, Gothenburg, Sweden

Two isoforms of squamous cell carcinoma antigen (SCCA) have an almost identical nucleotide sequence and are members of the serine proteinase inhibitor family. One of them, SCCA1 is a blocker of papain-like cysteine proteinases and the other, SCCA2, inhibits chymotrypsin-like serine proteinases. The aim of the study was to determine the concentrations of SCCA (total), SCCA1 and SCCA2 in the serum of patients with gastro-oesophageal reflux disease (GERD) and squamous cell carcinoma (SCC) in comparison to the control group. Moreover, to analyse whether any of the SCCA isoforms would provide more specific/sensitive clinical information than the total SCCA concentration.

Methods: The concentrations of these markers were estimated by ELISA assay in the same serum specimens taken from an individual patient before treatment.

Results: Twenty-five patients had GERD classified according to the Los Angeles scale and 45 suffered from oesophageal SCC in stage of clinical advancement I - IV. Higher than normal values of SCCA, SCCA1 and SCCA2 concentrations were found in 28.0%, 8.0%, 32.0% and 48.9%, 31.1% and 46.7% of cases of GERD and SCC, respectively. It is interesting that simultaneous elevated levels of SCCA and SCCA2 were found both in GERD and SCC (24.0% and 42.2% of cases, respectively).

Conclusion: The results demonstrate the differentiated concentrations of the studied markers; however it was shown that elevated SCCA levels were frequently accompanied by high levels of SCCA2. Mean concentrations of SCCA1 in GERD and SCC did not exceed the mean values in healthy donors. The results indicate that SCCA2 is a more useful serological marker in oesophageal diseases than SCCA1, however it is comparable with the total SCCA marker.

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CLASSICAL AND PROLIFERATIVE TUMOUR MARKERS AS PROGNOSTIC FACTORS OF GALL BLADDER, BILE DUCT AND CHOLANGIOCELLULAR CARCINOMAS – A PILOT STUDY

V. Liska, L. Holubec Jr., V. Treska, J. Vrzalova, T. Skalicky, A. Sutnar, S. Kormunda, J. Finek, O. Topolcan

Department of Surgery and Department of Oncology, Central Isotopic Laboratory 3, Medical School and Teaching Hospital Pilsen, Charles University, Prague, Czech Republic

The behaviour of proliferative and classical tumour markers in gall bladder carcinoma, bile duct carcinoma (Klatskin) and cholangiocellular carcinoma is not commonly known and could play an important role in diagnostic and treatment strategies and prognostic schemas. Further, it could help oncosurgeons and oncologist as an important additional diagnostic method. This paper presents a statistical analysis of classical (CEA, CA 19-9, CA72-4) and proliferative (TK, TPA, TPS, CYFRA) tumour markers in named carcinomas. The study tries to find the role of tumour markers in non-properly studied dieseases, where their importance is often marginalized.

Methods: The study included 43 patients who underwent explorative laparotomy without any surgical treatment or were treated by paliative or radical surgical procedures for gall bladder carcinoma, bile duct carcinoma (Klatskin) and cholangiocellular carcinoma (24, 8 and 11 patients, respectively) between the years 2000 and 2007 at the Department of Surgery, Medical School and Teaching Hospital Pilsen, Charles University Prague. The relation between particular tumour markers after none, paliative or radical surgical treatment in comparison with disease free survival (only for radical surgery) and overall survival was studied. The purpose was to describe the dependency of behaviour of serum tumour markers upon prognosis of patients. LogRank test and Wilcoxon test were used for statistical evaluation. Survival rate or disease free interval (DFI) was computed by the Kaplan-Meier method.

Results: The statistical analysis of tumour markers proved TK, TPS and CEA as promising prognostic factors of recurrence or poor prognosis for patients with gall bladder carcinoma. AFP was demonstrated as a prognostic factor for all named diagnoses after radical surgical treatment.

Conclusion: The results of the study suggest the importance of tumor markers in prediction of recurrence of disease after radical surgery or poor prognosis after paliative or no surgery for patients with gall bladder carcinoma, bile duct carcinoma and cholangiocellular carcinoma. In spite of the limitations of the presented pilot study, namely the small number of patients in particular disease groups, the presented results are promising and illustrate the importance of classical and proliferative tumour markers in diagnostic and treatment decision strategies. *This study was supported by MSM 0021620819 Research Project.*

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THE VALUE OF RADIOCHEMOTHERAPY FOR LOCALLY ADVANCED UNRESECTABLE EXOCRINE PANCREATIC CANCER

O. Micke[1,2], F. Bruns[3], U. Schäfer[2,4], N. Willich[2]

[1]Klinik für Strahlentherapie und Radioonkologie, Franziskus Hospital, Bielefeld, [2]Klinik und Poliklinik für Strahlentherapie und Radioonkologie, Universitätsklinikum Münster, [3]Klinik für Strahlentherapie und spezielle Onkologie, Medizinische Hochschule Hannover, [4]Klinik für Strahlentherapie, Klinikum Lippe, Germany

Combined radiochemotherapy has been established as standard treatment in locally advanced carcinoma of the exocrine pancreas. Unfortunately, the majority of patients continue to succumb to the disease process. Recently, there has been 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.

Methods: Between January 1994 and February 2005, a total of 148 patients with locally advanced irresectable pancreatic adenocarcinoma, histologically proven, were included in the study provided there were no distant metastasis, karnofsky performance status >70%, age >18 and <75 years and normal blood counts, renal and liver function. The median age was 61 years (range: 26-75 years); 88 patients were male and 58 were female. Two different treatment schemes were consecutively used: Between January 1994 and December 2001 all patients (n=110) received a combined radiochemotherapy, consisting of hyper-fractionated accelerated conformal radiotherapy and simultaneous application of 5-fluorouracil and folinic acid. Conformal radiotherapy was carried out under megavoltage conditions at 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/m^2 of 5-fluorouracil (5-FU) and 300 mg/m^2 of folinic acid (FA) were given intravenously. Chemotherapy was repeated monthly in all cases of no progressive disease after evaluation of radiological tumor response. From January 2002 to June 2008, for another 38 consecutive patients the chemotherapy regimen was changed to gemcitabine (Gem) (300 mg/m^2) and cisplatinum (Cis) (30 mg/m^2), followed by gemcitabine (1000 mg/m^2) every 2 weeks in all non-progressive patients. Regular follow-up examinations included abdominal ultrasound, CT-scans as well as the repeated measurement of serum CA 19-9 levels.

Results: Median overall survival analyzed by Kaplan and Meier method of the 5-FU/FA group was 10.5 months. The actuarial 1-year survival was 46.6%, the 2-years survival 20.1% and the 3-years survival 15.5%. Median time to progression was 8.6 months. The progression-free survival was 40.0% after one year, 18.8% after two years and 11.5% after three years. In the Gem/Cis group median survival was 13.4 months with a 1-year-survival of 55.5%, a 2-year-

survival of 24.8% and 3-years survival 20.5%. Median time to progression was 11.1 months. The progression-free survival was 48.8% after 1 year, 25.8% after 2 years and 13.5% after 3 years. The overall survival and the progression-free survival were significantly superior in the Gem/Cis group (p=0.03, resp. 0.048). Overall the treatment in the 5-FU/FA group was relatively well tolerated with primarily nausea/vomiting. In the Gem/Cis group, there was comparable feasibility with a slight tendency towards more severe side-effects. Uni- and multivariate analysis revealed CA 19-9 levels to be a significant, independent prognostic factor.

Conclusion: Radiochemotherapy as primary treatment in locally advanced pancreatic cancer is an effective and well tolerable treatment. The long-term efficacy is still limited. The integration of new chemotherapeutic agents like gemcitabine as well as prognostic and monitoring factors like CA 19-9 in the multi-modality treatment may give a more promising perspective in terms of better survival rates. Because of the narrow therapeutic index of gemcitabine-based radiochemotherapy schemes a feasible combination of radiotherapy treatment volume and gemcitabine dose must be found. In the light of recent controlled-randomized trials, the use of adjuvant radiochemotherapy is controversially discussed and not generally recommended.

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THE PREDICTIVE ROLE OF CA 19-9 KINETICS FOR TIME-TO-PROGRESSION (TTP) AND OVERALL SURVIVAL (OS) IN PATIENTS (PTS) RECEIVING PALLIATIVE FIRST-LINE CHEMOTHERAPY FOR ADVANCED PANCREATIC CANCER (PC)

M. Haas[1], S. Boeck[1], P. Stieber[2], R. P. Laubender[3], H. Buchner[3], C. Klose[4], F. Kullmann[5], S. Ostermaier[1], C. J. Bruns[6], U. Mansmann[3], V. Heinemann[1]

[1]Department of Internal Medicine III, [2]Department of Clinical Chemistry, [3]Institute for Medical Informatics, Biometry and Epidemiology, University Campus Großhadern, München, [4]Institute for Medical Biometry and Informatics, University of Heidelberg, [5]Department of Medicine I - Gastroenterology, Klinikum Weiden, [6]Department of Surgery, University Campus Großhadern, München, Germany

Previous studies showed contradictory results for a prognostic and/or predictive role of CA 19-9 tumor marker kinetics during chemotherapy for advanced PC.

Methods: We performed a retrospective, multicenter study in order to evaluate the role of CA 19-9 as a biomarker for TTP and OS in PC. Main inclusion criteria: histologically confirmed diagnosis of PC, treatment with first-line chemotherapy for advanced disease, pre-treatment CA 19-9 level of >5.2 U/ml. As CA 19-9 measurements were conducted in different laboratories using different commercial assays, we defined a subgroup of pts where CA 19-9 was assessed exclusively by the Elecsys® assay (Roche Diagnostics). For the analysis of CA 19-9 kinetics, at least one follow-up measurement between day 20 and 64 during first-line chemotherapy had to be available. A duration of chemotherapy of at least 40 days was demanded in order to avoid survival-time being biased by a too short or ineffective treatment. Pts were divided into two subgroups of CA 19-9 responders and non-responders by cut-offs of a 25% and 50% decline, respectively. OS and TTP were estimated with the Kaplan-Meier-Method, differences between the subgroups were analyzed by using the log-rank test (α=5%).

Results: One hundred and eighty-six pts were included for the analysis of CA 19-9 kinetics, 83 of them were tested with the Elecsys® method. Median age was 63 years, 90% of the pts were treated within prospective clinical trials. Median pre-treatment CA 19-9 was 1076 U/ml (range 5.7-100,000 U/ml), the median bilirubin was 0.6 mg/dl. Median OS and TTP were 9.8 months (mo) and 5.4 mo, respectively. In univariate analysis, pts with a CA 19-9 decline of at least 25% during chemotherapy lived significantly longer (11.9 mo *vs*. 8.2 mo, p=0.003) and had a significantly prolonged TTP (5.8 mo *vs*. 4.4 mo, p=0.018) than those with a lower decline or even CA 19-9 increase. Data for the Elecsys-measurements were comparable (OS: 13.4 mo *vs*. 8.6 mo, p=0.004; TTP: 7.0 mo *vs*. 2.6 mo, p=0.003). None of the analyses demanding a CA 19-9 drop of at least 50% reached the level of statistical significance.

Conclusion: An early CA 19-9 decline of 25% during first-line chemotherapy may predict OS and TTP in pts with advanced PC. Innovative statistical methods are required to improve our understanding of the utility of CA 19-9 as a predictive biomarker in PC.

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GENETIC ANALYSIS OF KRAS MUTATION STATUS IN METASTATIC COLORECTAL CANCER PATIENTS

K. Zavodna[1,4], M. Konecny[1], S. Spanik[2], S. Galbavy[3], M. Vizvaryova[1], E. Weismanova[1], J. Kausitz[1]

[1]Department of Clinical Genetics, [2]Department of Internal Medicine and [3]Department of Pathology, St. Elizabeth Cancer Institute, [4]Laboratory of Cancer Ganetics, Cancer Research Institute of Slovak Academy of Sciences, Bratislava, Slovakia

Colorectal carcinoma (CRC) represents a serious problem worldwide: in the Slovak republic about 2600 new CRC cases are diagnosed annually and its incidence is increasing. Colorectal cancer patients may succumb to the disease because of local recurrence or local formation of metastasis. Therefore, it is necessary to modulate therapeutic algorithms with new methods, leading to early diagnostic of CRC or changing the existing therapeutic procedures. Recent progress has been made in the understanding of EGFR pathways involved in CRC carcinogenesis, especially the role of Ras proteins. Ras proteins play an important part in the process of cell growth, differentiation and survival. Dysregulation of Ras protein by activating mutations, leads to the abnormal functional properties of cancer cells. Mutations in KRAS oncogene are frequently found in human cancers, particularly colorectal, pancreatic, biliary tract and lung cancer. The presence of the KRAS mutations in metastatic colorectal cancer patients correlates with a lack of response to certain EGFR inhibitor therapies, such as panitumumab and cetuximab. Consequently, screening for KRAS mutation status may be used as a prognostic marker, because CRC patients with KRAS positive tumors have a worse prognosis.

Aim and Methods: The aim of this study was to establish the methods for rapid and sensitive detection of KRAS mutation status of DNA in formaline fixed paraffin embedded (FFPE) tissues. Real Time PCR analysis (TheraScreen KRAS Mutation Test Kit) and sequencing analysis (optimised for the analysis of FFPE tissues) were used to detect somatic mutations in codon 12 and 13 of the KRAS gene. Both methods were used concurrently in the panel DNA isolated from from 25 colorectal tumor samples derived from FFPE tissues. The positive or negative results from all samples were identified by both methods independently.

Results and Conclusion: The KRAS mutations were presented in 8 of 25 patients (32%). The results demonstrate the utility of using the Real Time PCR analysis for detection of somatic KRAS mutations in clinical samples. However, it is also estimated that sequencing analysis for approximately 200 bp amplicon may be applied for KRAS mutations status screening, but with care of method sensitivity.

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CA 19-9 SERUM CONCENTRATIONS – ANALYSIS OF THE SERUM KINETICS DURING FIRST-LINE THERAPY OF PANCREATIC CANCER IN RELATION TO OVERALL SURVIVAL

R. Klapdor, M. Bahlo, A. Babinski, S. Klapdor

Internal Unit, ZeTDT GmbH, Hamburg, Gemany

In order to get an impression on the use of CA 19-9 serum concentration kinetics as a potential prognostic factor for survival, we analysed the data of 39 patients suffering from exocrine pancreatic cancer within the period of 1998-2000.

Patients and Methods: Thirty-nine patients with proven exocrine pancreatic cancer, age 32-72, N=30 suffering from liver metastasis were included in the study. CA 19-9 determinations were done in 4 weeks intervals (ES-700, Roche, Germany). Simultaneously the imaging procedures CT and/or MR were performed every two months. The following parameters of the CA 19-9 serum curves were used for the analyses: CA 19-9 serum concentration before start of the first-line chemotherapy, decrease of CA19-9 below 50% of the initial values (in days after start of treatment), time to nadir, time to new serum increase (PFS), as well as parameters of a new increase: increase of 25%, 50%, 100% and the relative increase of 3 consecutive serum CA 19-9 levels (% increase of serum CA 19-9/10 days during this period) above the first serum concentration possibly or retrospectively indicating the end of PFS. Chemotherapy treatment was started in n=27 patients following a locoregional/systemic approach with a combination of gemcitabine + mitomycin-C, as described earlier, in n=12 patients with *i.v.* applied gemcitabine or gemcitabine combinations with oxaliplatin or mitomycin-C. In all patients, treatment plan followed the EOSPC (efficacy orientated sequential polychemotherapy) concept. The second line treatment mainly consisted of 5FU/FA or combinations, and the third line therapy of irinotecan, irinotecan combinations or other gemcitabine combinations.

Results and Conclusion: The results did not show any relevant correlation between the measured/calculated parameters of the CA 19-9 serum concentration curves and the overall survival. However, our analysis suggests again a correlation between overall survival and the number of efficient treatment sequences, as published earlier (median survival in this study 14 months). Possibly, a previously by other authors observed correlation between CA 19-9 serum kinetics and survival is overlapped in our study by the results of the second and third line therapies in our patients. It is therefore concluded that in the case of EOSPC of pancreatic cancer patients the kinetics of the tumor marker serum concentrations during fist line therapy do not allow conclusions with respect to overall survival of the patients. Furthermore the observed new increase of CA 19-9 serum concentration by 27%/10 days (median) in case of new progression suggests that serum CA 19-9 determinations *e.g.* every two weeks would improve an early/earlier diagnosis of new tumor progression *e.g.* in order to: avoid unnecessary and ineffective treatment of the patients, to share costs for ineffective drugs, to reduce potential side-effects for the patients and to improve the possibility to try a second or third line treatment in pancreatic cancer diseases, which are even nowadays predominantly diagnosed in an

advanced stage. Our recent experience determining CA 19-9 serum concentrations in 2-week intervals (Kryptor, BRAHMS, Germany) seem to underline this concept.

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CYTOLOGY TO DETECT ANAL DYSPLASIA: A POSSIBLE MARKER FOR EARLY DETECTION OF ANAL PRE-CANCER LESIONS AND ANAL CANCER RISK ASSESSMENT

F. Bergauer, G. Spitzauer, A. Rost, A. Gingelmaier, A. Frank, A. Niedermeier, K. Friese, I. Mylonas

Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, LMU München, Germany

Female patients with cervical dysplasia or HIV positive, and HIV positive homosexual men (MSM) are probably at high risk of developing anal dysplasia (anal intraepithelial lesion: AIN low/high grade) and anal cancer. The study objectives were to assess whether anal cytology sampling is an adequate tool to generate evaluable results; to evaluate the prevalence of anal dysplasia in the different cohorts; and to correlate anal zytology to histology.

Methods: Patients from colposcopic and HIV outpatient units were examined. Anal cytology samples were taken with a moistened cotton swab from the anal canal, fixated and stained according to the Papanicolaou protocol. Speciman evaluation was perfromed by one experienced cytologist using the Munich and Bethesda nomenclature.

Results: Altogether 359 anal cytology samples were evaluated. There were 309 (86%) normal results and 50 showing anal dysplasia (12%) or unclear results (1.4%). Out of the 50 there were 31 (62%) indicative for low grade AIN and 14 (28%) indicative for high grade AIN. The MSM cohort had the highest overall risk of AIN (23%) and of high grade AIN (12%) compared to female HIV-positive patients (11% of overall AIN and 4% of high grade AIN) and patients with cervical dysplasia (11% of overall AIN and 2% of high grade AIN). From the 14 patients with samples indicative for high grade AIN biopsies were taken (anoscopically guided) for 9 cases, all showing AIN grade 3.

Conclusion: Anal cytology sampling seems to be a useful tool for detecting anal dysplasia. HIV-positive homosexual men are at the highest risk of developing anal dysplasia compared to female patients with cervical dysplasia and/or HIV-Infection. Anal cytology indicative of high grade AIN seems to be quite specific. Whether detection and treatment of anal dyplasia (as a possible pre-cancer lesion for anal cancer analogous to the CIN-cervical cancer concept) will help in reducing anal cancer incidence remains to be proved in further research efforts.

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ANAL CYTOLOGY AS A SCREENING TOOL FOR EARLY DETECTION OF ANAL DYSPLASIA IN HIV-INFECTED WOMEN

A. Gingelmaier, T. Weissenbacher, B. Kost, R. Kaestner, M. Sovric, I. Mylonas, K. Friese, F. Bergauer

Frauenklinik der Ludwig-Maximilians-Universität München, Germany

HIV-positive patients show a high rate of anal dysplasia and anal carcinoma but there is no gold standard for an early detection. Therefore the study objectives were the evaluation of an anal screening using anal/perianal cytology and, in the case of a positive result, relation to immune status, clinical symptoms of HIV infection and antiretroviral therapy.

Methods: In every HIV-positive female visiting the gynaecologic outpatient clinic an anal and perianal swab for anal cytology was taken. One experienced cytologist examined all specimens. Relevant details of the HIV related history like CDC classification, CD4 count, viral load, actual antiretroviral therapy *etc*. were documented.

Results: Altogether, 104 HIV-positive women were included in this study. The results of 13 (13.5%) anal cytologies were classified as suspect for low-grade or high-grade anal dysplasia and 6 of these showed a high-grade anal lesion in an anal biopsy. A total of 9 out of 13 also had cervical dysplasia and 12 were positive for high-risk HPV at the cervix. An total of 10 of these patients had already experienced clinical symptoms of their HIV infection and 7 of them showed a nadir of the CD4 count below 200 cells/µL. All but one were already prescribed with a highly active antiretroviral therapy.

Conclusion: In this pilot study anal screening using anal cytology showed 13.5% suspected anal dysplasia in HIV-positive women. All performed biopsies resulted in a high-grade anal lesion. The majority of these women had already an advanced disease and/or immune defect related to their HIV infection. In summary, anal cytology was found to be a useful tool for the early detection of anal dysplasia in high-risk patients such as HIV-positive women. How far this screening method contribute to the prevention of anal cancer has to be evaluated in further investigations.

14
RAS MUTATIONAL STATUS IS A BIOMARKER FOR RESISTANCE TO EGF-R INHIBITORS IN COLORECTAL CARCINOMAS

W. Dempke

University Medical School Munich, Germany

The epidermal growth factor receptor (EGF-R) has been validated as a therapeutic target in several human tumors, including colorectal cancer (CRC). Ligand occupancy of EGF-R activates the RAS/RAF/MAPK, STAT and PI3K/AKT signaling pathways, which together modulate cellular proliferation, adhesion, angiogenesis, migration and survival. The anti-EGF-R monoclonal antibodies cetuximab and panitumumab administered as monotherapy in CRC have shown response and disease stabilization rates of approximately 10-30%. Although EGF-R expression is used for patient selection, clinical experience shows that levels of EGF-R expression (measured by immunohistochemistry) do not predict clinical benefit. The ras proteins (K-, H-, and N-ras) are members of a large superfamily of GTP-binding proteins that play a complex role in the normal transduction of growth factor receptor-induced signals. Ras mutations in codons 12, 12, and 61 (found in 30-50% of CRC tumors) result in inhibition of GTPase activity, thus leading to the constitutive activation of the ras proteins, which may render tumor cells independent of EGF-R signaling and thereby resistant to cetuximab, panitumumab and EGF-R TKIs. Data from several recently published studies in patients with metastatic CRC (OPUS, CRYSTAL) clearly indicated that the benefit of cetuximab when added to chemotherapy was only restricted to patients with wild-type K-ras tumors. In those patients who were treated with tumors harbouring K-ras mutations (cetuximab plus chemotherapy), their clinical outcome was identical (or even worse in the case of oxaliplatin-containing regimes) to those patients who were treated with chemotherapy alone. Similar data have also been provided for panitumumab monotherapy in CRC. These results show that K-ras mutations predict a lack of clinical benefit of cetuximab and panitumumab therapies in CRC and indicate that K-ras status should be considered when selecting CRC patients as candidates for these antibodies. Moreover, the results from these studies should also trigger retrospective analyses of K-ras mutations from all available trials in CRC (as well as NSCLC and pancreatic cancer). Analysis of K-ras mutations may also be important in the cetuximab-containing N0147 and the PETACC-8 adjuvant studies in CRC. These studies would enable further establishment of the correlation between K-ras mutation and resistance to cetuximab and panitumumab in CRC patients.

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IMPROVED DIAGNOSIS OF COLORECTAL CANCER USING A COMBINATION OF FAECAL OCCULT BLOOD AND NOVEL FAECAL PROTEIN MARKERS

J. Karl, N. Wild, M. Tacke, H. Andres, U. Garczarek, W. Rollinger, W. Zolg

Roche Diagnostics GmbH, Germany

Novel faecal protein markers, detected in a proteomics based search were evaluated in combination with faecal occult blood testing for the diagnosis of colorectal cancer.

Methods: Six biochemical markers, including immunological faecal occult blood test, were measured in a collective of 551 samples (186 CRC, 113 advanced adenoma and 252 control patients) to establish the diagnostic performance of each marker and marker combinations. Results: Beside the known stool markers hemoglobin (=iFOBT), hemoglobin-haptoglobin, calprotectin and carcinoembryogenic antigen the novel faecal markers tissue inhibitor of metalloproteinase-1 (=TIMP-1) and S100A12 were tested. The best diagnostic performance was found for S100A12 with an area under the curve of 0.95, followed by TIMP-1 (0.92), hemoglobin-haptoglobin (0.92), hemoglobin (0.91), calprotectin (0.90) and CEA (0.66). Using Bayes Logistic Regression as a mathematical model, the highest sensitivity (88%) for the detection of CRC at 95% specificity was obtained with the marker pair S100A12 and hemoglobin-haptoglobin. Raising the specificity to 98%, the combination of S100A12, hemoglobin-haptoglobin and TIMP-1 resulted in a sensitivity of 82%, with the highest increase of sensitivity found in early tumor stages (UICC-stage I: 74% sensitivity *vs*. 57% of the best single marker).

Summary: Depending on the specificity selected, a marker pair, S100A12 and hemoglobin-haptoglobin, or a triple combination including TIMP-1, allowed the detection of colorectal cancer (including early stages) at significantly higher rates than can be obtained with iFOBT alone.

Gastroenterology II

16

REGIONAL 90YTTRIUM MICROSPHERE TREATMENT OF CHEMOTHERAPY-REFRACTORY COLORECTAL CANCER AND BREAST CANCER LIVER METASTASES

T.F. Jakobs, R.T. Hoffmann, K. Dehm, C. Trumm, J. Stemmler, K. Tatsch, C. La Fougere, T.K. Helmberger, M. F. Reiser

Department of Radiology, Department of Internal Medicine III, Department of Nuclear Medicine, LMU, Grosshadern Campus, Department of Radiology, Klinikum Bogenhausen, Munich, Germany

Aim: To present data for radioembolization treatment with 90Yttrium resin microspheres in patients with colorectal

cancer and breast cancer liver metastases for whom standard-of-care therapies had failed.

Methods: We retrospectively reviewed the case files of 41 patients with colorectal cancer liver metastases and 30 patients with breast cancer liver metastases who had previously failed polychemotherapy and underwent radioembolization *via* intra-arterial infusion of 90Yttrium resin microspheres administered as a single-session, whole-liver treatment at our institution between 2003 and 2008. Pre- and postprocedural imaging and laboratory follow-up results were available for 61 patients. After treatment, we assessed tumor response by CT/MRI using Response Evaluation Criteria in Solid Tumors (RECIST) -criteria, toxic effects noted clinically and by laboratory workup, and survival.

Results: The patients with colorectal cancer liver metastases (mean age, 61 years) received 90Yttrium microspheres having a mean activity of 2.1 GBq. Imaging follow-up at a median time after treatment of 2.9 months demonstrated partial response, stable disease, and progressive disease in 17%, 61%, and 9.8% of patients, respectively. Mean and median decreases in tumor marker levels of 18.8% and 27.9%, respectively, were noted. Median follow-up time was 7.9 months. Median overall survival was 10.5 months, with superior survivals for patients with low levels of tumor involvement (11.7 months), tumor marker response (19.1 months) and imaging response (29.3 months). Except for one instance of treatment-associated cholecystitis, no severe toxicities were observed. The median age of patients with breast cancer liver metastases was 58 years with a mean activity of 1.9GBq of 90Yttrium being delivered in a single-session of whole-liver treatment. Follow-up at median 4.2 months after treatment demonstrated partial response, stable disease and progressive disease in 61%, 35% and 4%, respectively. Median follow-up time was 14.2 months. Median overall survival was 11.7 months. Median survival of responders versus non-responders and patients with and without extrahepatic disease was 23.6, 5.7 months and 9.6, 16 months, respectively. One patient's death was attributed to treatment induced liver disease.

Conclusion: Radioembolization can be performed with acceptable toxicity in patients with colorectal cancer and breast cancer liver metastases who fail standard-of-care polychemotherapy. This treatment's anti-tumor effect is supported by a decrease in tumor size and tumor marker levels. Further investigation is warranted to confirm a survival benefit.

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RADIATION ONCOLOGY IN GASTROINTESTINAL TUMORS – STATE AND TRENDS

O. Micke[1,2], A. de Vries[3]

[1]Department of Radiotherapy and Radiation Oncology, University Hospital, Münster, [2]Department of Radiotherapy and Radiation Oncology, Franziskus Hospital, Bielefeld, Germany, [3]Department of Radiotherapy, Feldkirch Hospital, Austria

In recent years, there is a growing number of controlled clinical trials investigating the value of (chemo-)radiation in various gastrointestinal cancers. Radiation has been shown to be a potent agent in enhancing tumor control of locally advanced cancer and to improve disease-free and overall survival in several entities. However, radiotherapy in the treatment of some gastrointestinal tumors remains controversial because of the marked radiation sensitivity of neighbouring organs frequently compromising application of high doses of ionizing radiation. Chemoradiation in combi-nation with surgery enhances tumor control of locally advanced rectal cancer disease and has been shown to improve disease-free and overall survival in rectal cancer. Preoperative chemoradi-ation, as compared with postoperative chemoradiation, improved local control and was associated with reduced toxicity but did not improve overall survival. In esophageal adenocarcinoma, survival was prolonged with preoperative chemoradiation in a meta-analysis. In gastric cancer, post-operative chemoradiation can be considered after limited lymphadenectomy.

Conclusion: Pre-operative radiotherapy or pre-operative chemoradiation may be considered in in-dividual cases, but should not be used routinely for all gastrointestinal carcinomas, except for rectal carcinoma, where preoperative chemoradiation was established as standard treatment. In many studies, preoperative radiotherapy/chemoradiation yielded promising results and merits validation in large controlled trials.

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TREATMENT OF ADVANCED EXOCRINE PANCREATIC CANCER: STATE AND TRENDS

R. Klapdor

Internal Practice, Center of Experimental Tumor Marker Diagnosis and Therapy (ZeTDT GmbH), Hamburg, Germany

Gemcitabine monotherapy is generally accepted as first-line-therapy in localy advanced pancreatic cancer. Since 2007 combination of Gemcitabine + Erlotinib ("small molecule") has been recommended for metastatic diseases. In addition the "S3-Leitlinien" (Zeitschrift Gastroenterologie 45, 2007, 487-523) open the possibility to start with a combination of Gemcitabine + Oxaliplatin because of a higher or slightly increased rate of partial responses in patients with a good clinical situation. Furthermore, again in contrast to the "S3-

Leitlinien" published 2004, the "S3-Leitlinien" published 2007 also discusses a potential benefit effect on survival of a 2nd –line therapy, *e.g.* with 5-FU/FA, Oxaliplatin, Irinotecan, or Taxotere, based on non- randomised or prospective randomised clinical studies which indicated a prolongation of survival by a 2nd -line-therapy after failure of or after a new progress after Gemcitabine monotherapy. These new "Leitlinien" published in 2007 seem to support our concept formulated about 10 years ago. We follow the concept of EOSPC (efficacy orientated sequential polychemotherapy), if needed also in combination with other multimodal approaches (palliative endoscopic surgery, locoregional therapy, best supportive care, especially effective pain therapy and consequent substitution with pancreatic enzymes or enteral / parenteral nutrition). This concept is based on the fact that there are some cytostatics with an efficacy in the range of, or only slightly lesser than Gemcitabine like 5-FU/FA, Irinotecan, Mitomycin-C, Taxotere and that combinations of Gemcitabine with *e.g.* Mitomycin-C and Oxaliplatin seem to increase the anti tumour effects of these drugs. On the other hand this concept is based on the modern possibilities for earlier detection of the tumour response to therapy and earlier detection of a new progress in order to hold patients in a clinical situation allowing a 2nd - or 3rd - line therapy. Our concept resulted in an overall survival rates for metastasised and advanced local diseases of 16 months (median) and 13 months (median) for metastasized tumours as well as in a median survival of 18 months for the subgroup of metastasized patients with more than one active drug treatment (about 45 % of the metastasized patients) in the course of their disease.

We suggest that the survival data for pancreatic cancer patients based on scientific prospective randomised clinical studies reporting 1st-line treatments and survival do not reflect the actual possibilities. On the other hand, clinical prospective studies should be planned no longer as 1st- line-concepts but at least as 2nd- or 3rd- line- concepts allowing the evaluation of new drugs or new drug combinations in a 1st - , 2nd - or 3rd – line treatment.

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EN BLOC RESECTION FOR LOCALLY ADVANCED CANCER OF THE PANCREAS: IS THERE A BENEFIT?

M. Bockhorn, J.R. Izbicki
Department of General, Visceral and Thoracic Surgery, University Medical Centre, Hamburg, Germany

Until the early 1990's, the presence of portomesenteric invasion was generally accepted as a contraindication for curative surgery. However, institutional experiences gained in several high-volume centres evidenced that advanced pancreatic carcinoma infiltrating major peripancreatic vessels can be safely treated leading to increasingly aggressive surgical approaches. Therefore, the apodictic assumption that involvement of mesenteric, portal and splenic veins as well as visceral arteries indicates irresectability is no more sustainable. Aim of our study was to assess in-hospital complication rates and survival duration after *en bloc* vascular resection.

Materials and Methods: Of 1099 patients subjected to pancreas-resection, 128 patients underwent resection of the portal vein (VR+) and 29 patients underwent arterial en-bloc resection (AEBR). These were compared to 449 patients (VR-) without vascular reconstruction.

Results: One hundred twenty-eight VR+ patients underwent portal or superior mesenteric vein resection. In-hospital morbidity and mortality rates of VR+ patients (39.7%/4.0%) nearly equaled that of VR-patients (40.3%/3.7%). Of the patients with AEBR 18 patients received a reconstruction of the hepatic artery (HA), 8 a reconstruction of the celiac trunc (CT) and 3 a reconstruction of the superior mesenteric artery (SMA). Patients with vascular resection had significantly longer operative times ($p<0.0001$), required more blood units ($p<0.0001$), and had longer hospital stay than standard patients ($p=0.049$). Morbidity and mortality in patients with vascular resection were higher comparable to patients with standard pancreatic resection ($p<0.03$). Median survival was 15 months (11.2-18.8) in VR+ patients, 12.5 months in AEBR patients and 16 months (14.0-17.9) in those without vascular resection ($p=0.86$), which was significantly longer than in patients treated with a palliative bypass, who survived only 7.5 months ($p<0.014$).

Conclusion: Despite higher morbidity and mortality rates in patients with vascular resection, median survival of patients with vascular invasion is superior to that of patients who undergo palliative therapy. Therefore extended resections even with portal vein or arterial resections are justified as long as alternative therapies are absent.

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MOLECULAR TARGETED THERAPY WITH ANTIBODIES IN GASTROINTESTINAL CANCER

U. Vanhoefer

Zentrum für Innere Medizin, Marienkrankenhaus, Hamburg, Germany

Anticancer strategies implicate molecular targets like the Vascular Endothelial Growth Factor (VEGF) or Epidermal Growth Factor (EGF) receptor.

The vascular endothelial growth factor has a crucial role in the angiogenesis of various solid malignancies, especially in

advanced colorectal cancer. Oxaliplatin or irinotecan-based combination chemotherapy (IFL, FOLFOX, CAPOX) with the anti-VEGF mAb bevacizumab showed superiority in terms of progression-free survival (PFS) and overall survival (OS) in the first- and second-line setting of advanced colorectal cancer compared to chemotherapy alone.

The epidermal growth factor (EGF)-receptor has also being investigated as a target for anticancer therapy in a variety of malignancies. The BOND trial demonstrated that the chimeric anti-EGFR mAb cetuximab reverses clinical resistance to irinotecan in advanced colorectal cancer. Panitumumab, a human anti-EGFR mAb, also showed clinical activity in the salvage therapy of colorectal cancer.

In randomized trials the addition of cetuximab to chemotherapy (FOLFIRI or FOLFOX) increased the progression-free survival and remission rate in the front-line setting. However, the Dutch Colorectal Cancer Group (DCCG) showed that the addition of cetuximab to the combination of chemotherapy (CAPOX) and bevacizumab was inferior in terms of progression-free survival and response rate.

Furthermore, retrospective analyses of recent trials (CAIRO-2, Crystal, OPUS-trial) clearly demonstrate no benefit in using cetuximab alone or in combination therapy in patients with mutated *k-ras* tumors. Therefore, cetuximab or panitumumab should only be used against wild type *k-ras* tumors. With the increase in treatment options, patients have greater opportunity to receive more individualized treatments specific to their disease. The role of molecular targeted therapies in gastrointestinal cancer will be discussed.

Gynecology I

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CA27.29 AS A TUMOUR MARKER FOR RISK EVALUATION AND THERAPY MONITORING IN PATIENTS WITH PRIMARY BREAST CANCER

B. Rack, P. Hepp, C. Schindlbeck, D. Mouarrawy, U. Groh, D. Finas, A. Hönig, M. W. Beckmann, W. Lichtenegger, H. Sommer, W. Janni, K. Friese

Ludwig-Maximilians-Universität, München, Klinikum Bremerhaven Reinkenheide, Hochwald Krankenhaus, Bad Nauheim, Universitätsklinikum Schleswig-Holstein, Campus Lübeck; Universitätsklinik Würzburg, Universitätsfrauenklinik Erlangen, Universitätsklinikum Charité, Berlin, Germany

Several trials show that the use of tumor markers (TM) leads to an early diagnosis of tumor dissemination in breast cancer patients. Whether earlier induction of treatment improves the prognosis of these patients is still under discussion. In the SUCCESS Trial CA27.29 is evaluated before and after adjuvant chemotherapy, as well as after 2 and 5 years in 3754 patients.

Methods: The SUCCESS Trial compares FEC-Docetaxel (Doc) *vs*. FEC-Doc-Gemcitabine (Doc-G) regime and two *vs*. five year treatment with zoledronate in patients with primary breast cancer (N+ or high risk). CA27.29 has been measured with ST AIA-PACK CA27.29 reagent using MUC-1 for AIA-600II. The cut-off for positivity of CA27.29 is 24 U/mL.

Results: Patients (n=2669) were examined prospectively before and after chemotherapy, of which 22.0% had a marker >24 U/mL (n=587, mean 19.00, range 3.04-410.00) before and 39.6% (n=1058, mean 23.34, range 2.70-330.76) after chemotherapy. The correlation between both values was significant ($p<0.0005$). While 16.9% showed elevated CA27.29 before and after therapy, 23.0% of pre-therapy positive patients were negative for CA27.29 afterwards. A total of 70.9% of initially negative patients stayed negative whereas 29.1% became positive after treatment. Before treatment the prevalence of elevated CA27.29 was equally distributed between the FEC-Doc and the FEC-Doc-G arms. After treatment 34.1% in the FEC-Doc arm showed an increased level *vs*. 45.6% in the FEC-Doc-G arm. The correlation analysis showed no significant coherence between hormonal status (ER: $p<0.323$; PR: $p<0.078$), HER2/neu status ($p<0.308$), grading ($p<0.565$) and CA27.29 level. However, tumor size ($p<0.020$) and the nodal status ($p<0.022$) were significantly associated with CA27.29 levels.

Conclusion: These results indicate a close relation between CA27.29 and the tumour mass at primary diagnosis. Whether this marker will be useful for treatment monitoring and to tailor more individualized treatment options will be shown by further follow-up in the SUCCESS trial.

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THE CLINICOPATHOLOGICAL AND PROGNOSTIC RELEVANCE OF PYRUVATE KINASE M2 AND PAKT EXPRESSION IN BREAST CANCER

C. Benesch[1], C. Schneider[1], H.-U. Voelker[2], M. Kapp[1], H. Caffier[1], J. Dietl[1], U. Kammerer[1], M. Schmidt[1]

[1]Department of Gynaecology and Obstetrics and [2]Department of Pathology, University of Würzburg, Germany

Cancer cells have been described to depend on glucose utilisation. Activation of Akt and up-regulation of pyruvate kinase M2 (M2-PK) are attributes of tumour glycolysis.

Methods: In order to evaluate the prognostic relevance of glycolytic markers in breast cancer, the expression of pAkt and M2PK was analysed in 160 tissue samples. The staining

results were compared with clinicopathological characteristics and survival data.

Results: Expression of pAkt was detected in 58% and of M2PK in 70% of breast cancer samples. pAkt expression was accompanied with shorter survival time. In contrast, M2PK expression was significantly higher in patients surviving breast cancer for more than 10 years.

Conclusion: Surprisingly, M2PK occurrence seems to be a favourable prognostic factor and the role of M2PK in breast cancer progression has to be further explored. M2PK might evolve to be of prognostic relevance and predictive for future therapeutics affecting glucose metabolism.

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IMPROVED OVERALL AND DISEASE-FREE SURVIVAL IN PRIMARY BREAST CANCER PATIENTS WITH HAEMOGLOBIN LEVELS ABOVE 12 G/DL AT FIRST DIAGNOSIS: RESULTS OF 1720 BREAST CANCER PATIENTS

F. Edler von Koch, N. Ditsch, J. Gallwas, I. Rühl, S. Rückert, M. Mosner, B. Toth, S. Kahlert

Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, Universität München, Germany

Anaemia is a common factor in cancer patients. For several tumour entities diminished haemoglobin (Hb) levels before adjuvant treatment have a negative prognostic value. It has previously been reported that breast cancer patients who developed anaemia during adjuvant therapy showed a higher relapse rate.

Methods: Between 1990 and 2004, 1720 patients with primary invasive, non-metastatic breast cancer underwent surgery and adjuvant treatment at …. Baseline Hb levels were measured at the institute of clinical chemistry before patients underwent surgery. Patients who received primary systemic therapy were excluded from this study. Data about local and distant recurrence and survival were prospectively collected.

Results: One thousand five hundred and eighty six patients (92.2%) had a baseline Hb >12g/dL, 134 patients (7.8%) had a baseline Hb<12g/dL. Patients with a baseline Hb below 12g/dL showed a significantly reduced disease free survival (DFS) with a median DFS- time of 78.8 (95%CI: 49.7-107.9) *vs*. 124.8 (95%CI: 111.1-138.5) months (p=0.0085) and a significantly reduced overall survival (OAS) with a median OAS- time of 132.4 (95%CI: 63.7-201.1) *vs*. 143.2 (95%CI: 138.7-147.7) months (p=0.0028). Distant metastasis free survival (MFS) was significantly reduced in patients with Hb<12 g/dL as well, showing a median MFS-time of 90.3 (95%CI: 64.5-116.1) *vs*. 143.2 (95%CI: 134.1-152.3) months (p=0.0024). Multivariate analysis identified a Hb below 12g/dL as an independent prognostic factor in patients with primary breast cancer.

Conclusion: Only a minority of patients presented during the last years had a baseline Hb below 12g/dL. This study showed that Hb-level before primary breast cancer treatment is a strong prognostic factor for DFS, MFS and OAS, independent from other factors. It is not clear at this point if these results are caused by interactions of breast cancer cells and erythropoesis or by other non-specific effects.

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THYROID HORMONES AND ANTIBODIES IN BREAST CANCER PATIENTS

N. Ditsch[1], S. Liebhardt[1], F. von Koch[1], M. Lenhard[1], M. Vogeser[2], C. Spitzweg[3], J. Gallwas[1], B. Toth[1]

[1]Department of Obstetrics and Gynecology, [2]Department of Clinical Chemistry and [3]Department of Internal Medicine, LMU Munich, Germany

The association between breast cancer and thyroid dysfunctions is controversial. Although there are studies indicating a possible relationship between hypothyroidism and breast cancer, others show otherwise. Furthermore, estrogen-like effects of thyroid hormones on breast cancer cell growth *in vitro* have been reported. So far, most studies were performed in breast cancer patients after surgery. The aim of this trial was the investigation of thyroid function of breast cancer patients before and after vacuum-biopsy of the breast, chemotherapy, radiation or hormonal treatment.

Patients and Methods: One hundred and forty eight women living in the same geographical region were included in this prospective open trial, 81 of them with newly diagnosed breast cancer (TNM stage: DCIS=13; T1=32; T2=28; T3/4=5), 27 with benign breast disease and 38 healthy controls with normal mammography and breast ultrasound. Thyroid history was reported and blood results (normal range) of serum-free thyroxine (fT4: 0.9-1.7 ng/dL), serum-free triiodthyronine (fT3: 2.5-4.3 pg/mL), thyroid-stimulating hormone (TSH: 0.44-3.80 µU/mL), antiperoxidase antibody (TPO: <40 IU/mL), thyrotropin receptor antibody (TRAK: <1.0 IU/mL) and thyroglobulin (Tg: <100 IU/mL) antibodies were determined.

Results: Tumour stage was mostly specified as pathologic but also partially as clinical stage because of planning of a neoadjuvant therapy. Eighty seven percent of patients were oestrogen- and progesterone-receptor positive. TSH was highest in the control group (mean±sd; 1.5±1.2) without reaching significance. FT3 and fT4 levels were highest in breast cancer patients (fT4: 1.3±0.2; fT3: 3.0±0.45) differing significantly from controls (fT3 and fT4: p<0.001) as well as patients with benign breast tumours (fT4: p=0.017). With

regard to TRAK antibodies breast cancer patients showed highest levels (2.3±0.08) differing significantly from women with benign breast tumours (p=0.048). Women with benign tumours showed highest concentrations of Tg antibodies (141±318), whereas TPO antibodies were highest in breast cancer patients (104±169). However both antibodies did not reach significance.

Conclusion: Beside the small study population significant differences of fT3/fT4 as well as TRAK levels were observed between breast cancer patients, women with benign breast tumours and healthy controls. Whereas fT3/fT4 and TSH were within the normal range, thyroid antibodies showed significant differences in the study population. Further studies including larger patient numbers especially focusing on breast cancer patients before treatment are required.

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A PHASE II TRIAL OF ORAL VINORELBINE AND CAPECITABINE IN ANTHRACYCLINE PRETREATED PATIENTS WITH METASTATIC BREAST CANCER

J. Finek, L. Holubec Jr., T. Svoboda, L. Sefrhansova, I. Pavlikova, M. Votavova, M. Sediva, S. Filip, R. Kozevnikova, S. Kormunda

University Hospital Pilsen, University Hospital Bulovka Prague, University Hospital Hradec Kralove; 4DTC Centre Prague, Czech Republic

Objectives: Optimal chemotherapy (CT) for advanced breast treatment should be effective, well tolerated and convenient. In this study the efficacy and safety of the fully oral combination of oral vinorelbine (Navelbine Oral) plus capecitabine (Xeloda) in metastatic breast cancer (MBC) patients pretreated with anthracycline is evaluated.

Methods: In this phase II multicenter study, the above combination was given as a first or second line for MBC. Treatment schedule was: oral vinorelbine 60 mg/m^2 day 1 and day 8 plus capecitabine 1000 mg/m^2 twice daily from day 1 to day 14, every 21 days.

Results: One hundred and fifteen patients were included in this trial. Median age was 58 years (range: 40-75). All patients had received prior anthracycline based chemotherapy. The combination was well tolerated, with most notably only 0.8% of patients presenting with febrile neutropenia. In the ITT population, objective response was achieved in 65 patients (56.5%). Complete response was achieved in 22 patients (19.1%); partial response in 43 patients (37.4%); stable disease in 36 patients (31.3%); progressive disease was observed in 14 patients (12.2%). After a median follow up of 10.0 months, median progression-free survival was 10.5 months and median survival was 17.5 months.

Conclusion: Oral vinorelbine-capecitabine shows very promising activity and low toxicity in MBC treatment with high compliance of the patients. For patients who are prescribed oral chemotherapy, taking their medication at home is one of the main advantages and is highly preferred. Oral vinorelbine-capecitabine combination has been approved by the Czech Cancer Society as standard treatment of MBC. This study was supported by research project VZ MSM 0021620819.

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DO OLDER WOMEN (>80 YEARS) HAVE UNFAVORABLE HISTOLOGICAL CHARACTERISTICS OF PRIMARY BREAST CANCER?

E. Chatzaki[1,3], I. Mylonas[2], K. Friese[2], B. Gerber[1], V. Briese[1]

[1]Department of Obstetrics and Gynaecology, University Rostock, [2]First Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University Munich, Germany, [3]Onassio Hospital, Athens, Greece

Although the majority of breast cancer patients is more than 65 years old, few data exist about the pathological features of breast cancer in very elderly patients. The aim of this study is to evaluate the histological characteristics of breast cancer in women aged over 80 years.

Methods: The data from all women over 80 years of age, who were diagnosed with primary breast carcinoma at the gynecologic clinic of the University of Rostock in Germany during the period 1990-2005, were reviewed. The histological type, TNM classification, histological grade, hormone receptor status and the vascular invasion of their tumors were investigated.

Results: One hundred and fourty women with a median age of 82.83 years (minimum: 80.06, maximum: 95.51) were diagnosed with primary breast carcinoma, 6 of them (4.3%) having simultaneous bilateral tumors at the time of diagnosis. From the 146 tumors of the cohort, only 7 were carcinomas *in situ*, 6 ductal carcinomas *in situ* (DCIS) and 1 lobular carcinoma *in situ* (LCIS), while the rest (95.2%) were invasive carcinomas. Among the invasive carcinomas, ductal carcinoma of no special type (IDC/NST) was seen in 69.2%, lobular (ILC) in 4.8%, mixed ductal and lobular in 6.2%, while special types of invasive breast carcinoma (mucinous, papillar, medullar, tubular, cribiform) were noted in 15.1%. There were 38 (27.3%) invasive carcinomas with associated DCIS and only 3 (2.2%) with associated LCIS.

According to the TNM classification for breast cancer from the UICC the primary tumor size was most often as T2 (32.9%) assessed, followed by T1c (30.1%). Fourty eight point six percent and 62.3% of cases respectively had no regional lymph node metastasis and no distant metastasis. The most common histological grade was grade 2 (47.9%), followed by grade 1 (26.7%). The estrogen receptor (ER) and the progesterone receptor (PR) status were assessed respectively in 120 and 118 carcinomas. Around 71% of the tumors were ER-positive and 56% PR-positive. Lymphatic vessel invasion (LVI) was present in 48.6% of cases and blood vessel invasion (BVI) in 11.6%.

Conclusion: Although it is believed that older women have more advanced tumor stages at the time of diagnosis, in this cohort the majority of the tumors were assessed as stage I and II according to the TNM stage grouping for breast cancer. Special types of invasive breast cancer, that are considered as having a favorable prognosis, are seen quite often in this age group. The frequency of ER-positive and PR-positive tumors in these patients is high, another parameter with a good prognostic value.

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APPENDICES OF THE NIPPLE AND AREOLA OF THE BREAST IN NEUROFIBROMATOSIS TYPE 1 PATIENTS ARE NEUROFIBROMAS

R.E. Friedrich, C. Hagel

Maxillofacial Surgery and Neuropathology, University Hospital, Hamburg, Germany

Neurofibromatosis type 1 (NF1) is an autosomal dominant inherited disease. The disease is assigned to the heterogenous group of phacomatoses being defined by obvious pigmentary disorders of the integument. In NF1 multiple café-au-lait spots are characteristic affections of the skin. The hallmark of the disease is cutaneous or subcutaneous tumors that are derived from the peripheral nerves' sheath. These tumors are individually variable in number and size and usually appear during or after puberty. The tumor can develop at almost any site. Tumors arising in patients with the genetic background of NF1 in the areola region of the breast often appear similar to the nipple. In some cases the tumors are even appendices of the nipple. In order to elucidate the nature of these organoid structures a systematic analysis was performed of consecutive NF1-patients with tumors of the areola and nipple.

Materials and Methods: Analysis of 83 specimens from 55 patients with NF1 was performed [females: 42, males: 13, mean age of the total group: 41.6 years (yrs), range: 4-62 yrs, mean age of females: 40.9 yrs, range: 4-62 yrs, mean age of males: 43.5 yrs, range: 12-61 yrs]. All patients were surgically treated for tumor reduction of neurofibromas affecting the skin, including tumors of the areola and nipple. All patients fulfilled the current diagnostic criteria of NF1 (NIH, USA). Tissues of NF1 patients with apparent extensive affections of the breast who received debulking procedures for disfiguring invasive or superficial plexiform neurofibroma were excluded. All surgically treated patients who had macroscopically defined small tumors, often of a fungoid or nipple-like shape, that originated inside the areola were included. Histological description was performed according to the WHO criteria for nerve sheath tumors.

Results: All specimens were entirely neurofibromas with no residues resembling segments of glandular structures. Histological tumor types in females were: dermal (n=23), diffuse (n=13), dermal-diffuse (n=2), with high or low cellularity (1 each) and plexiform-diffuse (n=2). In males, 6 cases each proved to be either of diffuse or dermal type. In one case the tumor showed a plexiform-diffuse growth pattern. Following ablative surgery, no local recurrence was noted so far (follow-up control up to 10 yrs). Multiple tumors of an individual arising in this region showed the same differentiation. No tumor showed signs of a malignant degeneration. Cellularity was extremely low in tumors of this region.The detection of tumor cells inside dense extracellular matrix often required immunohistochemistry (S-100 protein).

Conclusion: Tumors arising in the areola and nipple area somehow mimicking an accessory nipple are all neurofibromas. They occur in both men and women and occasionally in children. Local excision of neurofibroma is adequate to relief patients from an often unsightly appearance.

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INVESTIGATIONS ON THE IMPACT OF FLAX ROOT EXTRACT TO THE EXPRESSION OF THE TREFOILFACTOR1 AND OTHER PROTEINS IN THE HUMAN BREAST CANCER CELL LINE MCF7

A. Krys[1], A. Schlichting[2], S. Abarzua[3], D.U. Richter[1], V. Briese[1]

[1]Department of Obstetrics and Gynaecology, Faculty of Medicine, University Rostock, [2]STC Soil Biotechnology, Groß Lüsewitz, [3]Institute of Biological Science, Faculty of Natural Science, Rostock, Germany

A variety of studies on the effect of flax plants on breast cancer have been postulated. The impact of flax root on the expression of the Trefoilfactor1 (TFF1 or PS2) in the breast cancer cell line MCF7 is examined in this study. TFF1 is a common serum marker and prognostic factor in breast cancers with positive expression of hormone receptors. TFF1 plays an increasingly decisive role as a protooncogene in these cells

and is supposed to operate with a similar mechanism to a growth factor. TFF1 is probably involved in motility, migration, growth and survival of the cells in breast cancer.

Materials and Methods: The flax root extract was produced by the extraction process of Luyengi et al and was analysed by mass spectrometry. In previous pilot studies the toxic and proliferative potency of the extract was determined (LDH cytotoxicity test, MTT and BrdU proliferation tests). The conventional human breast cancer cell line MCF7 was used for all tests. Estradiol acted as positive control and tamoxifen as negative control in all investigations. The main content of this study is the immunohistochemical examination of the effects of the flax root extract to the expression of the receptors αER, βER, PR and TFF1.

Results: The LDH cytotoxicity test showed a toxic development of 70% in high concentrations of the extract. In the MTT test there was a proliferative tendency of 30% in higher concentrations and of 15% in very low and high concentrations in the BrdU test evidence. A decrease in the proliferation of 15% was found in the BrdU test for average concentrations of the extract. The receptors of αER, βER, PR and TFF1 could be immunohistochemically visualized. It was conspicuous that the morphological behaviour of the MCF7 cells changed after addition of high extract concentrations to the glass slide in immunohistochemistry. Discussion: The flax root extract does not appear to affect the expression of TFF1, nor the other receptor proteins in immunohistochemistry. The toxic and proliferative tendencies in the different tests and the change in cell morphology in the immunohistochemical experiments, suggest that there could be evidence for a special influence of the extract at an unidentified molecular level. That is why in further studies the effect on normal cells should be examined. In conclusion, flax root is still not a sufficiently tested field in basic research but there are promising possibilities in the prevention or treatment of breast cancer.

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ONLINE MONITORING OF CELLULAR METABOLISM FOR STUDYING ANTICARCINOGENIC EFFECTS OF *LINUM* PHYTOESTROGEN EXTRACTS IN THE HUMAN MAMMALIAN CARCINOMA CELL LINE MCF 7

S. Abarzua[1], E. Thedinga[3], S. Drechsler[3], K.Fischer[1], N. Pietschmann[1], D.-U. Richter[2], R. Ehret[3], B. Piechulla[1], V. Briese[2]

[1]Department of Biological Sciences and [2]Department of Obstetrics and Gynaecology, University of Rostock, Rostock; [3]Bionas GmbH, Rostock, Germany

Background: Phytoestrogens are naturally occuring, plant-derived, non-steroidal phytochemicals. The major structural classes of phytoestrogens are the isoflavones and lignans found at high levels in various plants such as soybeans, clover or flax. Numerous *in vitro* cell culture studies and *in vivo* animal experiments demonstrated that phytoestrogens can inhibit tumor growth. The aim of this study was to isolate phytoestrogens from the flax root of *Linum usitatissimum* and to test their effect on cellular acidification (glucose metabolism), respiration (oxidative phosphorylation) and adhesion (cell impedance) in the human mammalian carcinoma cell line MCF 7 using the Bionas® 2500 *analyzing system*. This system allows continuous monitoring of cellular processes at a high temporal resolution compared to conventional end-point assays.

Materials and Methods: Flax root extracts were prepared according to a special lignan extraction procedure at concentrations of 0.01, 1, 20, 50, 100, 150, 200 and 1000 µg/mL. Online monitoring of the metabolism of the human mammalian carcinoma cell line MCF 7 was performed with the Bionas® 2500 *analyzing system*. Using this system metabolically relevant data including cellular acidification, oxygen consumption and adhesion of cells were registered continuously over 8 hours on six sensor chips (200,000 cells/chip) in parallel at different concentrations of flax root extracts. The molecular-chemical composition of the flax root extract was analyzed by pyrolysis-field ionisation mass spectrometry (Py-FIMS). HPLC-MS analysis was performed to identify the compounds of the isolated phytoestrogens from the flax root extract. Results: The extracts from flax roots of *L. usitatissimum* reduced the cellular acidification, respiration and adhesion in a dose-dependent manner relative to the control. At concentrations of >150 µg/mL these cellular processes were significantly inhibited and oncocidal effects in the MCF 7 cell line were observed. Analysis of flax root extracts by Py-FIMS revealed primarily phenols and lignans while HPLC-MS analysis demonstrated more representatives of lignans compared to isoflavones (aglycones as well as glycosides).

Conclusion: The Bionas® 2500 *analyzing system* allows a multiparametric online monitoring of cellular processes and can be used to detect the mode of action of anticarcinogenic substances in the cellular metabolism.

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DETECTION OF OLIGOMETASTATES IN BREAST CANCER BY USING TUMORMARKERS

I. Ertl[1], V. Heinemann[2], I. Bauerfeind[3], D. Laessig[2], D. Nagel[1], D. Seidel[1], P. Stieber[1]

[1]Institute of Clinical Chemistry, [2]Medical Department III, [3]Gynecological Department, University Hospital of Munich, Campus Grosshadern, Munich, Germany

With the aim of early detection of metastatic disease, breast cancer patients are monitored in a prospective non randomized trial in intervals of 6 weeks by using kinetics of CEA and CA 15-3.

Methods: Among the 516 patients participating in our trial we analysed the clinical data of those patients who developed distant metastases (n=42). A reproducible increase of CEA (Abbott, AxSYM) or CA 15-3 (Roche, Elecsys) >= 100%, corresponding to a specificity of 100%, was the indicator for metastatic disease.

Results: Fourty-two patients developed distant metastases. Twenty-five patients (60%, of these 83% hormone receptor positive and 47% HER2 positive primary tumors) showed the previously defined increase of CEA or CA 15-3 at the time of first distant metastasis (true-positive). Seventeen patients (40%, of these 67% hormone receptor positive, 25% HER2 positive) did not show an increase in tumor marker levels (false-negative). At further progression, an increase of markers was determined in 7 of the 17 false-negative patients (remaining 23% false negative). The marker-positive patients showed a median disease-free interval until recurrence of 49 months (range 14-198). In 15 patients CA 15-3, in 7 CEA, and in 3 CEA and CA 15-3 increased after a median time of 10 months (range 1.5-30). All marker-positive patients were asymptomatic at the time of recurrence. Fourteen of the true-positive patients (56%) suffered from metastases in one site (7 liver, 4 bone, 2 lymph node, 1 lung), 7 in two sites and 4 of multiple metastases. None of the patients with oligometastases became progressive at another site within the following six months and all received treatment within one month after first metastases.

Conclusion: All marker positive patients were asymptomatic at the time of detection of metastatic disease. 56% of these patients showed metastases in only one site. Whether an organ defined specific treatment can lead more frequently to complete remission of the patients has to be shown.

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TUMOR MARKER KINETICS OF CEA AND CA 15-3 CORRELATE WITH RESPONSE IN PATIENTS UNDERGOING CHEMOTHERAPY FOR METASTATIC BREAST CANCER

D. Laessig[1], V. Heinemann1, M. Untch[2], S. Kahlert[2], I. Bauernfeind[2], D. Nagel[3], P. Stieber[3]

[1]Medical Department III, [2]Gynecological Department, [3]Institute of Clinical Chemistry, University Hospital, Campus Grosshadern, Munich, Germany

The aim of this retrospective analysis was to determine the correlation between tumor marker kinetics (TMK) like CEA and/ or CA 15-3 and imaging during chemotherapy of metastatic breast cancer (MBC) patients.

Materials and Methods: TMK (CEA, AxSYM, Abbott; CA 15-3, Elecsys, Roche) were evaluated in MBC patients (n=77) at the beginning of chemotherapy for metastatic breast cancer (pre-treatment value=A), after 20-30 days (1st intermediate value=B), after 40-60 days (2nd intermediate value=C) and at the time of staging with imaging techniques (D). Response to treatment was assessed by UICC criteria. Two criteria for progressive disease and two criteria for non-progressive disease based on TMK were established. The 1st criterion for progression required that the increase from A to C had to be greater than 25%, and the increase per day from A to C had also to be greater from A to B. The 2nd criterion for progression required that D should be >25% than A and also an increase of C to D was required. The 1st criterion for non-progressive disease required that the decrease from A to C had to be greater than 25% and C had to be lower than B. The 2nd criterion for non-progressive disease required D <25% than B and D had to be lower than C.

Results: Fifty-four (70%) patients showed a correlation of TMK and imaging results. In 10 (13%) patients no correlation was obtained, and in 13 (17%) patients no biochemical statement was possible because of divergent TMK. Using our criteria a sensitivity of 70.2% was reached.

Conclusion: We could show a correlation between TMK and imaging results. After validation in a prospective trial, using our criteria in clinical practice could improve therapeutic monitoring and reduce radiation exposure in patients with MBC.

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SERUM TUMOR MARKERS IN BREAST CANCER: RETROSPECTIVE VIEW AND OUTLOOK

R. Molina

Laboratory of Clinical Biochemistry, Hospital Clinic, Barcelona, Spain

Numerous serum tumor markers have been described for breast cancer, including members of the MUC-1 family of mucin glycoproteins (*e.g.* CA15.3, BR 27.29, MCA, CA 549), carcinoembryonic antigen (CEA), oncoproteins (*e.g.* HER-2/c-erbB-2) and cytokeratins [*e.g.* tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS). In spite that the majority of these tumor markers are being studied for more than 20 years, their clinical utility is not well establish. The majority of studies using tumor markers evaluate the tumor marker sensitivity and specificity in the different stages, but they do not evaluate the possible relationship with therapy, the most important point for the oncologist.

Clinical Applications. Diagnosis: Their lack of organ-, and tumor specificity and low sensitivity in early stage disease invalidate the use of tumor markers for either screening or early diagnosis. Nevertheless, tumour marker determination may complement patient staging: high levels of CA15.3 (*e.g.* >50 u/mL) and/or CEA (*e.g.* >20 ng/mL) in patients thought to have localised disease suggest the presence of unsuspected metastatic disease. *Prognosis:* Several studies have demonstrated shorter DFS and OS in patients with high pre-operative levels of these markers. However, it has not yet been demonstrated if the use of tumour markers as indicators of recurrence can lead to improvement in either patient DFS or OS. *Early diagnosis of therapy:* Serial tumor marker measurements can contribute usefully to early diagnosis of distant metastatic disease. However, the basic hypothesis that early diagnosis of recurrences may prolong survival is not well established.

Patients with advance disease – Disease monitoring. The sensitivity of tumor markers is significantly higher in patients with advanced disease and is related to the site of recurrence. By using combinations of several markers (*e.g.* CA15.3, CEA and cytokeratins) it is possible to increase the sensitivity to at least 90% in patients with distant metastases. The most important clinical application of tumor markers in metastatic breast cancer is in monitoring response to therapy.

Predictive factor. Her-2/neu serum levels is the most promising predictive factor in advanced breast cancer. A clear relationship has been found between pre-treatment HER-2/neu serum levels and/or HER-2/neu serum levels at follow-up and tumor response in most of the studies published. Nonetheless, the advantages of the use of HER-2/neu in serum compared to these values in tissue, the cut-offs necessary to suggest treatments, etc. remain under debate and thus, further studies are required to clarify these questions.

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HER-2/NEU IN TISSUE AND SERUM AT TIME OF PRIMARY DIAGNOSIS OF BREAST CANCER

M. Dresse[1], D. Mayr[2], V. Heinemann[3], S. Kahlert[4], I. Bauerfeind[4], D. Nagel[1], D. Seidel[1], P. Stieber[1]

[1]Institute of Clinical Chemistry, [2]Institute of Pathology, [3]Medical Department III, [4]Gynecological Department, University Hospital, Campus Grosshadern, Munich, Germany

HER2 in breast cancer tissue is a marker of high prognostic and predictive relevance. Soluble HER-2, the extracellular domain of the HER-2/neu receptor which is shed into the blood, has been suggested to be a helpful tumor marker. We investigated, whether there exists a relationship between the concentration of HER-2/neu Shed Antigen in serum (s-HER-2/neu) and HER-2/neu in breast cancer tissue and whether this relationship could be used for diagnostic purposes.

Patients and Methods: s-HER-2/neu was measured at time of primary diagnosis in the pretherapeutical sera of 525 breast cancer patients with known HER-2/neu-status in breast cancer tissue. 33 of the patients (6.3%) had distant metastases (M1). The HER-2/neu- status in tumor tissue was determined by immunohistochemistry (HercepTest, Dako, Germany), followed by subsequent FISH analysis (Pathvysion, Abbott, USA) in case of score 2+. Dako-Score 3+ or 2+ and gene amplification in FISH analysis were regarded as HER-2/neu-positive. HER-2/neu shed antigen was analysed by Immunoassay (ADVIA Centaur, Siemens Medical Solutions Diagnostics, Germany).

Results: For patients without distant metastases (M0) as well as patients with distant metastases (M1) we observed a correlation of s-HER-2/neu with HER-2/neu-status. The median s-HER-2/neu-concentrations were 11.7 ng/ml (13.2 ng/ml) for the HER-2/neu-negative resp. –positive patients in the M0 group ($p<0.001$) and 11.9 ng/ml (16.0 ng/ml) in the M1 group ($p=0.01$). We performed a ROC-analysis within the M0 group of patients in order to investigate the diagnostic capacity of s-HER-2/neu concerning its predictive value for the HER-2/neu-status: using a cut-off value of 30 ng/ml the HER-2/neu-status was always positive, corresponding to a specificity of 100% and a sensitivity of 7.7%.

Conclusion: There is a correlation between high s-HER-2/neu-values and a positive HER-2/neu-status. Pretherapeutical s-HER-2/neu-values ≥30 ng/ml were only observed in HER-2/neu-positive patients. HER-2/neu shed antigen in serum can add up to the HER-2/neu-status and might reveal false-negative tissue findings.

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EFFECTS OF PHYTOESTROGEN EXTRACTS ISOLATED FROM FLAX ON HORMONE PRODUCTION OF TROPHOBLAST TUMOUR CELLS JEG 3 AND BEWO

D.-U.-Richter[1], S. Abarzua[2], M. Chrobak[2], B. Piechulla[2], C. Scholz[3], C. Kuhn[3], S. Schulze[3], M.S. Kupka[3], K. Friese[3], U. Jeschke[3]

[1]Department of Obstetrics and Gynaecology and [2]Department of Biological Sciences, Rostock University, Rostock; [3]First Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University, Munich, Germany

Phytoestrogens belong to polyphenolic compounds whose major biological role is to prevent plants from stress. They have similar effects as endogenous estrogens in humans and several potential anticarcinogenic activities have been described recently. In this study the effects of crude extracts from flax (*Linum usitatissimum*) on production of progesterone and estradiol in trophoblast tumour cell line Jeg 3 and BeWo were tested.

Methods: Isoflavone and lignan extracts from flax plant *Linum usitatissimum* were obtained using different extraction methods. The isolated extracts were incubated in different concentrations on trophoblast tumour cells (Jeg 3, BeWo). Untreated cells were used as controls. Aliquots were removed at designated times and tested for estradiol and progesterone production. In addition the effect of the phytoestrogen extracts on the expression of estrogen receptors α and β as well as on progesterone receptor were tested.

Results: Down-regulation of both estradiol and progesterone production after stimulation with different concentrations of lignan extracts from flax plant *Linum usitatissimum* were observed in Jeg 3 cells. In BeWo cells a down-regulation of progesterone production but an up-regulation of estradiol production were seen. Expression of ERα is up-regulated in Jeg 3 and BeWo cells after stimulation with lignan extracts, whereas ERβ expression is down regulated in both cell lines. PR expression is down regulated in BeWo cells whereas lignan extracts from flax plant leaves showed up-regulation of PR in Jeg 3 cells.

Conclusion: The results obtained in this study suggest that isoflavone and lignan extracts from flax plant *Linum usitatissimum* can reduce the production of progesterone in BeWo and Jeg 3 cells and modulation of estrogen production in both cell lines. In former studies it was shown that proliferation of trophoblast tumour cells was significantly reduced after phytoestrogen treatment. In addition, low doses of phytoestrogens induced a higher hCG production in both cell lines tested. In summary, with this *in vitro* study it is shown that isoflavone and lignan extracts from flax plant inhibit progesterone production in trophoblast tumour cells. Isoflavone and lignan extracts from flax plant could be useful candidates for special diet programs for prevention or treatment of steroid hormone sensitive tumours.

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A BIOMARKER ALGORITHM OF HE4 AND CA125 FOR DIFFERENTIAL DIAGNOSIS IN WOMEN PRESENTING WITH ADNEXAL MASS

O. Nilsson[1], W.J. Allard[1], R.G. Moore[2], St. J. Skates[3]

[1]Fujirebio Diagnostics Inc., Malvern, PA, [2]Women and Infants Hospital, Brown University, Providence, RI, [3]Massachussets General Hospital, Harvard Medical School, Boston, MA, U.S.A.

Ten percent of all women will be diagnosed with an adnexal mass in their lifetime and approximately 20% will be found to have epithelial ovarian cancer. Women with ovarian cancer have improved survival when their surgery is performed by gynecologic oncologists at experienced institutions. A dual marker algorithm using the HE4 and CA125 concentration together with menopausal status was developed to improve the triage of women presenting with an adnexal mass.

Methods: Fourteen biomarkers were evaluated in two pilot studies and only HE4 added sensitivity to CA 125 at fixed specificity. This dual-marker combination was applied to data from 487 sera drawn prior to surgery in women with adnexal mass to develop a logistic regression model that incorporated menopausal status. The algorithm was validated in a prospective, multicenter trial at 12 USA sites that enrolled a total of 566 patients with adnexal mass scheduled for surgery at gynecologic oncology practices. All women provided informed consent and all surgical biopsy specimens were reviewed centrally to confirm the pathological findings.

Results: Leave-one-out cross-validation analysis showed that only HE4 provided increased sensitivity compared to CA 125 alone. The logistic regression model derived from the pilot studies was validated by showing that the lower limit of the 95% confidence interval for sensitivity was >80% at a set specificity of 75%. In addition, at 75% specificity the algorithm provided an overall sensitivity for EOC and LMP of 89%, with sensitivities of 94% for post-menopausal women and 76% for pre-menopausal women.

Conclusion: Of the 14 biomarkers studied, only HE4 provided complementarity to the current standard, CA 125. Application of the dual-biomarker algorithm to clinical practice could reduce referrals to gynecologic oncology centers by 50% overall, including 75% of women.

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HE4: AN APPROPRIATE MARKER FOR THE PRIMARY DIAGNOSIS AND FOLLOW-UP OF OVARIAN CANCER

M. Casova, Z. Novotny, O. Topolcan, J. Vrzalova, L. Holubec, J. Finek, M. Prazakova, Z. Rokyta

Faculty Hospital and Medical School in Pilsen, Charles University, Prague, Czech Republic

Objectives: CA125 is the main marker used in both the primary diagnosis and follow-up of epithelial ovarian

tumours. The disadvantage is its low specificity. The aim of this study was to compare the new tumour marker HE4 with those commonly used in practice.

Methods: Fifty five patients with ovarian cancer were monitored along with a control group of 50 patients diagnosed with non-malignant gynaecological disease (pregnancy, endometriosis, ascites). The following tumour markers were analyzed in both groups: HE4, CA125, TK, TPA, TPS and Monototal. The sensitivity of these markers is always expressed at a specificity of 95%.

Results: In the group of patients with ovarian cancer significantly high values were found for HE4, CA125 and TK ($p<0.001$). HE4 showed the highest sensitivity (73%), while CA125 and TK were both at 64%. The combination of HE4 and TK had the highest sensitivity at 80%. In patients with non-malignant gynecological disease the elevation of HE4 above cut-off was not observed.

Conclusion: HE4 is an appropriate marker for the primary diagnosis and follow-up of ovarian cancer and with low false positive results. *This study was supported by research project VZ MSM 0021620819.*

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DETERMINATION OF GLYCODELIN EXPRESSION IN BENIGN, BORDERLINE AND MALIGNANT CHANGES OF THE OVARIES

A. Tsvilina[1], C. Kuhn[1], D. Mayr[2], S. Kunze[1], I. Mylonas[1], U. Jeschke[1], K. Friese[1]

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

Glycodelin is a glycoprotein with a molecular weight of 28 kDa. Due to its different glycosylation, several glycodelin molecules have been described, including glycodelin A (amniotic fluid). The precise function of glycodelin is still not well understood, although immunosuppressive and contraceptive functions, as well as its use as a marker of morphological differentiation have been demonstrated. The aim of this study was to assess the expression of glycodelin in benign and malignant tumors of the ovary.

Materials and Methods: Paraffin sections of 187 ovarial cancer specimens (including 132 serous, 22 endometriode, 17 mucinous, 12 clear cells and 4 borderline tumors) and 70 benign changes in the ovary were immunhistochemical analyzed with a monoclonal antibody GdA (MAk) and a peptide polyclonal antibody (PAk) against glycodelin. These separate antibodies recognise different epitopes.

Results: Benign tumors express significantly lower glycodelin than malignant tumors. In particular, serous benign tumors showed, compared to serous malignant ovarian tumors, a significantly low glycodelin expression for both analysed antibodies. Mucinous tumors did not demonstrate significant glycodelin changes. Interestingly, endometroid carcinomas showed the highest glycodelin expression of all malignant tumors, being significantly higher compared to endometriotic foci in normal ovary. Moreover, ovarian cancer of surgical stage FIGO III-IV demonstrated a lower glycodelin expression of FIGO I-II stage cancers.

Conclusion: Glycodelin is a glycoprotein with immuno-supressive function. The increased expression of this glycoprotein in malignant tissue of the ovary could be related to a "immuno escape" mechanism. In particular, serous and endometroid tumor tissue showed a strong glycodelin expression. In addition to its immunosupressive function, glycodelin seems to be an important marker of morphological differentiation in ovarian cancer. The use of glycodelin as a tumor marker in ovarian carcinomas is currently under investigation.

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TRANSKETOLASE-LIKE 1 (TKTL1) EXPRESSION IN ENDOMETRIAL CANCER IS INCREASED

M. Krockenberger, U. Kämmerer, M. Kapp, J. Dietl, A. Honig

Universitätsfrauenklinik Würzburg, Germany

Malignant cells of many tumors ferment glucose even in the presence of oxygen (aerobic glycolysis). TKTL-1 and p-AKT are assumed to be key proteins for this switch from oxidative glycolysis to non-oxidative fermentation. Glycolysis implies that cells need more glucose. Therefore the glucose transporter GLUT 1, which facilitates glucose uptake of cells and is known to be expressed in cancerous tissue, was also investigated.

Methods: TKTL-1, GLUT1 and p-AKT expression was evaluated immunhistochemically on paraffin embedded biopsy material of 14 benign and 38 malignant endometrial tissue samples. TKTL-1 mRNA levels were evaluated by RT-PCR in endometrial cancer cell lines Ishikawa and Hec1a.

Results: In endometrial carcinomas, TKTL-1, GLUT1 and pAKT expression was significantly elevated in comparison to benign endometrial tissue. There was a significantly weaker TKTL-1 expression in highly differentiated G1 tumors compared to (welche Tumoren???). In human endometrial cancer cell lines Ishikawa and HEC-1A, TKTL-1 mRNA was clearly detectable.

Conclusion: TKTL-1, GLUT1 and pAKT expression points to the glycolytic phenotype of this cancer entity.

Given the TKTL-1 expression across all different histologies of endometrial cancer, it seems a rather general characteristic of malignant transformed endometrial cells. Therefore TKTL-1 could serve as a target for future cancer treatments given its predominant expression in malignant tissue.

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EXPRESSION PATTERN OF THE ACTIVATED LEUKOCYTE CELL ADHESION MOLECULE ALCAM/CD166 AS A DIAGNOSTIC AND PROGNOSTIC MARKER IN ENDOMETRIAL CANCER

J. Briese[1,2], F. Doeleke[3], K. Milde-Langosch[4], M. Sajin[5], AM. Bamberger[3]

[1]Department of Pathology and Tumor Biology, University of Leeds, United Kingdom; [2]Department of Obstetrics and Gynaecology, University of Greifswald, Germany; [3]Section on Endocrinology and Metabolism of Ageing and [4]Department of Gynaecology, University Clinic, Hamburg, Germany; [5]Department of Morphopathology, University of Bucharest, Romania

The activated leukocyte cell adhesion molecule (ALCAM/CD166) is an immunoglobulin superfamily cell adhesion molecule. Whilst expressed in a wide variety of tissues and cells, ALCAM is restricted to subsets of cells usually in dynamic growth and migration processes and has been implicated in tumorigenesis and tumor progression. Over the past decade, alterations in expression of ALCAM have been reported in several human tumors, like melanoma, prostate, breast and bladder 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 pattern of ALCAM in the normal human endometrium and in endometrial carcinomas for the very first time.

Methods: In the present study, immunohistochemistry with specific antibodies was performed on a series of 20 normal endometrial samples, 15 endometrial hyperplasias and 40 endometrial carcinomas to investigate the expression pattern and cell-type specific localization of ALCAM and to correlate it with clinico-pathological data. In addition, Western blot was performed on normal human endometrium and endometrial neoplasia.

Results: Strong ALCAM expression with a consistent cytoplasmic localization was observed in epithelial and glandular cells of normal human endometrium in 80% of the samples of the proliferative and secretory phase (score 8-12). Most of the hyperplasias showed nearly the same result. Moderate (score 4-7) cytoplasmic and membranous ALCAM expression could be observed in 50% of the low grade endometrioid carcinomas. With increasing malignancy grade, increasing areas with low ALCAM expression level or complete loss of ALCAM expression could be observed. Down-regulation (score 0-3) occurred preferentially in 40% of the analyzed high grade endometrioid carcinomas as well as in serous and clear cell carcinomas. The results were confirmed by western blotting.

Conclusion: The data suggest that ALCAM expression is disturbed in endometrial carcinoma, which might indicate a role of ALCAM in the progression of this disease. At present, it is only possible to speculate about the molecular mechanisms underlying these results. The expression pattern of ALCAM in endometrial tissue indicates that it might play a role in the pathogenesis of endometrial cancer and seems to be useful as an additional independent diagnostic and prognostic marker for such lesions.

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HPV-DNA IN NORMAL AND MALIGNANT HUMAN ENDOMETRIAL TISSUE

T.J.F. Blankenstein, M. Montag, S. Schulze, C. Kuhn, K. Friese, I. Mylonas

First Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University, Münich, Germany

In contrast to the abundance of reports about the association of HPV and cervical carcinoma, studies focussing on the potential role of HPV in the pathogenesis of endometrial adenocarcinoma are rare and contradictory. The aim of this study is to test for HPV-DNA expression in a series of normal human endometrial tissue and endometrial adenocarcinoma.

Materials and Methods: Thirty six tissue specimens from patients with endometrial adenocarcinoma and 10 normal endometrial tissue samples were analysed using the GenPoint HPV DNA Probe Cocktail. Three cervical carcinoma cell lines with a defined HPV copy number per cell (SiHa, HeLa, CaSki) and two HPV-negative cell lines (AC-1M32, MCF-7) served as control for HPV expression.

Results: A clear hybridisation signal could neither be detected in normal endometrial tissue nor in 36 endometrial carcinoma samples (22 endometrioid, 5 mucinous, 2 serous, 2 clear cell and 5 mixed adenocarcinomas).

Conclusion: In this study HPV-DNA could not be detected in normal endometrial tissue and endometrial adeno-carcinomas. Therefore, HPV does not seem to be a major cause in the carcinogenesis of human endometrial adenocarcinomas.

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IS LYMPHADENECTOMY A PROGNOSTIC MARKER IN ENDOMETRIOID ADENOCARCINOMAS OF THE HUMAN ENDOMETRIUM?

N. Bassarak, T. Blankenstein, N. Shabani, D. Dian, K. Friese, I. Mylonas

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

Pelvine and/or paraaortal lymphadenectomy is performed during operation of endometrial cancer and is recommended by the FIGO. Although metastatic lymph nodes are important prognostic parameters, it has been controversially discussed if a performed pelvic lymph node dissection has itself a prognostic impact in the treatment of endometrial cancer, especially in endometrioid adenocarcinomas. Therefore, this study evaluated whether a performed lymphadenectomy has a prognostic impact in patients diagnosed with an endometrioid adenocarcinoma.

Materials and Methods: Three hundred and two endometrial carcinoma cases were analysed for the benefit of a performed lymphadenectomy. Tumor characteristics were analysed again with respect to surgical and pathological stage and only endometrioid adenocarcinomas were included in this study.

Results: Two hundred and fourteen patients with a histological diagnosis of endometrioid adenocarcinomas were included in this study. Of these patients 171 (79.9%) were classified as FIGO stage I, 15 (7.0%) as FIGO stage II, 21 (9.8%) as FIGO stage III and 7 (3.3%) as FIGO stage IV. One hundred and thirty four (62.6%) of the patients had a histological grade 1 tumor, while 56 (26.2%) and 24 (11.2%) demonstrated a histological grade 2 or grade 3, respectively. Lymphadenectomy was performed in 151 (70.6%) patients. Only 11 (5.1%) patients demonstrated metastatic disease in the lymph nodes. The performance of a lymphadenectomy resulted in a significant better cause-specific and overall survival ($p<0.05$), while the progression-free survival was not affected by this operative procedure.

Conclusion: The performance of an operative lymphadenectomy resulted in better survival of patients with endometrioid adenocarcinomas. Although just a tendency in affecting the progression-free survival was observed, a significant survival benefit for the cause-specific and overall survival was demonstrated. Therefore, a pelvine and/or paraaortal lymphadenectomy should be performed during operation even in endometrioid adenocarcinomas.

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EXPRESSION OF INHIBIN/ACTIVIN SUBUNITS (ALPHA, BETA-A AND BETA-B) IN NORMAL AND CARCINOMATOUS CERVICAL TISSUE

J. Jückstock, T. Blankenstein, S. Kunze, U. Jeschke, K. Friese, I. Mylonas

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

Inhibins are dimeric glycoproteins composed of an alpha-subunit and one of two potential beta-subunits, (betaA (bA) or betaB (bB)), showing substantial functions in human reproduction and in endocrine-responsive tumours. In this study the expression of these different subunits was examined in normal and pathological cervical tissue.

Methods: Normal cervical tissue (n=10) and cervical adenocarcinomas (n=6) in archival specimens were examined by immunohistochemistry.

Results: Immunoreactivity of inhibin-alpha could be demonstrated in glandular cervical epithelium, while squamous epithelia cells did not express this subunit. Interestingly no analyzed cervical adenocarcinoma showed any staining reaction of this subunit. Both inhibin-bA and -bB subunits were seen in glandular epithelium of both normal and pathological cervical tissue. However, squamous epithelia cells also expressed these subunits, but with a lower intensity.

Conclusion: In this preliminary study an immunohistochemically detected expression of inhibin-alpha, -bA and -bB subunits in normal as well as in pathological cervical specimens is demonstrated. Inhibin molecules are therefore potentially useful serological markers in cervical cancer. The subunits are expressed immunohistochemically to a certain levels, thus suggesting possible functions in normal and pathological cervical tissue. Moreover, inhibin-alpha is considered a tumour suppressor in several gynaecological malignancies, including endometrial and ovarian cancer. Whether this holds also true for cervical cancer will be evaluated in future studies.

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EXPRESSION OF GALECTIN-1 IN VULVAR NEOPLASIA

N. Kohrenhagen[1], U. Voelker[2], J. Dietl[1], U. Kammerer[1]

[1]Department of Obstetrics and Gynecology and
[2]Department of Pathology, University of Wuerzburg, Germany

Galectin-1, a member of the β-galactoside-binding family, is widely expressed in epithelial and im-mune cells. It is involved in many normal and pathological processes, such as cancer progression, metastasis and immunobiology. The grade of expression of galectin-1 varies between different cancer cells and the corresponding normal tissue. Therefore, it has

been described as a marker for tumor progression according to the cancer type. Unfortunately, there is no information available on the expression of galectin-1 in vulvar cancer.

Methods: In this study, the expression of galectin-1 was examined in 75 formalin fixed paraffin-embedded vulvar tissues: 10 normal vulvar specimen, 10 vulvar intraepithelial neoplasia type 1 (VIN I), 15 VIN II and 20 VIN III lesions, respectively, and 20 invasive squamous cell carcinomas of the vulva.

Results: Immunohistochemical analyses showed that the intensity of the galectin-1 expression on stromal cells next to the cancer cells in-creased according to the pathological grade ($p<0.001$). The epithelial cells were always negative for galectin-1.

Conclusion: These results suggest that galectin-1 expression increases with the histopathological grade of vulvar tissues and it can be concluded that this increase is associated with the progression of vulvar neoplasia.

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OVEREXPRESSION OF POLYCOMB PROTEIN BMI-1 IN HUMAN SPECIMENS OF BREAST, OVARIAN, ENDOMETRIAL AND CERVICAL CANCER

A. Hönig, C. Weidler, S. Buchholz, F. Köster, M. Krockenberger, S. Häusler, M. Becker, J. Wischhusen, J. Dietl, J. B. Engel

Universitätsfrauenklinik, Würzburg MSZE, Germany

Background: The identification of factors for malignant transformation is crucial to identify new therapeutic targets for cancer therapy. BMI-1 belongs to the group of polycomb proteins and induces suppression of DNA transcription. Thus, it is an important survival factor for malignant and benign tissue stem cells. In addition, BMI-1 is a known antagonist of tumor suppressor gene p16 and therefore can indirectly contribute to malignant transformation. This study investigates the expression of the BMI-1 protein in ovarian, endometrial, cervical and breast cancer.

Materials and Methods: BMI-1 expression was investigated in SKOV-3 human ovarian cancer cells as well as in human ovarian cancer specimens by RT-PCR and Western-blot analyses. Additionally, surgical biopsies of human ovarian, endometrial, cervical and breast cancer were immunohistochemically evaluated for expression of BMI-1. Additionally, immunohistochemistry was used to evaluate a correlation between BMI-1 and p16. Samples of the corresponding benign tissues were used as controls.

Results: Human SKOV3 ovarian cancer cells express BMI-1 mRNA as well as the BMI-1 protein. BMI-1 protein was detected in surgical biopsies of ovarian cancer by immunohistochemistry and western blotting analyses. In breast, cervical and endometrial cancer BMI-1 protein expression was shown using immunohistochemistry. In breast cancer tissue samples BMI-1 protein expression was markedly pronounced in the invasive front. BMI-1 expression did not correlate with the expression of p16 in samples of cervical cancer. Expression of BMI-1 was less pronounced in benign tissues corresponding to the respective cancers, as shown by immunohistochemistry.

Conclusion: Overexpression of BMI-1 in ovarian, endometrial and breast cancers as compared to benign tissue suggests that this protein contributes to malignant transformation in these tumor entities. This notion is further supported by the distinctly increased expression in the invasive front of breast cancer tissue samples. Surprisingly, the expression of BMI-1 did not inversely correlate with the expression of its functional antagonist p16. However, these results have to be re-evaluated with more sophisticated methods such as real-time PCR and Western blot analysis.

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WHICH BIOMARKERS ENHANCE THE EFFICIENCY OF CA 125 IN THE DIAGNOSIS OF OVARIAN CANCER?

A. Burges[1], M. Lenhard[1], D. Mayr[2], D. Nagel[3], P. Stieber[3]

[1]Department of Obstetrics and Gynecology, [2]Department of Pathology, [3]Department of Clinical Chemistry, Ludwig-Maximilians-University - Campus Grosshadern, Munich, Germany

Due to the lack of organ specificity of most biomarkers and the lack of tumor specificity of all biomarkers, serological diagnostic oncology mainly focuses on follow up care of cancer patients using a single marker with the best profile of specificity and sensitivity for this purpose. It becomes evident that malignant diseases lead to a significant release or non release of multiple biomarkers and that the sum of this information leads to an increase of their diagnostic or differential diagnostic capacities.

Patients and Methods: We investigated retrospectively the sera (stored at –80˚C) of 109 healthy women (81 premenopausal, 28 postmenopausal), 396 patients with various benign gynecological disorders (endometriosis, uterus myomatosis, ovarian cysts, benign adnex tumors, bleeding disorders, cervical dysplasia (226 premenopausal, 170 postmenopausal)), and 147 patients suffering from ovarian cancer (low malignant potential (LMP): 16; ovarian cancer (OC) stage I: 23, stage II: 15, stage III: 83, stage IV: 10 (32 premenopausal, 115 postmenopausal)) at time of

diagnosis before first treatment using the following parameters: CA 125 (Abbott, ARCHITECT, USA), CYFRA 21- 1 and CA 72-4 (Elecsys, Roche, Germany), as well as HE4 (Elisa, Fujirebio, USA).

Results: All 4 biomarkers investigated showed higher concentrations in the benign diseases as compared to healthy individuals and again a higher release as compared to both control groups in borderline tumors (LMP). In ovarian cancer patients the strongest release could be observed for CA 125 (median 393 U/ml, 95th percentile:8321 U/ml), the median being >20-fold higher than in the benign disease group. CA 72-4 was released to the highest extent in mucinous epithelial carcinomas, CA 125 and HE4 in serous epithelial carcinomas. A correlation with tumor size and stage could be observed for all 4 markers.

Conclusion: CA 125 is the best single marker in the diagnosis of ovarian cancer. The combined analysis with one or several of the other biomarkers leads to a significantly superior profile of diagnostic efficacy of ovarian tumors.

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SERUM CA125 AND CA72-4 IN OVARIAN BORDERLINE TUMORS

M. Lenhard[1], S. Nehring[1], D. Nagel[2], D. Mayr[3], A. Kirschenhofer[1], L. Hertlein[1], K. Friese[1], P. Stieber[2], A. Burges[1]

[1]Department of Obstetrics and Gynecology, [2]Department of Clinical Chemistry, [3]Department of Pathology, LMU, Campus Grosshadern, Munich, Germany

Aim: To assess the prognostic value of CA 125 and CA 72-4 in patients diagnosed with an ovarian borderline tumor (BOT).

Materials and Methods: All women diagnosed and treated for BOT between 1981 and 2008 at our institution were included in this retrospective study. Preoperative serum CA 125 (Architect, Abbott and Elecsys, Roche) and CA 72-4 (Elecsys, Roche) were analyzed with reference to patient characteristics and clinical data including histology and ascites at primary diagnosis, relapse and survival.

Results: 101 patients had serum tumor marker measurement and surgery at our institution. Relapse occurred during the follow up period in five patients (4.95%), and five deaths (4.95%) were documented. Median age at primary diagnosis was 53 years (range 18-88 years). Serum tumor marker CA 125 was elevated at median with 37.1 U/ml (range 2.6-1996.0 U/ml) and CA 72-4 with 1.9 U/ml (range 0.2-396.0 U/ml), being significantly different from a healthy female control group (n=109) with a median CA 125 of 13.5 U/ml (range 4.0-49.7 U/ml) and CA 72-4 of 0.8 U/ml (range 0.2-20.6 U/ml) and being moreover significantly different from a control group of ovarian cancer patients (n=130) with a CA 125 of 401.5 U/ml (range 12.5-11891 U/ml) but without a significant difference for CA72-4 (median 3.85 U/ml, range 0.3-10068 U/ml). Histology revealed a serous tumor in 63% of patients, mucinous in 36%, and endometrioid in one case, but tumor markers did not differ in neither group. 62.2% of all patients were diagnosed at early tumor stage pT1a. Patients with a pT1a tumor stage had significantly lower tumor marker values for CA 125 but not for CA 72-4 compared to those with higher tumor stages (CA 125 29.9 U/ml for pT1a *vs*. 50.9 U/ml; p=0.014). There was a tendency for elevated levels of CA 125 but not for CA 72-4 in the presence of ascites, endometriosis or peritoneal implants at primary diagnosis. Regarding the prognostic value of serum tumor markers CA 125 or CA 72-4, there was a statistically significant finding for elevated CA 125 in patients with recurrent disease (251.0 U/ml *vs*. 34.65 U/ml, p=0.012).

Conclusion: Serum CA 125 and 72-4 median values in ovarian borderline tumor patients differ in healthy controls and ovarian cancer patients. CA 125 but not CA 72-4 correlates with tumor stage at primary diagnosis and tends to be elevated in the presence of ascites, endometriosis or peritoneal implants. Moreover, CA 125 at primary diagnosis seems to be of prognostic value for recurrence.

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USE OF CA-125 IN THE MANAGEMENT OF OVARIAN CANCER

S. Mahner[1], J. Sehouli[2], L. Wölber[1], F. Jänicke[1]

[1]University Medical Center, Department of Gynecology, Hamburg, [2]Charité University Medical Center, Department of Gynecology, Berlin, Germany

CA-125 is a high molecular weight membrane glycoprotein that was first described as an antigen increased in more than 80% of patients with epithelial ovarian cancer. It is shed from the surface of the tumor cells and can be detected in the serum. Due to the close correlation of CA-125 serum concentrations and tumor volume, it is an accepted tool to monitor response to systemic therapy in ovarian cancer patients. The prediction of response is comparable to radiological therapy monitoring according to RECIST criteria. However, experience is currently limited to conventional systemic therapy and targeted therapeutic agents like bevacizumab can potentially alter the serum levels of CA-125. Despite radical surgical and chemotherapeutic treatment, the majority of patients with ovarian cancer develop recurrent disease within two to three years. Because of the high likelihood of disease progression,

most women are closely monitored after completing treatment. In case of disease recurrence, serum concentration of CA-125 usually rises 3-4 months prior to the development of symptoms or clinical and radiological signs of relapse. Although a cure of patients with relapsed ovarian cancer is rarely possible, it is still unclear whether an early reinduction of therapy on the basis of rising marker levels results in extended survival. Potential benefits of an early treatment of recurrent disease could be the delay of symptoms and an improved survival. The toxic side-effects and decreased interval without treatment in an otherwise asymptomatic patient are potential disadvantages. Depending on the duration of response to previous therapy and the length of the treatment free interval, patients should be counselled on the advantages and disadvantages of serial measurement of CA-125 during follow up. The use of CA-125 measurement in the screening and early detection of ovarian cancer remains inconclusive. A large randomized controlled trial of ovarian cancer screening in the UK (UKCTOCS) is currently investigating the role of CA-125 serum levels and vaginal ultrasound in 200,000 postmenopausal women. If this screening strategy is shown to be effective, CA-125 measurement will likely be implemented in future ovarian cancer screening programs.

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(MIS)MANAGEMENT OF YOUNG PATIENTS WITH BORDERLINE TUMORS OF THE OVARY – RESULTS OF A MULTICENTER SURVEY OF 328 CLINICS IN GERMANY

J. Sehouli, A. Coumbos, R. Chekerov, D. Schädel, G. Oskay-Özcelik, W. Lichtenegger, W. Kühn

On behalf of the North-Eastern German Society of Gynecological Oncology (NOGGO), Berlin, Germany

Borderline tumor (BOT) of the ovary represents a characteristic type of ovarian malignancy, but show no destructive stromal invasion. Thus, the borderline tumor – in contrast to that of an invasive ovarian carcinoma – has generally an excellent prognosis.

Over a period of 12 months, a questionnaire about the therapy management of BOT was sent by post to a total of 1135 clinics in Germany, including all university hospitals, hospitals of tertiary or secondary medical care and public community general practitioner's clinics. For women in the reproductive group with desire to have children the resection of the concerned ovary followed in 92%, and even in 19% a tumor/ovarian cystectomy was carried out. The contralateral ovary was usually checked by biopsy by 53% of the participants, but not surgically resected. Much less often peritoneal biopsies (67%) and peritoneal wash-cytology (86%) were performed. Omentectomy was performed in 81% of the universities, but only in 57% of the tertiary care units, 31% of the secondary care units and as few as 17% of the public general practitioner's centers ($p<0.05$). Peritoneal biopsies were performed at 81% and 80% of the university departments and tertiary care hospitals respectively, but at only 64% of the secondary care and 39% of the general practitioner's hospitals. After completion of the wish to have children, 47% of the clinics recommended a completion of the surgical resection of the involved ovary and/or contralateral adnexectomy. No significant difference was found between the different institutions ($p>0.05$).The results of our multicenter survey underline that high grade of unsureness and hence resulting in variability exists still now in the diagnostic and therapeutic management of BOT. The educational activities of all oncological societies and working groups are essential and have to be intensified.

Metabolic Tumor Control

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TKTL1 FERMENTATION METABOLISM – NEW OPTIONS FOR THE DIAGNOSIS AND THERAPY OF CANCER

U. Giese[1], J.F. Coy[1,2]

[1]Tavargenix GmbH, Würzburg, [2]Tavarlin AG, Darmstadt, Germany

Despite considerable progress in the early diagnosis and treatment of cancer the mortality, especially that of patients with metastasing tumours, is still very high. In 1924 the German Nobel prize laureate and biochemist Otto Warburg described one principal difference between tumour and normal tissue. The cancer cell is able to degrade glucose to lactate even in the presence of oxygen. This effect, known as the Warburg effect or aerobic glycolysis, is nowadays, according to recent research, understood as a change of the cancer cell metabolism to a mainly glucose derived but oxygen independent energy metabolism. With the discovery of the enzyme Transketolase-like 1 (TKTL1), one of the key enzymes of the non-oxidative part of the Pentose Phosphate Pathway, it is now possible to understand the biochemical principles of the Warburg effect. Aggressive tumour cells use this pathway to generate energy from glucose in the form of ATP as well as important cellular building blocks like ribose-5-phosphate, acetyl-CoA and fatty acids independently from the mitochondria. This changed pathway for the generation of energy is accompanied by an elevated resistance to radical and

apoptosis inducing therapies like radiation and chemotherapy. Furthermore the elevated lactic acid produced by this energy metabolism protects the cancer cell from the attack of the body's immune system and in addition leads to matrix degradation which results in enhanced invasiveness and metastasis. This changed energy metabolism, by activation of the TKTL1 enzyme, has gained high clinical importance, since most of the cancer patients die because of invasive cancer cells and metastasis. A number of studies have shown that an expression of TKTL1 is predictive for this changed energy metabolism of the cancer cell and is also a marker for poor survival of cancer patients. Because TKTL1 positive tumour cells, compared to normal cells, have a 20 to 30 times higher uptake of glucose and are very often not capable of utilizing fatty acids as energy source, they are highly dependent on glucose supply. This gives rise to the option to attack these tumour cells by a targeted change in nutrition. Feeding experiments of nude mice, transplanted with human TKTL1 positive aggressive tumours, with an omega-3-fatty acids and MCT-oil rich, but carbohydrate reduced diet show encouraging results. A targeted change in nutrition (TAVARLIN®) helps to attack the achilles heel of these TKTL1 positive aggressive cancer cells. This has also been shown during the past 2 years in tumour patients where usual treatment options have partly failed. Developing small molecule inhibitors of the enzyme TKTL1 will enable the direct influence on this energy metabolism. Now, the availability of cell based test systems to detect elevated TKTL1 levels in circulating tumour cells and in tumour tissues, sets the basis for a predictive diagnosis and a monitoring of the success of cancer therapies. Altogether the development of small molecule TKTL1 inhibitors, the availability of a specialised diet (TAVARLIN®) and the availability of a diagnostic system to detect and monitor TKTL1 levels in circulating tumour cells and tumour tissue, form the basis of a new pharmacodiagnostic concept that makes a targeted and individual cancer therapy possible.

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A KETOGENIC DIET IMPROVES QUALITY OF LIFE IN SOME PATIENTS WITH ADVANCED METASTATIC TUMOURS

M. Schmidt[1], N. Fetzer[1], I. Strauss[1], J. Coy[2], J. Dietl[1], U. Kammerer[1]

[1]Department of Gynaecology and Obstetrics, University of Wuerzburg, [2]Tavartis GmbH, Otzberg, Germany

Tumour cells are considered to utilise glucose as main source of energy supply. Inhibitors of glycolysis are not yet available for clinical trials. Therefore, a pilot study was performed to investigate the feasibility of a ketogenic diet and its influence on the quality of life of patients with advanced metastatic tumours.

Methods: Fifteen patients with measurable metastatic tumours of any origin and no conventional therapeutic options who were interested in participating in the study were included. The patients were instructed to follow a low-carbohydrate (less than 70g per day) diet and were provided with a supply of food additives to simplify the 3-month intervention period. Tumour staging, quality of life, serum and general health parameters were determined at baseline and at the 3 month follow-up. The effect of dietary change was monitored by measuring urinary ketone bodies.

Results: Two patients did not tolerate the diet and dropped out of the study within 2 weeks. Among those who accepted the diet, three patients had a progress, two felt worse and eight stated an unchanged or improved quality of life during the intervention period. Except from temporary constipation and fatigue, no severe adverse side-effects were observed nor any changes in cholesterol or blood lipids.

Conclusion: These pilot data suggest that a ketogenic diet might increase quality of life in some patients with advanced metastatic tumours. Whether a patient benefits from the diet or not is yet to be predicted. A randomised controlled trial is needed to determine the influence of the diet on tumour growth.

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SELENIUM SUBSTITUTION DURING RADIOTHERAPY OF SOLID TUMOURS – LABORATORY DATA FROM TWO OBSERVATION STUDIES IN GYNAECOLOGIC AND HEAD AND NECK CANCER PATIENTS

R. Mücke[1], J. Büntzel[2], K. Kisters[3], F. Bruns[4], M. Glatzel[5], K. Schönekaes[3], U. Schäfer[3], O. Micke[6]

[1]Department of Radiotherapy, St. Josef Hospital Wiesbaden, [2]Department of Otolaryngology, Südharzkrankenhaus Nordhausen, [4]Department of Radiotherapy, University Hospital Hannover, [3]AKTE, [5]Department of Radiotherapy, Zentralklinikum Suhl, [6]Department of Radiotherapy, Franziskus Hospital Bielefeld, Germany

Objective: Selenium is an essential cofactor of the enzyme glutathionperoxidase (GSH-Px), which is important for the endogenous detoxification of free radicals. A reduced activity of GSH-Px is related to increased toxicities due to radiation therapy which is integrated in the primary cancer treatment. Therefore selenium substitution may be a new supportive strategy to diminish radiation-associated side-effects.

Materials and Methods: The selenium blood concentrations of 121 radiotherapy-patients were measured in two randomized

observation studies (81 gynecological tumours, 40 head and neck tumours). Measurements (atomic absorption spectrometry) were performed in serum and whole blood (WB) samples before (begin RT), during (half RT), immediately after (end RT) and 6 weeks after radiotherapy. In case of decreased selenium levels in WB, 63 patients (mean age 63.83±9.23 yrs) received selenium substitution (500 μg sodium selenite at RT-days, 300 μg at weekend) and 64 patients (mean age 63.03±10.47 yrs) were evaluated as control group without any selenium substitution. Both groups were well balanced according to tumour localization and stage. Reference values were 85-162 μg/L WB-selenium, and 65-135 μg/L serum-selenium.

Results: The following WB selenium (Se) levels were measured (Se-group *vs*. control group, *U*-test): begin RT 64.17±13.98 μg/L *vs*. 64.50±14.47 μg/L (p=0.869); half RT 92.48±26.68 μg/L *vs*. 65.80±18.04 μg/L (p<0.001); end RT 93.78±25.90 μg/L *vs*. 64.06±17.54 μg/L (p<0.001); 6 weeks after RT 74.01±20.06 μg/L *vs*. 69.66±17.83 μg/L (p=0.183). The serum levels were as follows: begin RT 59.18±13.49 μg/L *vs*. 61.99±15.72 μg/L (p=0.427); half RT 104.75±31.41 μg/L *vs*. 62.37±16.23 μg/L (p<0.001); end RT 100.63±31.12 μg/L *vs*. 62.29±16.11 μg/L (p<0.001); 6 weeks after RT 72.73±26.53 μg/L vs.64.17±17.22 μg/L (p=0.170).

Conclusion: The used dosage of 500 μg sodium selenite per day is sufficient to treat the selenium deficiency during the radiotherapy. After the end of substitution the patient returns to his individual selenium status.

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LIMITED EFFECTS OF SELENIUM IN THE PREVENTION OF RADIATION-ASSOCIATED TOXICITIES – RESULTS OF A RANDOMIZED STUDY IN HEAD NECK CANCER PATIENTS

J. Büntzel[1], D. Riesenbeck[2], M. Glatzel[3], R. Berndt-Skorka[4], T. Riedel[5], O. Micke[3], R. Mücke[3], K. Kisters[3], K. G. Schönekaes[3], F. Bruns[6]

[1]Department of Otolaryngology, Suedharzkrankenhaus Nordhausen, [2]Department of Radiotherapy, Prosper Hospital Recklinghausen, [4]Department of Radiotherapy, Municipal Hospital Neubrandenbrug, [3]AKTE, [5]Department of Radiotherapy, Municipal Hospital Bielefeld, [6]Department of Radiotherapy, University Hospital Hannover, Germany

Objective: To study the effects of substitution of selenium in the activation of the selenium dependent enzyme glutathione peroxidase which is important for the scavenging of free radicals. Until today only limited data are available regarding the clinical impact of selenium on the toxicities due to free radical producing therapies, *e.g*. irradiation or chemotherapy.

Materials and Methods: Thirty nine patients (8 female, 31 male) with advanced head and neck cancer were included to a randomized phase II study. The mean age was 63.52±9.31 years. Tumour localizations: oral cancer 15 patients, oropharynx 19 patients, hypopharynx 5 patients and CUP 1 patient. Group A (n=22) received 500 μg sodium selenite at the days of radiotherapy and 300 μg sodium selenite at holidays or weekend. Group B (17) was irradiated without any selenium substitution. Both groups were well balanced according to age, gender, localization and stage of the tumour. The RTOG grade of radiation-associated toxicities was evaluated once per week.

Results: The following serious toxicities were observed (group A *versus* group B): dysphagia 22.7% *vs*. 35.3%, loss of taste 22.7% *vs*. 47.1%, dry mouth 22.7% *vs*. 23.5% and stomatitis 36.4% *vs*. 23.5%. A statistical trend (Fisher's exact test) is only seen in the area of loss of taste (p=0.172). The analysis per week (Student *t*-test) had shown a significant reduction of dysphagia in the selenium group at the last week of irradiation.

Conclusion: The small randomized trial has shown limited effects of selenium in the prevention of ageusia (loss of taste) and dysphagia due to radiotherapy because of head and neck cancer.

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SELENIUM IN SUPPORTIVE TREATMENT FOR GLIOBLASTOMA MULTIFORME TREATED WITH CONCOMITANT RADIOTHERAPY AND TEMOZOLOMIDE

O. Micke[1,2,7], F. Bruns[3], R. Mücke[4], J. Büntzel[5], K. Kister[6], U. Schäfer[2,7,8]

[1]Klinik für Strahlentherapie und Radioonkologie, Franziskus Hospital Bielefeld, [2]Klinik und Poliklinik für Strahlentherapie und Radioonkologie, Universitätsklinikum Münster, [3]Klinik für Strahlentherapie und spezielle Onkologie, Medizinische Hochschule Hannover, [4]Strahlentherapie Wiesbaden, [5]Klinik für Hals-, Nasen- und Ohrenheilkunde, Südharz-Krankenhaus Nordhausen, [6]Innere Medizin I, St. Anna-Hospital Herne, [7]Klinik für Strahlentherapie, Klinikum Lippe, Germany, [8]German Working Group Trace Elements and Electrolytes - AKTE

The overall prognosis of patients with resected or inoperable glioblastoma multiforme (GBM) is generally very poor. The establishment of the concomitant application of radiotherapy and temozolomide in the primary or postoperative treatment of GBM lead to a marked increase of progression-free and overall survival for the first time. One of the major toxicities compromising patients' quality of life in combined radiotherapy

and temozolomide chemotherapy is the development of radiation-associated brain edema. The aniti-edematous effect of selenium is well known from early historical studies. The results of an own exploratory study (Micke *et al*. 2003) involving 48 patients suggest that selenium has a positive effect on radiation-associated secondary lymphedema in patients with limb edemas as well as in the head and neck region, including endolaryngeal edema. The majority of patients showed a reduction in edema characteristics. It was also found that 65% of patients with interstitial grade III or IV endolaryngeal edema, who normally would require tracheotomy for treatment, could avoid surgical intervention. Based on these clinical results an exploratory study was initiated using sodium selenite with combined radiotherapy and temozolomide chemotherapy to improve the tolerability of treatment.

Methods: A total of 45 patients with newly diagnosed GBM received temozolomide (75 mg/m^2/day) concurrent with 60 Gy of conventional radiotherapy according to the EORTC study scheme. As a supportive treatment 350 µg/m^2 body surface sodium selenite were applied during the radition treatment and one week before and after radiotherpy. Number, type and grade of treatment-associated side-effects as well as episodes of symptomatic brain edema and need of corticosteroids were recorded. Patients' quality of life (QOL) was evaluated by 10 point visual analogue scale (VAS).

Results and Conclusion: Overall the treatment was well tolerated. No episodes of symptomatic brain edema were observed. In all patients the steroid doses were reduced. No steroid dependency was observed. The overall toxicity was limited to hematotoxicity, some mild episodes of nausea were observed. QOL improved in all patients during selenium treatment. These exploratory results show that selenium addition to combined radiochemotherapy with oral temozolomide is well tolerated and has high patient compliance. The data suggest that selenium may improve the QOL of patients and the tolerability of the treatment scheme. Therefore, it may offer a new perspective as a supportive agent in multimodal treatment of GBM.

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COGNITIVE DYSFUNCTION, BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND IL-6 LEVELS IN CANCER PATIENTS WITH DEPRESSION

C.F. Jehn, D. Kuehnhardt, A. Dieing, S. Lehenbauer-Dehm, P. Schmid, K. Possinger, C. B. Flath, D. Lüftner

Medizinische Klinik mit Schwerpunkt Onkologie und Hämatologie, CCM, Charité, Universitätsmedizin, Berlin, Germany

Increased pro-inflammatory cytokines (IL-6) and decreased brain-derived neurotrophic factor (BDNF) levels have been implicated in the pathophysiology of depression. Depression has been shown to be associated with cognitive dysfunction. The objective of this study was to assess the correlations between cognitive dysfunction, IL-6 and BDNF in cancer patients with depression.

Methods: Clinical depression was assessed in 55 patients with cancer according to DSM-IV criteria and by the Hospital Anxiety and Depression Scale (HADS). A HADS-D score of >11 was considered sufficient for the diagnosis of clinical depression. Cognitive function was assessed by the Auditory Verbal Learning Test (AVLT). Plasma concentrations of IL-6 and BDNF were measured at 8 AM the same day.

Results: The mean age of the 55 patients was 60 years, 77% were female and 23% male. There was no difference in age or Karnofsky-index between depression *vs*. no depression. Depression was associated with higher IL-6 levels (14.8 *vs*. 3.7 pg/mL; $p<0.01$). Long term memory was reduced in patients with depression ($p<0.01$). There was no difference in BDNF levels between both groups ($p=0.16$). There were correlations between HADS-D score and IL-6 (r=4.11; $p=0.002$), IL-6 and BDNF(r=–0.42; $p=0.001$), BDNF and long term memory (r=0.48; $p=0.02$). No correlations were found for level of depression (HADS-D score) and BDNF levels (r=–0.20; $p=0.14$).

Conclusion: In patients with advanced cancer clinical depression is associated with increased IL-6 concentration and reduced long term memory. There seems to be no association between BDNF and depression in cancer patients. However there is a positive correlation between the cognitive function, at least for long term memory, with BDNF.

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METABOLISM IN CANCER PATIENTS

J. Arends

Tumor Biology Center, Albert-Ludwigs-Universität, Freiburg, Germany

Every second cancer patient has lost weight before the disease is diagnosed and in advanced cancer 75% of patients are malnourished. Weight loss is relevant and associated with decreased well being, less tolerance for and diminished effect of cancer therapies and shortened overall survival. Anorexia and insufficient energy intake are the major direct causes of weight loss. Appetite and gastrointestinal functions are disturbed by a wide spectrum of factors. At the center, however, are typical metabolic derangements, characterized by the activation of systemic proinflammatory pathways.

Cancer cachexia thus differs fundamentally from starvation and is recognized by insulin resistance with raised insulin and glucose levels, lack of ketosis and relatively high proteolysis. Metabolic analyses in cachectic patients reveal normal energy expenditure with low glucose and high fat oxidation capacity. Nutritional therapy in cancer cachexia should respect these findings and should be coupled with anti-inflammatory concepts. Recent data suggest glucose dependence and apoptotic decay in glucose-free media for poorly differentiated cancer cells. This has initiated further studies to use metabolic interventions (*e.g.* ketogenic diet) to energetically deprive tumor tissues. Future metabolic and nutritional treatments in cancer cachexia should aim at supporting metabolic needs of host tissues while simultaneously depriving tumor cells of vital substrates.

Lung

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IMPROVING TREATMENT OF ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC)

M. Reck

Department of Thoracic Oncology, Großhansdorf, Germany

After introduction of new third generation regimens in treatment of advanced NSCLC, first line therapy has reached a plateau with a realistic median survival of 7.4-11.3 months and a 1-year survival rate of 31%-41%. Several approaches to improve first-line treatment by modification of the schedules or addition of further agents have failed to show efficacy. Four phase III studies including more than 4,000 patients showed no benefit in survival or progression free survival by adding an EGFR-Tyrosinkinase inhibitor (EFGFR-TKI) to standard chemotherapy. However, recently one randomized phase III trial showed a significant increase in survival by adding the EGFR-antibody cetuximab to cisplatinum based chemotherapy (FLEX trial). Furthermore the combination of the anti-VEGF antibody bevacizumab to platinum based chemotherapy also significantly increased efficacy of treatment in randomized phase III trials in patients with non-squamous NSCLC (ECOG4599, AVAIL). Another approach showed a potential correlation between histological subtypes and improved efficacy of selected drugs. Pemetrexed in combination with cisplatin was superior to gemcitabine and cisplatin in patients with non-squamous cell histology, probably related to differential expression profiles of thymidilate synthetase (TS) (Scagliotti, J Clin Oncol 2008). In addition there are lot of clinical data which show an increase in activity by using new agents in a sequential way. By introduction and approval of innovative drugs like pemetrexed or molecular agents like erlotinib and gefitinib second and even third line therapy becomes an ever more important therapeutic strategy in the treatment of NSCLC. Facing the growing spectrum of therapeutic options one might predict that a sequential therapy might be an important approach for using the maximal therapeutic potential of each drug. The question of the optimal point in time for starting a new therapy is not yet defined. Further studies for optimizing the therapy are on the way but up to now there are a lot of valid data which demonstrate that an effective treatment of NSCLC incorporates more than only a first-line therapy. Future trials will be designed to define the optimal sequence and to identify potential predictive factors for the selection of patients who might benefit best from a specific therapy.

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THE IMPORTANCE OF MONOTOTAL FOR PRIMARY DIAGNOSIS OF PATIENTS WITH NON-SMALL CELL LUNG CANCER (NSCLC)

O. Topolcan, L. Holubec, J. Safranek, M. Prazakova, J. Vrzalova, M. Casova, S. Svobodova, J. Dresslerova, Vladislav Treska, M. Pesek, J. Finek

Faculty Hospital and Medical School in Pilsen, Charles University, Prague, Czech Republic

The aim of the study was to evaluate the clinical importance of MonoTotal for diagnosis and follow-up of non-small cell lung cancer (NSCLC) in comparison with other cytokeratins conventionally used as tumor markers.

Methods: MonoTotal assay was measured using IRMA technology in the serum of 120 patients with NSCLC stages Ia-IIIb. As a control group, the sera of 80 people of corresponding age, with no history or evidence of cancer or lung disease, were used. The results were compared with other soluble cytokeratin fragments conventionally used in the diagnosis and management of NSCLC: TPA, TPS and CYFRA 21.1 (TRACE method).

Results: Statistically significant differences were found between the control group and patients with NSCLC in all cytokeratins: TPA ($p<0.0001$), TPS ($p<0.0002$), CYFRA 21.1 ($p<0.0001$) and MonoTotal ($p<0.0001$). Similar significant differences were found between particular histological types (adenocarcinoma or squamous cell carcinoma). Sensitivity during the pre-operative period, at 95% specificity for cytokeratins, was as follows: MonoTotal 71%, TPA 53%, CYFRA 21.1 51% and TPS 25%. In particular subgroups, higher sensitivities were noticed in

patients with squamous cell carcinoma: MonoTotal 76%, TPA 63%, CYFRA 21.1 65% and TPS 27%. Sensitivities were significantly lower in the subgroup of patients with adenocarcinoma.

Conclusion: MonoTotal is a more sensitive tumor marker for NSCLC than the cytokeratin markers currently used in routine clinical practice, especially in patients with squamous cell carcinoma. MonoTotal is also important for prognosis (disease free interval and overall survival). This study was supported by the grant IGA MZCR 9343-3 and research project VZ MSM 0021620819.

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THE TMI ALGORITHM IMPROVES THE DIAGNOSTIC AND PROGNOSTIC VALUE OF TUMOR MARKERS IN NSCLC AND IS OF PROGNOSTIC SIGNIFICANCE IN SCLC PATIENTS

T. Muley, C. Traschütz, H. Hoffmann, H. Dienemann, F. J. F. Herth, M. Meister

Thoraxklinik am Universitätsklinikum Heidelberg, Germany

Tumor markers in lung cancer such as CEA, CYFRA 21-1 and NSE have been shown to provide prognostic information but are only of limited diagnostic value. Combinations of at least 2 markers might considerably improve both the prognostic and the diagnostic value of the markers. An algorithm has recently been successfully introduced, combining CEA and CYFRA 21-1 for the new parameter TMI (tumor marker index) for risk evaluation in early stage NSCLC [TMI=Square root of (marker1/diagnostic cut-off marker1 × marker2/diagnostic cut-off marker2)]. In a prospective study of 114 p-stage I patients, TMI (cut-off 0.54/cut-off 0.58) proved to be a significant predictor of recurrence (HR 7.4 and 9.9, respectively) even after a short median follow-up period of only 8 months (p=0.026/p=0.007). In a cohort of 328 NSCLC and 151 benign lung diseases the discriminatory power of TMI(CEA/CYFRA) to differentiate NSCLC from benign diseases was shown to be superior compared to the individual markers in ROC analysis (AUCs: TMI 0.82; CEA: 0.74; CYFRA 21-1: 0.77). In an adopted formula based on NSE and CEA or NSE and CYFRA 21-1 corresponding TMIs were tested for their prognostic significance in 52 operated SCLC patients. In univariate analysis a combination of both TMIs was able to detect patients with an increased risk of death by a factor of 2.9 (p=0.031) if one TMI was increased over cut-off and by a factor of 6.6 (p=0.018) if both TMIs were elevated. Combining individual tumor markers by the TMI algorithm proved to be a useful tool for prognostic evaluation in NSCLC and SCLC. In addition the diagnostic value of TMI was shown to be superior to that of the individual markers at least in NSCLC.

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DIAGNOSTIC AND PROGNOSTIC VALUE OF MESOTHELIN IN PATIENTS WITH MALIGNANT PLEURAL MESOTHELIOMA IN COMPARISON TO BENIGN ASBESTOSIS AND LUNG CANCER

T. Muley[1], H. Hoffmann[3], H. Dienemann[3], F. Herth[4], M. Meister[2], J. Schneider[1]

[1]Translational Research Unit, [2]Department of Surgery and [3]Department of Pneumology and Respiratory Medicine, Thoraxklinik, University of Heidelberg; [4]Institute and Policlinic for Occupational and Social Medicine, Justus-Liebig University, Giessen, Germany

The diagnostic and prognostic value of soluble mesothelin-related proteins (SMRP) in sera from different patient groups were investigated.

Patients and Methods: SMRP was measured in patients with newly diagnosed malignant pleural mesothelioma MPM (n=100), MPM patients at tumor relapse (n=29), primary lung cancer (LC) (n=139) and benign asbestosis (n=75) using Mesomark™ –ELISA kit.

Results: SMRP concentrations were significantly higher in MPM compared to benign asbestosis (p<0.001) or LC (p<0.001). The median values were 1.4 nM, 0.9 nM and 0.8 nM, respectively. The best statistical cut-off was found to be 1.35 nM resulting in a sensitivity of 53% and a specificity of 82.7%. Receiver operator characteristic (ROC) curves gave an area under curve (AUC) of 0.72 for the discrimination between MPM and non-MPM patients (p<0.001). No significant differences in SMRP levels were found among histologies and stages of MPM. The highest median SMRP levels (4.2 nM) were measured in 29 MPM patients with relapse/progression (75.8% >1.35 nM). Univariately, SMRP discriminated significantly (p<0.003) between favorable (n=71, median survival (MS): 17.1 months; 1-yr-S: 63.1%) and worse prognosis (n=20, MS: 8.4 months, 1-yr-S: 32%) at 3.5 nM. In multivariate analysis histology, therapy and SMRP were shown to be independent prognostic factors in all MPM patients (hazard ratio for SMRP: 1.96; p=0.025). However, sub-type driven re-analysis showed only a trend in epithelial MPM.

Conclusion: SMRP add limited information to the diagnosis of MPM. However, SMRP might be a useful measure in treatment monitoring of MPM. The prognostic impact of SMRP in MPM is not conclusive and needs further evaluation.

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MENTAL NEUROPATHY (NUMB CHIN SYNDROME) AS THE FIRST SYMPTOM IN METASTATIC MEDIASTINAL DISEASE

R.E. Friedrich

Oral and Maxillofacial Surgery, University Hospital, Hamburg, Germany

Neuropathy of the mental nerve, also referred to as numb chin syndrome, is a rare finding that demands accurate differential diagnosis. The finding of a numb chin in a patient with no history of trauma or other obvious causes of nerve damage (*e.g.* oral cancer), is more often associated with cancer either as the first symptom or during the course of the disease, than with benign conditions (*e.g.* multiple sclerosis). This is the case report of a 69 year-old male who, 4 weeks prior to admission to hospital, experienced a progredient hypesthesia of the right side of the corner of the mouth and chin, associated with intermittent phases of pain. On admission the patient located the pain at the mental foramen with centripedal projection along the nerve route inside the mandible. A computed tomogram of the brain had excluded cerebral lesions and a plain chest radiograph had demonstrated age-dependent findings but no space occupying lesions. The patient was teethless and the pain could have been triggered by his prosthesis. An orthopantomogram revealed the mental foramen on the top of the mandible and a symmetrically depicted mandibular canal. A revision of the nerve was decided in order to displace the nerve from direct chewing pressure. However, after osteotomy and exposing the inferior alveolar nerve in the mandibular canal, a greyish mass invading the nerve and running inside the tube was detected. Histological investigation of the biopsies revealed a small cell carcinoma, probably of bronchial origin (sporadically EMA-positive). A computed tomogram of the chest revealed an extensive mediastinal tumor (7.6×6.2×8 cm^3, multiple retrosternal, pretracheal and subcarinal lymph nodes) with further metastases to the kidney and liver. Clinical diagnosis was small cell bronchial carcinoma (extensive disease, stage grouping II B, Marburg classification). Palliative chemotherpy was non-effective and the patient deceased with evidence of tumor progression.

Conclusion: Numb chin syndrome is rare and not well-appreciated as a serious finding in the field of oncology. Indeed, dysesthesia of the mental nerve is frequently associated with obvious dental findings (poor retention of dental prosthesis, diseases of the dental apices affecting the inferior alveolar nerve) or in certain cases the history of trauma passes the differential diagnosis in the accurate direction. This report demonstrates that the cause of a numb chin needs to be clarified, has to be taken seriously and requires thorough diagnostics, including the surgical revision of the affected nerve.

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CEA, CYFRA 21-1, NSE AND PROGRP IN THE DIAGNOSIS OF LUNG CANCER – A MULTIVARIATE APPROACH

C. Gruber[1], R. Hatz[2], D. Nagel[1], J. Reinmiedl[3], P. Stieber[1]

[1]Institute of Clinical Chemistry, [2]Department of Surgery, University, Campus Großhadern, München, [3]Asklepios Lungenfachkliniken, Gauting, Germany

We studied retrospectively the single and combined diagnostic value of CEA, CYFRA 21-1, NSE and ProGRP, which were routinely analysed in patients with lung tumors of unkown origin at the date of hospitalisation.

Methods: The embedding criteria were the determination of CEA (AxSYM / Abbott), CYFRA 21-1 (ElecSys / Roche) and NSE (Kryptor / Brahms) for each patient. We examined 1747 patients, whereof 1325 suffered from lung cancer (=LC, small cell lung cancer SCLC: n=194, non small cell lung cancer NSCLC: n=1015, others: n=116), 318 from benign lung diseases and 104 from lung metastases due to another primary. As ProGRP (ELISA ALSI/IBL) became routine just some years ago this marker was evaluated in less patients.

Results: 99.8% of LC patients released at least one of the 4 biomarkers (>median of healthy controls) and for the discrimination between benign disease (BD) and malignant lung disease each marker reached 100% tumor specificity at high value levels (CEA: 20 ng/ml; CYFRA 21-1: 40 ng/ml; NSE: 45 ng/ml; ProGRP: 250 pg/ml). At a specificity of >99%, ProGRP reached the highest diagnostic efficacy for SCLC with 57% true positive results, CEA had the highest capacity (17%) to detect malignant lung tumors in general and adenocarcinomas of the lung (29%). CYFRA 21-1 was dominant for squamous cell carcinomas (12%). Combination of the 4 markers leads under the postulation of this high specificity of >99% to 50% true positives for malignant lung tumors, 44% for NSCLC, 36% for squamous cell carcinomas, 53% for adenocarcinomas and 78% for SCLC.

Conclusion: In cases of lung tumors of unknown origin the combined use of CEA, CYFRA 21-1, NSE and ProGRP is useful for the differentiation between benign and primary or secondary malignant disease and even allows the assignment to histological subtypes.

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LUNG CANCER BIOMARKERS: PAST, PRESENCE AND FUTURE

Petra Stieber

Institute of Clinical Chemistry, University of Munich–Campus Großhadern, Germany

Since decades it has been known that malignant lung tumours, also in early stage of disease, lead frequently to a significantly increased release or reduction of various blood components. Thus, already more than 50 years ago, common laboratory parameters like hemoglobin, platelets, urea, albumin and LDH have served mostly as prognostic factors for this disease. Carcinoembryonic antigen CEA became available more than 40 years ago and was recognized to be frequently released in lung cancer, especially in adenocarcinomas of the lung. The squamous cell carcinoma antigen (SCCA) as a quite specific marker for squamous cell carcinomas, but with a relatively low frequency of increased release, became available in the early eighties, almost at the same time when neuron specific enolase (NSE) as a frequently released marker by small cell lung cancers could be measured in serum. The combination of these 3 markers served for several years as tools for diagnosis or follow up care of lung cancer patients. The availability of CYFRA 21-1 (cytokeratin 19-fragment) in the early 90ties improved the diagnostic situation of lung tumors clearly as this protein is abundant in the lung and is released by any kind of lung cancer with a high probability. At the same period of time when CYFRA 21-1 came up, ROC curves were established for the first time in diagnostic oncology as this was the first means to be able to compare various markers under the same, fair conditions and irrespective of cut-off drawbacks. In addition to the diagnostic or differential diagnostic capacity of CYFRA 21-1 in lung tumors this protein was described repeatedly to be of independent prognostic relevance in non small cell lung cancer. Moreover, CYFRA 21-1 combined with nucleosomes, as cell death products, revealed to be able to judge upon systemic treatment efficacy or failure after the first cycle of chemotherapy. Since the early 90ties Pro Gastrin Releasing Peptide (ProGRP) was established in Japan but became available in Europe only 10 years ago. Due to its high specificity for small cell lung cancer and stage-independent high sensitivity for this disease this biomarker improved significantly the situation of diagnosis of lung cancer in blood. From a diagnostic point of view ProGRP would be suitable as a screening test for small cell lung cancer. If we succeed within the near future either to detect a new biomarker specific for non small cell lung cancer *via* genomics or proteomics approaches or – what is much more probable – if we succeed to combine the right markers in the right way leading to a high diagnostic capacity of the panel to detect non small cell lung cancer early, then we could start with large scale screening studies combining ProGRP with this panel of markers for NSCLC. Moreover, it is likely that we will be able to measure in blood predictive factors for treatment selection as well as predictive factors for the individual cardiovascular risk and thus for treatment selection and that we will be able to judge early and specifically about treatment success or failure.

Quality Control

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CENTRE INDEPENDENT DETECTION OF MALIGNANCY BY MEANS OF CLASSIFICATION WITH ROC-BASED DATA TRANSFORMATION

N. Bitterlich[1], Th. Muley[2], J. Schneider[3]

[1]Medizin and Service GmbH Chemnitz, [2]Thoraxklinik am Universitätsklinikum, Heidelberg, [3]Institut und Poliklinik für Arbeits- und Sozialmedizin, Universitätskliniken Gießen/Marburg, Germany

The detection of malignancy by means of tumour marker profile analysis is a current challenge. A fuzzy-based analysis approach leads to an increase of sensitivity and a high specificity for lung cancer diseases. However, the handling of measurement values stemming from different centres requires the normalisation of data to allow jointly analyse comparable marker profiles. Measurement results are influenced by laboratory methods and structure of study populations. Different cut-off values preclude a direct comparison of data from various centres. Receiver operating characteristic (ROC) curves describe the data structure in more detail as they are independent from cut-off values. Any correlations may be analysed using the so-called decision guaranty of a measurement value. The goal of this examination is to create a centre-comprehensive computer-based decision support for the detection of lung cancer diseases.

Methods: A total of 771 data sets from 287 patients with benign diseases and 484 patients with non-small cells lung carcinomas of all stages were provided by the centres Heidelberg (HD; 160/326) and Gießen (GI; 127/158), respectively. Analyses for CEA, CYFRA 21-1 and NSE were performed. The HD data sets were mostly derived (more than 50%) from operated lung cancer patients (stages I to IIIA), while GI data sets stemmed from patients of a occupational medicine high-risk population. Also with regard to clinical stage classification populations were not comparable (GI data with only about 35% of the stage I to IIIA). Also the cut-off values at 95% specificity were different. A fuzzy-based pattern classification developed for the GI population is applied to the HD data sets.

Results: Comparison of the centres demonstrates as expected differences in the sensitivity for each marker due to of the different tumour stages. CYFRA is the most sensitive single marker (sensitivity HD: 35%, GI: 65% at 95% specificity). For CEA there are smaller differences in sensitivity (HD: 35%; GI 43%). The single marker NSE has only in GI a small additionally classification ability (32%). However it does not play a practicable role in the HD data sets (10%). By combination of the most sensitive single marker CEA and CYFRA using a fuzzy-based classification algorithm the sensitivities at 95% specificity increase by about 10% in both centres independently. In GI also the combinations CEA/NSE and CYFRA/NSE result in an increase in sensitivity. But in HD this approach does not convey a benefit using the HD data sets. The diagnostic performance (sensitivity and specificity) do not change, if the classification algorithm is based on transformed data, such as decision guaranty values, rather than on the original measurement data. Applying the classification algorithm optimised for the GI data to the HD data in impacts the achievable diagnostic performance, due to the different of the different application situation. Based on transformed data, the structural differences can be largely eliminated. The rate of data sets with different estimation of malignancy by comparison the HD specific algorithm and the from GI transformed one decreased to less than 5%.

Conclusion: This example demonstrates the possibility to transfer classification algorithms, if these are based on cut-off -independent procedures. A ROC-curve-based evaluation of malignancy can eliminate both, differences between laboratory methodologies, as well as structural differences between patient populations of centres. In this way classification algorithms can lead to better diagnoses of lung cancer independent of the individual condition of development.

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CONSEQUENCES FOR THE JUDGMENT OF TUMOR MARKER TIME COURSES IN FOLLOW-UP DUE TO POOR BETWEEN-TEST COMPARABILITY

M. Zwirner, K. Korte, N. Bewarder

Department of Obstetrics and Gynecology, University of Tuebingen, BIOREF GmbH, Mömbris, Germany

One of the most confusing aspects for untrained clinicians and a still unsolved problem is the poor between-test comparability of results when the same tumor marker is measured with different test methods. Such deviations can be of relevance interpreting a markers course in follow-up.

Materials and Methods: Data from current proficiency studies based on BIOREF reference materials including CA125 and CA153 as biomarkers as well as data derived from external quality assessment (EQA) studies from the German society of laboratory medicine were investigated for between-test (-method) deviations. The proficiency studies with BIOREF material are based on monoanalyte reference materials which are comparable to patient serum, whereas the EQA-material is a non-physiological serum cocktail with spiked analytes inside. Comparing the deviation of method means, maximum deviation of means ranging from 18% to 38% were found for several CA125 tests depending on the concentration range in the BIOREF control material. The maximum deviation of method means for the same biomarker in the EQA scheme however was 214% and 225% respectively.

Conclusion: A clinically relevant consequence will arise if a change in test systems is necessary. This can result in a drift of an individual patient's baseline. Here a stable and a long since available reference material such as that from BIOREF can help for better interpretation. A second effect will be exhibited in a longitudinal marker course time. By mixing results from different tests one can simulate all of the possible marker courses such as progressive disease or remission as well intermitting course. Keeping in mind that a 100% marker increase can indicate a progression with high probability a switch of test methods should be well considered. In consequence, further effort should be made to improve the comparability of different test methods which detect the same biomarker.

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TUMOR BIOLOGICAL ACTIVITY ASSESSMENT

O. Topolcan

Faculty Hospital and Charles University, Prague, Medical Faculty Plzen, Czech Republic

The current approach to tumor marker assessment for primary diagnosis, follow-up and therapy monitoring involves the ROC of the individual markers from the multicenter study results. This study proposes the opposite approach: to assess the optimal combination of tumor markers based on the clinical requirements.

Results: Here, the optimal combination of tumor biological activity parameters for the most frequent cancer diseases is proposed based on the analysis of retrospective and prospective monitoring of tumor markers and growth factors. These recommended guidelines, including optimal laboratory methods, are based on 30,000 laboratory tests of biological activity parameters performed over a period of 5 years.

Conclusion: A combination of at least 4 parameters seems to be the most optimal: traditional tumor markers, cytokeratine fragments, markers of proliferation and growth factor. The choice of combination of optimal parameters differs according to the histology type of tumor and the clinical purpose of the diagnoses (primary diagnostics, early detection of disease progression during follow-up, therapy monitoring, *etc*.) From a laboratory point of view, methods of multiplex analysis seem to be optimal. *This study was supported by research project VZ MSM 0021620819*.

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25 JAHRE TUMORMARKER (TM) CA19-9/CA 125 – VOM RADIOIMMUNO-ESSAY (RIA) ZUR MODERNEN VOLLAUTOMATEN AUS DER SICHT DER LABORANTIN

M. Bahlo

Zentrum für klinische und experimentelle Tumormarker Diagnostik und Therapie (ZeTDT GmbH), Hamburg, Germany

Vor nunmehr 25 Jahren konnten die Tumormarker CA 19-9 und CA 125 als durch monoklonale Antikörper definierte tumorassoziierte Antigene in die klinische Diagnostik eingeführt werden.

Durch die Entwicklung vom ersten manuellen CA 19-9 RIA von Centocor über den manuellen Enzymimmunoassay bis hin zum Vollautomaten mit automatischer Verdünnung und Barcode-Lesen (z. B. TRACE-Technik) ist die Bestimmung der Tumormarker zuverlässig und schnell geworden (z. B. Volumenerkennung, kurze Inkubationszeiten etc.). Anfängliche Probleme, wie Chargenunterschiede, HAMA-Interferenzen und High-Dose-Hook-Effekte sind inzwischen durch moderne Bestimmungsverfahren, wie z. B. EKLIA, MEIA, IFA äußerst selten geworden.

Bei einem Methodenwechsel einer Tumormarkerbestimmung hat sich eine Validierung mit 21 Seren unterschiedlicher Höhe bewährt. Bei Tumormarkerverläufen sollte im Falle eines Methodenwechsels mindestens ein Tumormarker-Wert gleichzeitig mit alter und neuer Methode bestimmt werden, um eine falsche Aussage zu vermeiden. Es hat sich gezeigt, dass zur laboreigenen Qualitätskontrolle zusätzlich zu den Kit-Kontrollen mindestens eine laboreigene Serumkontrolle mitbestimmt werden sollte. Die Teilnahme an externen Ringversuchen (4x im Jahr) sichert die Qualität des Labors.

Durch engen Kontakt von Labor, Arzt und Hersteller konnten unplausible Tumormarker-Ergebnisse schnell aufgedeckt und erkennbare Probleme gelöst werden. Der Aufbau einer Serumbank (–30˚ bis - 80˚C) ermöglicht es, auch ältere Tumormarker-Ergebnisse jederzeit zu überprüfen. Weiterhin hatten Arzt und Labor lernen müssen, auf Abnahmemodalitäten (Serum oder Plasma), richtigen Transport (evtl. gekühlt) und genaue Beschriftung/ Barcodierung zu achten. Bewährt hat sich auch, die Ergebnisse im Rahmen der Verlaufskontrolle, nach einer Operation oder unter einer Tumortherapie, zusätzlich zu aktuellen Befundübermittlung in einer Verlaufskurve aufzuzeigen und zusätzlich die jeweilige Bestimmungsmethode anzugeben.

Angesichts dieser Entwicklung und der engen Zusammenarbeit von Arzt, Labor und Hersteller konnten sich die beiden Tumormarker in den letzten 25 Jahren insbesondere im Rahmen der Verlaufsdiagnostik und Therapiekontrolle einen festen Platz in der Klinik erwerben.

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USE OF TUMOR MARKERS IN EUROPE AND OVERSEAS: CONFORMITIES AND CONTROVERSIES

M.J. Duffy

Department of Pathology and Laboratory Medicine, St Vincent's University Hospital, Dublin, Ireland

The most widely used tumor markers include PSA for prostate cancer, CEA for colorectal cancer (CRC), AFP, HCG and LDH for germ cell tumors, HCG for trophoplastic tumors, CA 125 for ovarian cancer, CA 19-9 for pancreatic cancer, AFP for hepatocellulat cancer, CA 15-3/CEA, ER, PR and HER2 for breast cancer. For some of these markers, universal agreement exists as to their clinical application. Markers for which universal agreement exists include the use of AFP, HCG and LDH in determining prognosis in patients with advanced germ cell tumors, AFP and HCG in surveillance and monitoring therapy in patients with germ cell tumors, CEA as part of a postoperative surveillance programme in patients with diagnosed CRC, CA 125 in monitoring therapy in patients with ovarian cancer, HCG in the follow-up of patients with trophoblastic disease, ER and PR in selecting for endocrine sensitivity in patients with breast cancer and HER2 for identification of patients with breast cancer who are likely to benefit from Herceptin. Consequently, all the above markers are recommended by European and US expert panels such as the European Group on Tumor Markers (EGTM), the National Academy of Clinical Biochemistry (NACB), the European Society of Medical Oncology (ESMO) and the National Comprehensive Cancer Network (NCCN).

In contrast to agreement on the use of the above markers, disagreement exits as to the value of PSA in screening for prostate cancer, CA 15-3 and CEA in surveillance following a diagnosis of breast cancer and CA 125 in post-therapy

follow-up in patients with ovarian cancer. The primary reasons for disagreement in these situations is the lack of validation in a high-powered or level I evidence study such as a prospective randomized clinical trial or meta/pooled analysis of existing studies. Ideally, therefore, in order to have wide acceptance for a tumor marker, its clinical utility should be confirmed in a level 1 evidence study.

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QUALITY MANAGEMENT AND INFLUENCE ON BIOMARKERS IN LABORATORY PRACTICE

U. Hasholzner, W. Schultis

Synlab München, Germany

Laboratory data influence many decisions in medicine while being influenced by a lot of preanalytical tools which can vary in clinic and medical practice. If laboratory data are not of the quality needed for decision, the decision may be incorrect resulting in risk for human health or causing unnecessary costs and activities. (b/c) Good laboratory practice comprehends internal and external quality control, (*e.g.* RiLiBÄK=Richtlinien der Bundesärztekammer), validation of methods concerning analytical performance as well as clinical aspects and changing methods, and needs to consider many variables in the preanalytical, analytical and postanalytical process. A quality management system includes working with standardized protocols and a well-trained staff and continuous improvement. All materials, reagents, technical tools, hard- and software are adequate for the specific purpose. Monitoring as well as supervising may be internal and external. In general, clinical laboratory data are determined in the same house or area while external laboratories may be located for away. Therefore logistics have to fulfil more criteria, concerning preanalytical preparation, temperature and transport. The consequent use of a quality management systems as well as observing the wide spread criteria of all quality controls results in excellent data of biomarkers in laboratory practice.

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QUALITY CONTROL OF TUMOUR MARKERS

C.M. Sturgeon

Royal Infirmary of Edinburgh, England

Tumor marker guidelines developed by the National Academy of Clinical Biochemistry (NACB) (1) in the United States and the European Group for Tumor Markers (EGTM) (2) highlight quality requirements of particular relevance to the clinical laboratory. Establishing whether such guidelines are followed in routine practice is difficult, but some indication can be obtained through carefully designed local and national audit projects. External quality assessment (EQA) schemes, while focusing primarily on analytical aspects of tumor marker measurement, also provide a unique means of assessing practice and confirming trends. In the pre-analytical phase it is essential to ensure that the appropriate tumor marker test is requested, that specimen type and timing is suitable, and that clinicians are aware of possible effects of treatment or other conditions on the interpretation of results. PSA for example may be increased in men with urinary tract infections and prostatitis and its measurement should be postponed until the completion of successful treatment. In the analytical phase methods used should be well validated and their performance carefully monitored. Implementation of rigorous internal quality control (IQC) procedures and participation in well-designed EQA schemes should enable objective assessment of whether methods are performing according to specification. NACB and EGTM recommendations for IQC and EQA are applicable to most analytes, with several factors especially relevant to tumor markers. Excellent precision and reproducibility (intra-assay variability <5%; inter-assay variability <10%) are essential, especially at concentrations close to critical clinical decision points, *e.g.* when using PSA to select asymptomatic patients for biopsy. Long-term assay stability, which can readily be assessed by EQA schemes, should also be demonstrated. Data from EQA schemes confirm that there are still significant between-method differences in tumor marker results, with coefficients of variation >20% for some analytes. Poor calibration and differences in the specificity of antibodies used, as well as differences in method design, contribute to this variation. A number of international initiatives to address these issues are in progress, but for the present, considerable care must be taken when changing tumor marker methods or when interpreting cumulated results for patients who have been monitored using different methods. Differences in method design also influence method robustness to clinically relevant interferences, including the high dose hook effect and interference from heterophilic or human anti-mouse antibodies. Awareness of the potential for such interference is highly desirable, with active dialogue between laboratory and clinical staff facilitating early identification of erroneous results. In the post-analytical phase, tumor marker reports should include fully cumulated results, an appropriate reference interval and the assay method used, together with an indication of whether any change in marker level is significant. Laboratories should also regularly audit the tumor marker service provided. Occasional surveys undertaken through EQA schemes can complement such local audits and help to identify priorities for improvement.

Imaging Methods/PET/TM

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CLINICAL RELATIONS BETWEEN FDG-PET, CT AND TUMOR MARKERS IN LUNG CANCER

K. Kley, P. Oehr

Department of Nuclear Medicine, Bonn University, Germany

The aim of the study was to find out whether there is a synergy between PET, cumputed tompgraphy (CT) and tumor markers.

Methods and Results: Two different groups of lung cancer patients were studied. The first study (n=14) showed a positive- and a negative predictive value (PPV and NPV) of 81% and 80%, respectively, for FDG-PET, compared to 73.7% and 71.4% for CT. Both imaging procedures combined had 100% sensivity and specificity, and the localisation of PET-positive lymph nodes was improved by anatomic information based on CT. The second study investigated the relation between FDG-PET, CT and the tumor markers CEA, TPA, NSE and SCC in 28 patients. Both FDG-PET and CEA were positive in 43% of cases. In all other cases, FDG-PET was true positive and CEA false negative. For TPA the results were similar with 48% both positives and 52% only FDG-PET positive. Tumor markers were sometimes of advantage to CT detecting tumor recurrence much earlier, however, PET as functional diagnostics showed better sensitivity and overall results.

Conclusion: The results give evidence that combined FDG-PET/CT is superior to single FDG-PET or single CT investigations, and also superior to *in vitro* diagnostics with tumor markers. This does not exclude tumor markers from diagnostics of lung cancer at all, especially for longitudinal follow-up including short interval diagnostic check-up. A tumor marker determination *in vitro* is manyfold cheaper than a PET-CT, and the latter includes a high radiation dose of up to 25 mSv. According to these results a decrease in the conventional cut-off values of *in vitro* tumor markers is proposed in order to achieve a high sensitivity at cost of specificity, and to take advantage of the specificity of *in vivo* PET/CT in case of suspected tumor marker elevation.

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EFFECTS OF CHEMO- AND/OR RADIOTHERAPY IN COLON CANCER CELL CULTURES INVESTIGATED BY F-18 FDG AND CC-PET IMAGING, AND CLINICAL IMPLICATIONS

P. Oehr, B. Eickmeier, K. Kley, K. Bouchelouche

Department of Nuclear Medicine, Bonn University, Germany, Department of Nuclear Medicine, Odense University Hospital, Denmark

The aim of this study is to investigate the effects of irradiation and/or chemotherapy in colon tumor cells as a tool to understand the biological base and mechanisms of modulation of F-18 FDG accumulation in colon cancer cells.

Methods: Cell-lines were cultured as monolayers in plastic Petri dishes. Assays were made by incubation of the monolayer cultures with 7-50 µCi of F-18 FDG in phosphate buffered saline (PBS)-buffer pH 7.2. Cell bound F-18-activity was counted by 10 minute measurement of the photon emission in the gantry of a PET-scanner. The activity of each Petri dish was marked as a region of interest and evaluated as MBq/cm^3.

Results and Conclusion: Twenty four hours after irradiation with 30 Gy, there was a four-fold increased uptake of F-18 FDG per cell, which remained for at least 2 days. The activity of hexokinase per total cell protein increased from level 1 to 1.05 at 48 hours after irradiation. After either addition of dactynomycin, fluorouracil, or both at the time of irradiation, there was an inhibition of the irradiation effects for F-18 FDG uptake 24 hours later of approximately 50%, 75% and 90%, respectively, which even increased after 48 hours to more than 90% in all cases. Because irradiation and chemotherapy could lead to false positive or false negative results, depending on either irradiation or drug effects, or combined effects, it seems necessary to determine the point of time at which FDG-PET can be started after treatment.

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CHOLINE AS A PET TRACER FOR DETECTION OF MALIGNANCY IN PROSTATE CANCER

P. Oehr

Department of Nuclear Medicine, Bonn University, Germany

Contrary to FDG, choline is not as highly accumulated in the urinary tract, this advantage making it interesting for prostate cancer imaging. The goal is the intracellular accumulation of F-18 or C-11 labeled choline resulting from the choline transporter and phosphorylation by CK, which serves as the basis for assessing choline metabolism with PET imaging. Their uptake may also reflect a component of tissue perfusion. One shortcoming of radiological imaging modalities lies in the restricted ability to differentiate between benign and malignant changes. The use of choline PET dual phase imaging or optimized SUVmax can be useful for the differentiation between malignant and benign disease in prostate cancer. Choline PET combined to PET-CT and MRT

may improve the diagnostic safety for intra- and extra-prostatic diagnostics of primary prostate cancer in a multimodal approach, giving additional information regarding the localisation of main primary prostate cancer lesions. Localisation of prostate cancer in selected patients with increased risk of having the disease in view of having persistently elevated levels of PSA and negative prostate needle biopsy could become a target for choline PET. Search for positive choline PET-imaging should avoid prior biopsy unless there is a time gap of more than 2 weeks. Use of multimodal choline PET including PET-CT and MRT based diagnostics within one diagnostic procedure can be recommended to improve diagnostic security. Choline PET is not indicated for initial N-staging (but better than TRUS), and is not as useful in searching for occult lymph node metastases in clinically localized prostate cancer as radioactive sentinel guided pelvic lymph node dissection for the detection of small lymph node metastases. Choline PET-CT seems to become a superior indication for M-staging of bone metastases. It can be expected that this approach results in higher sensitivity and specificity than a bone scan, but caution should be taken in patients who are under hormone therapy to avoid false negative results. Last but not least, choline PET is an indication for detection of tumor recurrence in prostate cancer, even with PSA values below 5 ng/mL. On the basis of data already known for detection of recurrence, the indication of choline PET-CT for preoperative (re)staging advanced transitional cell carcinoma is supported as well.

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PET/CT AND TUMOR MARKERS FOR PRIMARY DIAGNOSTICS, THERAPY MONITORING AND FOLLOW-UP OF PATIENTS WITH VARIOUS MALIGNANCIES

M. Prazakova, Z. Novotny, O. Topolcan, L. Holubec, J. Vrzalova, P. Mrazkova, M. Casova, J. Finek, J. Zahlava

Faculty Hospital and Medical School in Pilsen, Charles University, Prague, Czech Republic

PET is the most dynamically developing imaging method in the entire field of medicine. One of the best indications for PET is any dynamic growth of tumour markers in patients with cancer disease. The aim of this study was to compare the accuracy of modern imaging methods (USG, CT, PET/CT) and conventionally used tumor markers for primary diagnostics, therapy monitoring and follow-up of patients with various malignancies, such as lung, colorectal and breast cancer and gynaecological malignancies.

Methods: The evidence of the roles of PET and PET/CT in different cancer diseases has been reviewed on the basis of literature data and experience from routine clinical practice. The authors also present, *via* own results and case reports, the use of PET/CT and tumor markers for detection of locoregional and distant metastases and evaluation of therapy response during follow-up.

Results: Diagnostic accuracy of imaging methods with tumor markers assessment was compared in case of patients with diagnosed and treated cancer diseases. For example in patients with ovarial cancer optimal correlation was achieved between the levels of CA 125 and PET/CT examination. The sensitivity of CA 125 at 95% of specificity for the diagnosis of relapse of patients with ovarian cancer was 80%. From imaging methods the best sensitivity (SN) and specificity (SP) was achieved by PET/CT (SP 100%, SN 90%). Sensitivities and specificities of other imaging methods were significantly lower (for example CT scan: SP 75%, SN 71%).

Conclusion: A combination of modern imaging methods (PET/CT scan) with recommended tumour markers allows for prompt changes to the chemotherapeutic schedule and also it enables early detection of tumor progression during follow-up. PET/CT is more accurate than conventional imaging for restaging many cancers after treatment. *This study was supported by the MSM 0021620819 Research Project.*

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TUMOR MARKER KINETICS AND SENSITIVE MEDICAL IMAGING: EFFECTIVE DIAGNOSTIC TOOLS FOR EARLY DETECTION OF ASYMPTOMATIC TUMOR RECURRENCE IN BREAST CANCER

P. Stieber[1], G. Schmidt[2], A. Baur-Melnyk[2], I. Bauerfeind[3], V. Heinemann[4]

[1]Institute of Clinical Chemistry, [2]Department of Clinical Radiology, [3]Department of Gynecology, [4]Medical Department III, University Hospital, Campus Grosshadern, Munich, Germany

Aim: To evaluate the diagnostic potential of controlled tumor marker monitoring in combination with sensitive imaging algorithms for the detection of early, asymptomatic tumor recurrence in patients suffering from breast cancer.

Materials and Methods: 21 female patients (mean age 61, 37-76 years) with a history of breast cancer underwent regular tumor marker monitoring for CEA (Abbott, AxSYM), CA 15-3 and CA-125 (Roche, Elecsys) within an intensified diagnostic aftercare algorithm. All examined patients were asymptomatic and primarily treated with a curative approach. After identification of individual marker base levels regular monitoring was performed within a 2-3 month interval. A reproducible 100% increase of single or combined markers was

considered pathologic and followed by a high resolution whole-body MRI (WB-MRI) examination on a 1.5 Tesla scanner (Magnetom Avanto, Siemens Medical Solutions, Erlangen). As imaging protocol, coronal T1w-TSE- and STIR-sequences, dedicated HASTE-imaging of the lungs, contrast-enhanced T1w- and T2w-TSE-sequences of the liver, brain, abdomen and chest were performed. Presence and distribution of local recurrence, lymph node involvement and distant metastatic disease were assessed. In the case of absent or inconclusive findings FDG-PET-CT was performed as a gold standard imaging method for verification. Additionally, all patients underwent WB-MRI follow-up examinations within 3 months after individualized therapeutic approaches.

Results: At the defined reproducible 100% increase of one or several biomarkers based upon the individual baseline values, a morphologically detectable tumor recurrence was found in 91% of patients (19/21). 48% (10/21) showed limited metastatic disease at the time of examination. 14% (3/21) of patients had local tumor recurrence, 24% (5/21) lymph node metastases and 67% (14/21) organ metastases. Anatomical distribution of organ metastases was 57% to the bone, 21% liver, 11% lung and 11% other sites. In 14% of patients (3/21) secondary malignant tumors were detected: in 2 patients with a pathologic increase of CA-125 a carcinoma of the ovaries and uterus was detected, in another patient with an increase of CEA a carcinoma of the parotid gland. In 2 patients no evidence of disease was found at initial imaging examinations, while one of them showed a tumor recurrence after 3-months.

Conclusion: Monitoring of CEA, CA 15-3 and CA-125 with analysis of marker kinetics, combined with state-of-the-art whole-body imaging techniques is highly effective for the detection of early tumor recurrence in clinically asymptomatic breast cancer patients. A 100% increase of CEA and/or CA 15-3 is highly specific for recurrence in breast cancer patients.

Immunohistochemistry - Circulating Cells

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REPRODUCIBILITY OF TUMOR MARKER CONTAINING MACROPHAGES ASSAY ON TWO DIFFERENT FLOW CYTOMETERS

D. Japink[1], M.N. Sosef[1], M.P.G. Leers[2], M. Nap[3]

[1]Department of Surgery, [2]Department of Clinical Chemistry and Haematology, [3]Department of Clinical Pathology, Atrium Medical Center Heerlen, Holland

Applicability of the flow cytometric analysis of tumor marker-containing activated macrophages (TMCM) to prostate cancer (measuring PSA) and colorectal cancer (measuring CEA) have been shown recently. Separation of normal and benign disorders from malignant disease using this technique is promising. To determine the robustness of the TMCM assay the reproducibility of the different steps in the assay still need to be assessed.

Materials and Methods: Duplicate EDTA-specimens (3 mL) were drawn from a mix of colorectal cancer patients in different stages of treatment (n=45) after informed consent. From this whole blood the buffycoat was extracted, divided over 5 tubes to obtain negative control (NC), fluorescence-minus-one (FMO) and 3 tubes stained for either CEA, TKTL-1 or M30. Except for the NC, all tubes were stained with anti-CD14-APC and anti-CD16-FITC, before a Fix-and-Perm step and an intracellular staining step with anti (CEA, TKTL1 or M30)-PE was performed. All 5 tubes from the duplicate specimens were then measured on a FACScanto I and a FACScanto II flow cytometer and analyzed using FACSDiVa-software. Up to the level of activated macrophages the assessment was performed in quadruplicate on each machine. All data were also obtained on both machines but each TMCM-assay could only be measured once on both machines, resulting in duplicate data only. Analyzed parameters in all tubes where: lymphocytes(LC), mononuclear cells (MNC), monocytes (MC) and AM in total numbers and parent-percentages. Data acquisition was set at a stopping gate for 10,000 MNC.

Results: The mean value of machine-comparing measurements over quadruplicate parent %-measurements showed a correlation of 82.2-100% for the inter-machine measurements analyzing the LC (Median %CV: 6.65%, %CV-range: 0.3-15.2%), MNC (Median %CV: 7,0%, %CV-range: 0.0-27.1%), MC (Median %CV: 2.1 %, %CV-range: 0.02-11.7%) and AM (Median %CV: 2.5%, %CV-range: 0.07-32.6%) cell groups. The TMCM groups showed a correlation in parent %-duplicate measurements from 71.1% to 80%. For anti-TKTL1 this was present in 80% of the cases (Median: 8.9% %CV-range: 0.5-40.0%), for anti-CEA and anti-M30 it was 71.1% (Median %CV: 9.9% and 11.1% respectively, %CV-range: 0-37.3% and 0.05-37.6% respectively).

Conclusion: Reproducibility of this flow cytometrical analysis of TMCM was shown successfully in 82-100% of the parameters. This percentage might improve further by enhancing the techniques used. For instance optimal analytical reagent-concentrations for this assay need to be analyzed in depth. Beside this, consequent high quality sample preparation and flow cytometer performance must be guaranteed. The present results are promising for improving the use of tumor markers in diagnosis and monitoring of disease compared to classic serum assays.

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EXPRESSION AND DISTRIBUTION OF TENASCIN IN RAT SUBMANDIBULAR GLANDS FOLLOWING IRRADIATION

S. Bartel-Friedrich[1], C. Lautenschläger[2], H.-J. Holzhausen[3], R.E. Friedrich[4]

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

Within the extracellular matrix (ECM), the tenascins form an intriguing protein family each of whose members displays distinct features. The first tenascin to be described, tenascin-C, is a potentially useful marker for tissue remodelling due to its limited distribution in adult and healthy tissue. It serves as an indicator of developing fibrosis, including radiofibrosis in tissues unaffected by cancer. The clinical impact of radiofibrosis is focused on late radiation reactions. To date, tenascin-C alterations in parotid and lacrimal gland tissues have been reported in the early phase (up to one month) after irradiation, predominantly using single-shot irradiation (IRR) protocols. The aim of this study is therefore to add to the sum of knowledge of tenascin-C expression in the late postirradiation period following a clinically relevant IRR protocol in submandibular gland tissues.

Methods: In 122 submandibular gland specimens from 61 Wistar rats the effect on tenascin-C expression profile and distribution pattern of 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) was investigated by means of immunohistochemical methods, semiquantitative assessment and multivariate analysis.

Results: Expression of tenascin-C showed slight to moderate dose-dependent alterations in the irradiated specimens. The expression differed in frequency and degree among the various tissue structures. The most striking finding was pronounced dose-dependent heterogeneity, with increases, decreases and fluctuations in staining. For example, the largely periacinar immune reaction in control glands decreased with increasing dose, while the intracellular staining increased. Age and time since IRR had no significant effect on immune reaction.

Conclusion: The staining of tenascin-C 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 can be attributed to late radiation effects. The altered expression of tenascin-C may play at least a partial role in late radiogenic dysfunction of the submandibular gland.

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CIRCULATING TUMOR CELLS (CTCS) IN PERIPHERAL BLOOD OF BREAST CANCER PATIENTS BEFORE AND AFTER ADJUVANT CHEMOTHERAPY - THE GERMAN SUCCESS-TRIAL

B. Rack, C. Schindlbeck, A. Schneeweiss, J. Hilfrich, R. Lorenz, J. Jückstock, M. Beckmann, K. Pantel, W. Lichtenegger, H. Sommer, K. Friese, W. Janni

Ludwig-Maximilians-University, Munich, University of Heidelberg, Henriettenstiftung, Hannover, Gemeinschaftspraxis Braunschweig, University of Erlangen, University Medical Center Hamburg, Charité University Hospital, Berlin, Germany

The prognostic significance and predictive relevance of CTCs in metastatic breast cancer has been previously demonstrated (Cristofanilli *et al*., NEJM 2004). Within the translational research project of the German SUCCESS trial the role of CTCs in the adjuvant setting is evaluated. If the presence of CTCs in early breast cancer would also predict an increased risk for relapse, this method might be used as an early marker for treatment efficacy and risk for disease recurrence.

Methods: Peripheral blood (23 mL) from 1500 N+ and high risk N- breast cancer patients was analysed before and after adjuvant taxane based chemotherapy. The presence of CTCs was assessed with the CellSearchSystem. After immunomagnetic enrichment with an anti-Epcam antibody, cells were labelled with anti-cytokeratin and anti-CD45 antibodies to distinguish between epithelial cells and leukocytes.

Results: In 10% of patients (n=143) >1 CTC was detected before the start of systemic treatment (mean 14, range 2-827). While 2 CTCs were found in 4% of patients, 3% had 3-5 CTCs and 1% 6-10 and >10 CTCs, respectively. The presence of CTCs did not correlate with tumor size (p=0.32), grading (p=0.36), hormonal status (p=0.28) or Her2/neu status of the primary tumor (p=0.82), but with the presence of lymph node metastases (p=0.003). Three of 74 individuals without malignant disease (4%) showed more than 1 CTC. After completion of chemotherapy, 9% of patients (n=130) presented with >1 CTC (mean 6, range 2-124). Of those initially CTC positive, 10% remained positive (n=15), whereas of those initially CTC negative, 8% returned with a positive test (n=115, p=0.42).

Conclusion: Detection of CTCs with the CellSearch system is an easily applicable and highly standardized approach, which can also be used in primary breast cancer patients. A relevant number of patients show circulating tumor cells in their blood after completion of adjuvant chemotherapy. Longer follow-up of the SUCCESS-trial has to be awaited to determine the prognostic relevance of these persisting tumor cells.

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DETECTION OF INTRACELLULAR CYTOKINES IN LYMPHOCYTES OF BREAST CANCER PATIENTS AFTER *IN VITRO* STIMULATION

L. Sanisló[1], T. Železníková[2], J. Chovancová[2], G. Šulíková[2], J. Sedlák[2], O. Babušíková[2], M. Hrubiško[1]

[1]Department of Clinical Immunology and Allergology, Clinic of Laboratory Methods of Examination, St. Elizabeth Cancer Institute, [2]Tumor Immunology Laboratory, Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovak Republic

T-lymphocyte cells (CD3+) produce several types of cytokines. The most important are type I cytokines, IL-2 and IFN-γ, which are responsible for cell-mediated inflammatory reaction, delayed-type hypersensitivity and tissue injury in infections and autoimmune disease. The cancer development and metastasis exhibit the down supression of the host immune system, including the down-regulation of T-lymphocyte function. The aim of this work is to evaluate the production of IL-2 and IFN-γ cytokines in T-lymphocytes in breast cancer patients.

Methods: Venous blood samples were collected from 28 breast cancer patients, of which 20 had early stage breast carcinoma and 8 patients had metastatic breast carcinoma. The control group consisted of 16 subjects with allergy anamnesis. Blood samples were taken after adjuvant chemotherapy but prior to adjuvant radiotherapy. The analyses were performed from 6 mL of peripheral blood. After 4 hours stimulation (phorbol 12-myristate 13-acetate, PMA; 2.5 ng/mL) intracellular cytokines in lymphocytes were measured with flow cytometry.

Results: In the control, non-metastatic and metastatic groups the mean±SD for CD3+ / IL-2+ cells were 23.8±9.5% *vs*. 30.6±19.8% *vs*. 13.6±12.1% and median were 25.0% *vs*. 25.8% *vs*. 9.2%. The mean±SD for CD3+ / IFN-γ+ cells were 31.2±12.8% *vs*. 24.9±12.9% *vs*. 20.4±22.8% and median were 27.3% *vs*. 25.6% *vs*. 12.6%.

Conclusion: The results show that reactivity of T-cells in cancer is ambivalent. Decreasing expression of IL-2 and IFN-γ on T-lymphocytes was observed only in metastatic breast cancer patients. Paradoxically, in patients with non-metastatic breast cancer, expression of IL-2 was higher than in the control group. These results document the ambivalent role of immunity in cancer. In the early stage cancer immunity is stimulated, but cannot stop the development of cancer (and metastases), and can even it can support it; on the other hand in advanced disease patients have decreased immunity.

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EXPRESSION OF CATHEPSIN-D IN HUMAN DUCTAL BREAST CARCINOMA *IN SITU*, INVASIVE CARCINOMAS, THEIR LYMPH NODE METASTASES, THEIR DISTANT METASTASES, CARCINOMAS WITH RECURRENCE AND IN RECURRENCE TISSUES

N. Shabani[1,2], I. Mylonas[1], C. Kuhn[1], C. Kunert-Keil[3], C. Schindlbeck[1], B. Gerber[4], O. Camara [2], I.B. Runnebaum[2], K. Friese[1], U. Jeschke[1]

[1]Department of Gynecology and Obstetrics, Ludwig-Maximilians-University, Munich, [2]Department of Gynecology and Obstetrics, Friedrich Schiller University Jena, [3]Ernst Moritz Arndt University of Greifswald, Institute of Pathophysiology, [4]Department of Gynecology and Obstetrics, University of Rostock, Germany

Breast cancer cells can invade and generate metastasis *via* either lymphatic or blood vessels. A critical factor for metastasis is cathepsin-D, a lysosomal protease. Cathepsin-D increases the incidence of metastasis and is involved in cell proliferation and inhibition of tumor cell adhesion. In this study the expression of cathepsin-D was analyzed in mammary carcinoma *in situ*, invasive breast carcinomas without metastasis, invasive carcinomas with their lymph node and distant metastasis and invasive carcinomas with local recurrence in breast cancer tissue.

Methods: A total of 37 paraffin embedded slides of: carcinoma *in situ* (DCIS, n=8 D), invasive carcinomas without lymph node metastases (n=9), invasive carcinomas with corresponding lymph node metastases (n=7), invasive carcinomas with corresponding recurrence (n=5) and invasive carcinomas with corresponding distant metastases (n=5) were investigated for cathepsin-D expression. For immunohisto-chemistry staining mouse IgG antibody was used (1 µg/mL).

Results: A strong expression of cathepsin-D in carcinoma *in situ* was demonstrated. Expression of cathepsin-D was moderate in invasive carcinomas without metastases. Expression of cathepsin-D was moderate in invasive carcinomas with corresponding lymph node metastases. Cathepsin-D expression was reduced in lymph node metastases compared to the primary tumor, in primary tumors with

recurrence, in recurrence tissue and in primary tumors with distant body metastases and in its metastases.

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

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CEA-, HER2/NEU-, BRCP- AND HSP27-POSITIVE MICROPARTICLES IN BREAST CANCER PATIENTS

S. Liebhardt, N. Ditsch, R. Nieuwland, A. Rank, F. von Koch, U. Jeschke, K. Friese, B. Toth

Departments of Obstetrics and Gynaecology, LMU, Munich, Germany; Clinical Chemistry, Academic Medical Center, Amsterdam, The Netherlands; Obstetrics and Gynaecology, Internal Medicine III, Ludwig-Maximilians-University, Munich, Germany

Several studies demonstrated increased concentrations of circulating cellullar microparticles (MP) in breast cancer patients. Furthermore, *in vitro* studies emphasize the central role of MP in cancer outgrowth and spreading. Platelet-derived MP are the most well-known sub-population of circulating MP, supporting coagulation in an active and passive manner. In this prospective case-control study, MP expressing tumor-specific antigens like CEA (CD66), Her2/neu, BRCP and Hsp27 were studied in breast cancer patients and women with benign breast tumor.

Methods: MP were determined by flow cytometry in women with breast cancer in different disease states (n=34) and women with benign breast tumor as control group (n=19). Neither patients nor controls smoked or had taken hormonal replacement therapy. The cancer patients were classified according to the TNM system into patients with a T1 tumor (<2 cm, n=19) and patients with T2 tumor (2-4 cm, n=15). Twenty three patients had negative lymph nodes (N0) and 9 had positive lymph nodes (N1).

Results: No significant differences were present concerning age, body mass index, haemoglobin, platelet- and leukocyte concentration. T1 patients had significantly higher levels of Annexin V+MP (p=0.004), CD66+MP (p=0.025), BCRP+MP (p=0.008) and Hsp27+MP (p=0.020) than the controls. With regards to nodal state, patients with N1 showed significantly higher concentrations of Annexin V+MP (p=0.042), CD66+MP (p=0.045), BCRP1+MP (p=0.025) and Hsp27+MP (p=0.034) than the controls.

Conclusion: This study is the first report on cancer-related MP. Out of the sub-populations of MP expressing tumor-specific antigens, CD66-, BCRP- and Hsp-positive MP showed significant differences between patients with positive lymph nodes and healthy controls. To specify the role of CD66-, BRCP- and Hsp-positive MP in breast cancer progression further studies enrolling larger patient groups are necessary.

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IMMUNOHISTOCHEMICAL STUDY ON AN EPITHELIAL-MYOEPITHELIAL INTERCALATED DUCT CARCINOMA TRANSPLANTED TO THE NUDE MOUSE

R.E. Friedrich, R. Klapdor, C. Hagel

Oral & Maxillofacial Surgery and Neuropathology, University, ZeTDT, Hamburg, Germany

Parotid gland carcinomas are rare. Among these carcinomas, the epithelial-myoepithelial carcinoma is extremely rarely diagnosed. The salivary gland registry of Hamburg University recorded 21 cases collected during the first 15 years after establishment, with no patients having died from this cancer (Hamper *et al*. 1989). A female with a history of 17 years of recurrent EMC of the parotid and evidence of distant metastasis was treated over a period of 2 years. Surgery was intended as a palliative measure. During debulking procedures small samples of the tumor were transplanted to the nude mouse. These tumors grew well on these animals and were immunohistochemically characterized after explantation. The cellularity/mm^2 varied between 3.470 and 7.410. The tumor was characterized by the typical bipolar pattern of tumor cells and broad stromal septae. All but one of 7 transplanted tumors were positive for KL-1. The proliferation index in terms of MIB-1 stained nuclei increased from 2% to 20% and was positively correlated to the expression of EGFR. IGF-1R, VEGF and FLK1 stained cells were found in all cases. The increase in EGFR and MIB-1 positive cells correlated with the clinical course of the patient who showed shorter periods of tumor recurrence prior to her death. These findings in EMC transplanted to the nude mouse demonstrate the feasibility of growing EMC *in vivo*.

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INVESTIGATION ON GLYCODELIN EXPRESSION IN CORRELATION TO GRADING, NODAL INVOLVEMENT AND STEROID-RECEPTOR EXPRESSION IN HUMAN BREAST CANCER PATIENTS

C. Scholz[1], B. Toth[2], E. Barthell[1], I. Mylonas[1], T. Weissenbacher[1], K. Friese[1,2], U. Jeschke[1]

[1]LMU, Department of Obstetrics and Gynaecology,
[2]Department of Obstetrics and Gynaecology-Grosshadern,
Munich, Germany

Glycodelin (Gd) is a glycoprotein, which is secreted in high quantities during early pregnancy and modulates local immune responses in order to allow implantation of the semi-allogeneic blastocyst into the maternal endometrium. GdA is expressed in normal glandular epithelium of the endometrium as well as in normal and malignant glandular cells in and outside of the reproductive tract. It exists in various glycosylation isoforms that influence their staining behaviour. Recently, GdA expression was demonstrated in normal and cancerous human breast tissue and was associated with better differentiation *in vitro*. In order to tailor endocrine- and antibody- therapies to those cancer patients that are likely to respond, additional markers that are associated with better differentiation and intact intracellular signalling are needed.

Methods: One hundred and twenty one paraffin-embedded in lobular and ductal breast cancer tissue blocks were stained and graded using the immune reactivity score (IRS) commonly used to evaluate steroid receptors in the routine histological work-up. A polyclonal anti-Gd IgG-antibody being specific for the core amino acid sequence of Gd was used, thus being independent of the different glycosylation isoforms known in breast cancer (Code: UCI-2). From all breast cancer specimens surgically treated in the 1st Department of Obstetrics and Gynecology of the Ludwig Maximilians University, Munich during the years 1991 to 2001, only those specimens that did not contain any carcinoma *in situ* were chosen, known to uniformly stain positive for Gd. Gd expression was correlated to known prognostic markers such as grading, nodal involvement and steroid-receptor positivity. Statistical analysis was performed using the non-parametrical Mann-Whitney *U*-test and in case of 3 or more groups its extension, the Kruskal-Wallis one-way analysis of variance by ranks was used.

Results: Gd was expressed in invasive lobular and ductal breast cancer. Expression of Gd in breast cancer was reduced upon de-differentiation. Upon axillary lymph node involvement a non-significant increase in Gd expression was noted. Staining intensity in steroid receptor positive breast cancer specimen was increased.

Table I. *A total of 121 breast cancer tissue slides were used. Eighty three (69%) were invasive ductal and 38 (31%) invasive lobular. Allocation of both tumor types according to grading is shown.*

	lobular (n/%)		ductal (n/%)	
G1	15	39.5%	9	10.8%
G2	16	42.1%	40	48.2%
G3	3	3.6%	34	41.0%
GX	4	10.5%	0	

Table II. *Glycodelin IRS is elevated in invasive ductal carcinomas compared to lobular carcinomas.*

Histology		N	Minimum	Maximum	Mean	Standard deviation
invasiv ductal	Glykodelin poly IRS	83	0	12	8.92	3.63
invasiv lobural	Glykodelin poly IRS	38	3	12	8.63	3.09

Glycodelin staining intensity is significantly reduced according to grading in G3 carcinomas compared to G1 carcinomas (p=0.027). Lymph node positive carcinomas showed non-significantly higher staining of glycodelin. Mean of carcinomas with lymph node metastasis was 9.12 (n=60, SD 3.39) *versus* 8.54 (n=61, SD 3.53) in lymph node free carcinomas.

Conclusion: Statistical significance was reached in this patient population in G3 carcinomas compared to G1 carcinomas. These results implicate that Gd might be an additional marker for the differentiation of breast cancer tissue, yet indicating an increased tendency towards lymph node metastasis. To which extent GdA could serve as an additional indicator for breast cancer survival is part of ongoing research.

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A WATER EXTRACT FROM *LINUM USITATISSIMUM* AND ITS EFFECTS ON THE HUMAN BREAST CANCER CELL LINE MCF 7 AND HUMAN FIBROBLASTS

M. Szewczyk, A. Schlichting, D.-U. Richter,
S. Abarzua, V. Briese

Universitätsfrauenklinik Rostock im Klinikum Südstadt,
Germany

Several studies documented the inhibition of hormone dependent tumour cell growth *in vitro* and *in vivo* after application of phytoestrogen containing soy and flax seeds. Furthermore inhibitory effects of alcohol extracts from different flax organs were described on chorion carcinoma and breast cancer cell cultures.

Methods: In the present study water extracts from stem, leaf and root of Linum usitatissimu were prepared. Different concentrations were tested in cell cultures of fibroblasts and the mammalian tumour cell line MCF 7. Cytotoxicity was measured by LDH test, cell viability by MTT test and cell proliferation by BrdU test. The molecular-chemical analysis of the extracts was performed with pyrolysis field ionisation mass spectrometry (Py-FIMS).

Results: At concentrations of 0.01-1000 μg/mL the leaf, stem and root extracts from L. usitatissimum did not show cytotoxic effects on fibroblasts (LDH). For the MCF 7 cell line a significant cytotoxicity was measured at higher stem (500; 1000 μg/mL) and leaf (1000 μg/mL) extract concentrations between 30 and 40%. The cell proliferation of MCF 7 was not significantly influenced by the stem water extract but by higher leaf and root concentrations of 500 and 1000 μg/mL. The growth inhibition was between 25% and 35% (BrdU). There was no significant inhibition of fibroblasts. The application of 500 and 1000 μg/mL of stem, leaf and root extracts resulted in a significant increase of metabolic activity (MTT test) of fibroblasts from 40% (stem and root) up to 140% (leaf). Similar results were measured for MCF 7, where the extract concentrations of 1000 μg/mL induced significant activation of cell vitality between 50% and 75%.

Conclusion: In this *in vitro* study an inhibitory effect of flax water extracts on the breast cancer cell line MCF 7 with a minor influence on fibroblasts was demonstrated. Concerning a possible administration the water extract would have advantages against methanol extracts. However, the test principles of BrdU and MTT showed contradictory results.

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THE ROLE OF PREDICTIVE MOLECULAR PATHOLOGY IN THE DEVELOPMENT OF TARGETED THERAPY AND ITS CLINICAL RELEVANCE

M. Dietel

Institute of Pathology, Charité, University Hospital, Berlin, Germany

Due to continuous technical developments and new insights into the high complexity of many diseases, in particular the pathogenesis of cancer, molecular pathology is a rapidly growing field gaining center stage in the clinical management of tumors as well as in the pharmaceutical development of new anti-cancer drugs. Activated signaling components are the targets for classical therapeutic agents and newly developed inhibitors. The application of the compounds in clinical trials has revealed promising results, however, the current diagnostic procedures available for determining which patients will primarily benefit from rational tumor therapy are insufficient. To read a patients' tissue as "deeply" as possible, information on the morphology and on genetic, proteomic and epigenetic alterations will be the upcoming task of surgical pathologists experienced in molecular diagnosis in order to provide the clinicians with information relevant for individualized treatment (Dietel and Sers, 2006, Dietel 2007).

DNA microarrays currently provide an important approach close to enter routine diagnosis. Technically advanced and well established microarray platforms can nowadays be evaluated by distinct bioinformatic tools capable of both identifying novel genes associated with disease development and also clusters of genes predicting clinical outcome of an individual tumor. DNA microarrays were efficiently used for the classification of tumor subtypes, the prediction of metastatic potential and drug response. Also the automatic high parallel analysis of proteins and complex protein lysates has developed rapidly. Here, the opportunity of routine early detection of cancers such as breast, prostate and ovary through proteomic patterns in the serum appears at the horizon. In addition, an improved analysis of tumor samples *via* antibody or reverse phase protein arrays is likely to provide the pathologist in the future with information about overexpressed signaling proteins, activated oncogenic signaling pathways and other cell functions, such as drug response (Györffy *et al*. 2005 and 2006) or the potential to metastasize. While expression microarrays and proteomic analysis rely on relatively unstable material incompatible with paraffin embedded tissue samples, investigation of DNA methylation using specialized high throughput platforms has revealed the potential of being used in future diagnostics. Each of these approaches on its own might not suffice to extract all information required for an efficient individualized diagnosis. Therefore, a "multiplex approach" combining the different biological levels DNA, RNA and protein may be necessary to functionally classify malignant tumors. This appears to become a major challenge for diagnostic pathologists.

Urology I - Prostate Cancer

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TUMOR MARKERS IN ACTIVE SURVEILLANCE FOR PROSTATE CANCER, PRIAS AND P-MARK

Ch.H. Bangma, R. van den Bergh

Department of Urology, Erasmus Medical Centre, Rotterdam

Prostate cancer incidence has been increasing in every European country over the last decade. This is partly due to increasing cancer awareness, resulting in a quest for PSA based screening in asymptomatic men. Furthermore, the number of elderly and their life expectancy has grown, and it is to be expected that by the year 2050 in Germany 37% of the population will consist of people over 65 years of age, approximately half of them being male. Over the last five

years the number of radical prostatectomies has increased by 50%, and a gradual down staging of the cancers removed has been observed. Over 80% of cancers are locally confined, and an increasing part appears to be indolent. While in clinical series the amount of small (<0.5 mL) well differentiated (Gleason <7) tumors has been reported to be 20%, in screen detected series from the general population this number is up to 50%. Data obtained from the European Randomized Study for Screening of Prostate Cancer (ERSPC) confirm that about half of screen-detected cancers are unlikely to become symptomatic during life. Observations from autopsy series show that the incidence of small cancers is even more than 6-fold the age related screening incidence.

This over-diagnosis of indolent cancers forces urologists to think about how to avoid invasive therapy in a subset of men with prostate cancer in order to increase their quality of life. This is currently done in various European countries including Germany within the PRIAS project (Prostate Cancer International Active Surveillance). In the protocol of this registration study, changes of PSA over time, and repeated prostate biopsies serve as parameters to monitor indolent disease, and adviseadvice to invasive treatment based on rigid criteria. As the aim of the study is to obtain information to improve current protocols by better selection of those tumours that remain silent in contrast to those that will grow aggressively, biomaterials are obtained from a subset of men within the PROCABIO project (Prostate Cancer Biomarkers). This project is based on the sampling protocols used within the completed European sponsored P-MARK project, and has raised one of the first international sample collections for research on prostate cancer.

The value of PSA changes over time in relation to tumour progression under active surveillance will be discussed, as well as some findings on candidate biomarkers in serum and in urine that need to be validated in this clinical setting.

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PCA3 REPRESENTS A CLINICALLY MEANINGFUL PREDICTOR OF PROSTATE CANCER AT REPEAT BIOPSY

A. Haese[1], F. Chun[1], A. de la Taille[2], H. van Poppel[3], M. Marberger[4], P. Mulders[6], C.-C. Abbou[2], A. Stenzl[5], H. Huland[1], M. Tinzl[4], M. Remzi[4], S. Feyerabend[5], M. Van Gils[6], A. Stillebroer[6], J. Schalken[6]

[1]University Medical Centre, Hamburg, Germany; [2]CHU Henri Mondor, Paris, France; [3]University Hospital Gasthuisberg, Leuven, Belgium; [4]Medical University, Vienna, Austria; [5]Uniklinikum, Tübingen, Germany; [6]Radboud University Medical Centre, Nijmegen, The Netherlands

The urinary prostate cancer 3 (PCA 3) marker represents a hopeful novel molecular marker to improve specificity of prostate cancer detection. Whether the urinary prostate cancer 3 (PCA 3) marker is capable to withstand most stringent uni- and multivariable analyses to display its discriminative as well as its informative character has been investigated for the first time.

Materials and Methods: A multi-institutional dataset consisting of 432 men subjected to a repeat prostate biopsy of 6 different European centers were used. Uni- and multivariable logistic regression models to predict presence of prostate cancer at repeat biopsy were fitted using age, DRE, PSA, %fPSA, prostate volume and PCA 3. Bootstrap-corrected predictive accuracy was quantified using AUC estimates in models with and without PCA 3. This method was selected with the intent of quantifying the increment in predictive accuracy, associated with the addition of PCA 3 to all base predictor variables. PCA 3 was coded as a cubic spline to allow non-linear effects and to obviate the limitations associated with the use of categorical cut-offs. Differences in predictive accuracy were compared using the Mantel-Haenszel test.

	Univariable analyses			Multivariable analyses			
				Base Model		Base Model + PCA 3	
Variables	*p*-value	OR	PA (%)	*p*-value	OR	*p*-value	OR
Age	0.004	1.051	0.578	0.035	1.042	0.243	1.024
PSA	0.001	1.063	0.600	0.003	1.07	0.007	1.064
%fPSA	0.063	0.974	0.578	0.637	0.992	0.477	0.988
DRE	<0.001	2.610	0.577	0.001	2.473	0.006	2.263
Prostate volume	0.024	0.990	0.563	0.002	0.982	0.015	0.985
PCA 3*	<0.001		0.663	-	0.006	-	
PA (%)		66.8	71.0				
Increment in PA (%)				+4.2			
p value				<0.001			

PA: predictive accuracy; OR: odds ratio; PSA: prostate specific antigen; %fPSA: percent-free prostate specific antigen; DRE: digital rectal examination; PCA 3: prostate cancer gene 3; *coded as cubic spline to allow non-linear effects.

Results: Prostate cancer was detected in 120 (27.8%) men. PCA 3 represented a statistically significant and independent predictor of prostate cancer at repeat biopsy ($p \leq 0.006$). Additionally, PCA 3 represented the most informative univariable predictor and was capable of increasing predictive accuracy in multivariable models by 4.2% which was highly significant ($p < 0.001$).

Conclusion: In the repeat biopsy setting, PCA 3 outperformed all traditional risk factors such as age, DRE or %fPSA or total PSA. It is demonstrated, for the first time, that

inclusion of PCA 3 into multivariable models increased predictive accuracy significantly. Thus, PCA 3 meets all criteria of a novel, clinically useful marker and should be considered in future clinical practice and applications such as nomograms.

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A [-2]PROPSA-BASED ARTIFICIAL NEURAL NETWORK (ANN) IMPROVES PROSTATE CANCER (PCA) DETECTION: PERFORMANCE OF PROPSA IN AN ARTIFICIAL NEURAL NETWORK

C. Stephan[1], H. Cammann[2], S. Deger[1], M. Schrader[1], K. Miller[1], M. Lein[1], K. Jung[1]

[1]Department of Urology, [2]Institute for Medical Informatics, Charité, Universitätsmedizin, Berlin, Germany

The aim of this study was to combine the new automated Access [-2]proPSA (p2PSA) assay with a percent free PSA (%fPSA) based artificial neural network (ANN) or logistic regression (LR) model to enhance discrimination between patients with prostate cancer (PCa) and with no evidence of malignancy (NEM) and to detect aggressive PCa.

Methods: Sera from 311 PCa patients and 275 NEM patients were measured with the p2PSA, total PSA (tPSA) and free PSA (fPSA) assays on Access immunoassay technology within the 0-30 ng/mL tPSA range. Four hundred and seventy five patients (264 PCa, 211 NEM) had a tPSA of 2-10 ng/mL. LR models and leave-one-out (LOO) ANN models with Bayesian regularization by using tPSA, %fPSA, p2PSA/fPSA (%p2PSA), age and partial prostate volume were constructed and compared by receiver-operating characteristic (ROC) curve analysis.

Results: The ANN and LR model each utilizing %p2PSA, %fPSA, tPSA and age, but without prostate volume, reached the highest AUCs (0.85 and 0.84) and best specificities (ANN: 62.1% and 45.5%; LR: 53.1% and 41.2%) compared with tPSA (22.7% and 11.4%) and %fPSA (45.5% and 26.1%) at 90% and 95% sensitivity. The %p2PSA furthermore distinguished better than tPSA and %fPSA between pT2 and pT3, and Gleason sum <7 and =7 PCa. The %p2PSA had the largest impact on the ANN and LR models.

Conclusion: The automated p2PSA assay offers a new tool to improve PCa detection and especially aggressive PCa detection. Incorporation of %p2PSA into an ANN and LR model further enhances the diagnostic accuracy to differentiate between malignant and non-malignant prostate diseases.

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POPULATION DEPENDENCY OF NEURAL NETWORKS

T. Köpke[1], C. Stephan[2], H. Cammann[3], A. Semjonow[1]

[1]Prostate Center, Department of Urology, Münster, [2]Department of Urology, [3]Institute for Medical Informatics, Charité – University Clinic, Berlin, Germany

Objectives: To verify the artificial neural network (ANN) "Prostata Class" in an external population. The %f-PSA based ANN "Prostata Class" was developed by the Charité Berlin (B) to increase the diagnostic accuracy of prostate cancer (PCa) detection and to avoid unnecessary prostate biopsies. The discriminative power of the ANN was investigated in an external patient population from Münster (MS) and compared it to B for t-PSA, %f-PSA, digital rectal examination (DRE) and transrectal ultrasound examination (TRUE). In a second step the diagnostic power of t-PSA, %f-PSA and ANN were compared between both populations. In a final step both populations were analysed and compared to identify possible variations in the clinical parameters.

Patients and Methods: Nine hundred and fifty one patients (PCa in prostate biopsy: 536; no evidence of malignancy (NEM) in prostate biopsy: 415) were included in the study. PSA was analysed with the Beckmann Access PSA assay. The individual risk of prostate cancer was calculated with the "Prostata Class" ANN according to age, t-PSA, %f-PSA, DRE and TRUE. The results were illustrated by ROC-curve analysis.

Results: In MS the areas under the curve (AUC´s) were 0.63 (t-PSA), 0.68 (%f-PSA), 0.66 (DRE), 0.62 (TRUE) and 0.72 (ANN). There was no statistical difference between both populations in the diagnostic power of t-PSA and %f-PSA for t-PSA >10 ng/mL (MS: 0.58, 0.74; B: 0.56, 0.74) while the AUC for the ANN was less in MS (MS: 0.74; B: 0.86). For t-PSA <10 ng/mL the proportions of the AUC`s according t-PSA, %f-PSA and ANN were comparable in both populations while the discriminative power of all three parameters was less in MS (MS: t-PSA 0.60, %f-PSA 0.65, ANN 0.72; B: t-PSA 0.70, %f-PSA 0.81, ANN 0.89). The mean t-PSA for t-PSA <4 ng/mL was lower in B while the mean %f-PSA in this group was significantly higher for patients with NEM in B than in MS. The DRE of patients with PCa was more often positive in B than in MS for the t-PSA range 4-10 ng/mL. For t-PSA >10 ng/mL the prostate volume of patients with PCa was higher in MS than in B while NEM patients in MS had more (false) positive DRE´s and a higher t-PSA than in B.

Conclusion: The ANN "Prostata Class" increased the diagnostic accuracy in comparison to t-PSA and %f-PSA for t-PSA <10 ng/mL in the external population of MS. It showed less discriminative power than in B while the individual variables had even less diagnostic accuracy in the population of MS compared with B. The different AUC´s for the ANN in both populations can well be explained by the different populations.

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CALCULATING PSA DOUBLING TIMES WITH AN ERROR ESTIMATION TOOL

A. Ponholzer[1], N. Popper[2,3], F. Breitenecker[3], H.-P. Schmid[4], W. Albrecht[5], W. Loidl[6], St. Madersbacher[1], M. Rauchenwald[1]

[1]Department of Urology and Andrology, Danubehospital, [2]"die Drahtwarenhandlung" – Simulation Services, [3]Institute for Analysis and Scientific Computing, Vienna, Austria, [4]Department of Urology, Cantonal Hospital, St.Gallen, Switzerland, [5]Department of Urology, Mistelbach, [6]Department of Urology, Hospital Barmherzige Schwestern, Linz, Austria

PSA-doubling time (PSA-DT) is an important indicator for progression and survival in men with prostate cancer. Two relevant limitations regarding PSA-DT determination may lead to inconsistent results: the variety of mathematical methods currently applied and the non-standardized handling of input variables.

Methods: The aim of this project was to develop a reproducible PSA-DT determination tool, which simultaneously provides a PSA-DT error-estimation.

Results: An internet-based PSA-DT calculation tool *via* nonlinear optimization implementing the least–square–error method using the last three PSA values was developed. PSA-DT calculation error is estimated *via* randomly disturbed measurement data streams (n=65) based on an assumed 15% PSA-variation.

Conclusion: Herein an adequately defined, open and reproducible PSA-DT calculation- and PSA-DT error-estimation-tool based on a standardized PSA-data input is presented.

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GENE FUSIONS IN PROSTATE CANCER

H. Rittenhouse

Gen-Probe Incorporated, Genetic Center Drive, San Diego, CA, U.S.A.

PCA3 is the most over-expressed cancer gene in prostate cancer. A molecular urine test for PCA3 has been developed which measures PCA3 mRNA expression in urine following a DRE to release cancer cells. The fusion of the prostate-specific, androgen-regulated TMPRSS2 gene (T2) with the ERG gene has been identified recently. The prostate cancer gene fusions have been shown to be prostate cancer specific and appear to be a frequent and early event in the development of prostate cancer, with distinct biology and an aggressive phenotype. Gene fusions can also be detected in urine and a recent study of gene fusions in urine sediments showed over 90% specificity for pros-tate cancer.

Method: Using an established procedure for collecting the urine sample and a RNA amplification method (Transcription-Mediated Amplification) the specimen informative rate is over 95%. Like PSA, PCA3 is prostate tissue specific. In marked contrast to serum PSA, PCA3 is not affected by prostate size and is correlated with tumor volume and prostatectomy Gleason score.

Results: In several independent prospective studies, PCA3 has been shown to be significantly more specific than PSA/Free PSA to predict biopsy and more accurate for the detection of biologically significant cancers. Specificity for cancer ranges from 70 to 85% in a number of studies. PCA3 correlated with tumor volume (p=0.008) and prostatectomy Gleason score (p=0.005) in a study of 96 patients. The mechanisms for serum PSA (gland integrity) and PCA3 (direct cancer cell detec-tion in urine) to detect cancer are completely independent. Studies have shown that the accuracy of PCA3 is independent of serum PSA values including serum PSA levels below 4.0 ng/mL. Recent studies are showing a significant synergy to utilize PCA3 and PSA together with other parameters to significantly increase predictive values for cancer detection. In a study at Sharp Memorial Hospital and Cedars Sinai Medical Center of 100 archived prostatectomy tissues the T2:ERG gene fusion was found in 66% of tumor foci in agreement with other studies. A single focus can have multiple splice variants for a gene fusion. A particular T2:ERG splice variant (T2:ERGc) was found to be highly correlated with seminal vesicle invasion (p<0.02), in agreement with the study of Wang *et al*. In a larger study of urine sediments, a specificity over 90% for prostate cancer has also recently been found; the clinical sensitivity was 35-40% reflecting the subset of patients with gene fusions.

Conclusion: PCA3 and gene fusions have high potential for biopsy decisions and prognosis of prostate cancer.

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TP53 GENE MUTATIONS IN PROSTATE CANCER PROGRESSION

Th.H. Ecke, H.H. Schlechte, K. Schiemenz, M.D. Sachs, S.V. Lenk, B.D. Rudolph, S.A. Loening

HELIOS Hospital, Department of Urology, Bad Saarow, Department of Urology, German Institute of Pathology, Charité Universitätsmedizin Berlin, Germany

The predictive value of TP53 mutations and prostate specific antigen (PSA) were assessed for tumor progression in prostate cancer (PCa) patients.

Methods: Ninety tumor tissue samples of patients with PCa from radical prostatectomy were analysed. Tumor progression was estimated biochemically by PSA level of 0.2 μg/L or by detection of metastases. Screening for TP53 mutations was performed by temperature gradient gel electrophoresis (TGGE) in an exon-specific manner. Sampling of follow-up data was carried out following medical protocols. Statistical analysis was done by uni- and multivariate techniques.

Results: TP53 mutations were detected in 32 of 90 patients (35.6%). Thirteen of 32 patients (40.6%) with TP53 mutations and 9 of 58 patients (15.5%) with TP53 wildtype showed tumor progression after a time of 25 and 45 months, respectively. Mutations in exons 7-8 are progression factors.

Conclusion: TP53 mutations in exon 7 and exon 8 are factors of tumor progression in PCa. Their contribution to tumor recurrence is more significant than tumor stage and pre-therapeutic PSA level.

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OUTCOME PREDICTION FOR PROSTATE CANCER DETECTION RATE WITH ARTIFICIAL NEURAL NETWORK IN DAILY ROUTINE

T.H. Ecke, P. Bartel, S. Hallmann, S. Gunia, S. Koch, J. Ruttloff, H. Cammann, M. Lein, M. Schrader, K. Miller, C. Stephan

Department of Urology, German Institute of Pathology, HELIOS Hospital, Bad Saarow, Institute of Medical Informatics, Department of Urology, Charité Universitätsmedizin, Institute for Urological Research, Berlin, Germany

The use of the artificial neural network (ANN) program "ProstataClass" of the Department of Urology and the Institute of Medical Informatics at the Charité – Universitätsmedizin Berlin in daily routine to increase prostate cancer (PCa) detection rate and to reduce unnecessary biopsies was evaluated.

Methods: From May 2005 to April 2007 a total of 204 patients were included in the study. The Beckman Access PSA assay was used and pre-treatment PSA was measured prior to digital rectal examination (DRE) and 12 core systematic transrectal ultrasound (TRUS) guided biopsies. The individual ANN predictions were generated with the use of the ANN application for the Beckman Access PSA and free PSA assays, which relies on age, PSA, %fPSA, prostate volume and DRE. Diagnostic validity of tPSA, %fPSA, and the ANN was evaluated by ROC curve analysis.

Results: PSA and %fPSA ranged from 4.01 to 9.91 ng/mL (median: 6.65) and 5% to 48% (median: 15%), respectively. Of all men, 46 (22.5%) demonstrated suspicious DRE findings. Total prostate volume ranged from 7.1 to 119.2 cm^3 (median: 35). Overall, 71 (34.8%) PCa were detected. Of men with suspicious DRE, 28 (60.9%) had PCa on initial biopsy. The ANN was 78% accurate in the original report. The AUC of ROC curve analysis was 0.51 for PSA, 0.66 for %PSA, and 0.72 for the ANN-Output, respectively.

Conclusion: The results in this independent cohort show that ANN is a very helpful parameter in daily routine to increase the PCa detection rate and reduce unnecessary biopsies.

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DD3/PCA3 (DIFFERENTIAL DISPLAY CODE 3) IN PROSTATE CANCER DIAGNOSIS (EXPERIENCE FROM THE CZECH REPUBLIC)

J. Klecka, L. Holubec, M. Pesta, O. Topolcan, M. Hora, V. Eret, M. Chottova-Dvorakova, M. Babjuk, K. Novak, J. Stolz

Department of Urology, University Teaching Hospital, Central Radioisotope Laboratory of Charles University, Department of Oncology, University Teaching Hospital, Department of Physiology, Charles University, Medical Faculty, Plzen, Department of Urology, University Teaching Hospital of 1st. Medical Faculty, Charles University, Praque, Czech Republic

Early diagnosis of prostate cancer (PCa) at an organ confined stage with following radical treatment is the only potentially curative approach in PCa. PSA is very helpful in early diagnosis, but the main disadvantage is a low positive predictive value, which results in a high number of useless biopsies. Therefore, new tests with better parameters are required. One promising parameter is PCA3, which is a prostate-specific non-coding mRNA that is highly over-expressed in prostate tumor cells. The aim of study was to evaluate the diagnostic potential of PCA3 for PCa diagnostics.

Materials and Methods: Altogether 199 patients were examined. For the group of patients with a suspicion of PCa one tissue specimen core was collected for PCA3 expression in tissue. According to histological verification 103 patients were diagnosed with benign prostatic hyperplasia (BPH), 12 patients with prostatic intraepithelial neoplasia (PIN) and 84 patients with prostate cancer. Total RNA were isolated, PCA3 and PSA expression quantified using RT-PCR method. The PCA3/PSA mRNA ratio distribution was determined for both subject groups. To assess the ability of the PCA3 assay to predict biopsy outcome, the % biopsy positive was determined for different PCA3/PSA ratio ranges and PCA3 Ct.

Results: In this study, the fraction of specimens yielding sufficient RNA for RT-PCR analysis was only 75%. It was

found that levels of mRNA expressions of PCA3 (Ct) were significantly higher ($p<0.045$) in patients with prostate cancer than in patients with benign prostatic hyperplasia. Further, statistically significant differences were found in the levels of mRNA expressions of PCA3 (Ct) between patients with benign prostate cancer and patients with prostatic intraepithelial neoplasia (PCA3 Ct, $p<0.023$).

Conclusion: The specificity of the PCA3 assay for prostate cancer seems to be prospective for early detection of prostate cancer and also for differential diagnosis between patients with BPH and patients with prostate cancer. This work was supported by grant IGA NR/8918 – 3.

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SOLITARY TESTICULAR METASTASIS FROM PROSTATE CANCER - A RARE CASE OF ISOLATED RECURRENCE AFTER RADICAL PROSTATECTOMY

S. Janssen[1], J. Bernhards[2], A.G. Anastasiadis[3], F. Bruns[1]

[1]Department of Radiotherapy, Medical School, Hannover, [2]Department of Pathology, Robert-Koch-Krankenhaus Gehrden, [3]Department of Urology, Großburgwedel Hospital, Germany

The prognosis of prostate cancer is mainly determined by the presence or absence of metastases. An isolated testicular metastasis of prostate cancer is rare.

Case Report: A 71-year old patient with prostate cancer presented with an increased serum PSA level of 2.07 ng/mL two and a half years after radical prostatectomy. Assuming a local recurrence in the prostatic fossa, a local external beam radiotherapy with 64.8 Gy was performed. Unfortunately, the PSA level rose again accompanied with a swelling of the left testis approximately one month after radiotherapy. A unilateral orchiectomy was then performed presenting an isolated testicular metastasis of the prostate cancer. After orchiectomy, the PSA decreased to <0.07 ng/mL. Two years later the patient is still tumour-free.

Conclusion: Careful clinical follow-up of patients with raising serum PSA level is important to recognize isolated, locally treatable metastastic disease. Particularly, rare metastatic sites like the testis or the epididymis should be taking into account before treatment of biochemical recurrence is initiated.

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CHROMOGRANIN A IN HORMONE INDEPENDENT PROSTATE CANCER

L. Holubec, J. Klecka, O. Topolcan, J. Vrzalova, M. Pesta, V. Eret, J. Finek, M. Hora

Faculty Hospital and Medical School in Pilsen, Charles University, Prague, Czech Republic

Focal neuroendocrine differentiation of cancer cells occurs in 10% of prostate cancer patients. Current knowledge suggests that endocrine differentiation of tumor affects conversion of hormone dependent tumor into the hormone resistant tumor. Chromogranin A seems to be a promising tissue and serum marker of hormone resistant prostate cancer, particularly in term of prognostic and predictive value.

Methods: Results are presented in case reports. Importance of several parameters (PCA3/DD3, PSA, fPSA, Chromogranin A) is discussed in relationship to prognosis and prediction of prostate cancer.

Results: Elevated values of Chromogranin A are related to the lower grading of the tumor in prostate cancer males. Patients with lower PSA levels and higher Chromogranin A levels may be resistant to hormone therapy and they may therefore show worse prognosis.

Conclusion: Combination of serum PSA and Chromogranin A levels seems to be useful for prediction and prognosis monitoring in patients with hormone independent prostate cancer. This study was supported by IGA NR 8918-3 grant and MSM 0021620819 Research Project.

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BPHA (BPSA) IMPROVES DETECTION OF PROSTATE CANCER IN AN ARTIFICIAL NEURAL NETWORK

C. Stephan[1], H. Cammann[2], S. Deger[1], M. Schrader[1], K. Miller[1], M. Lein[1], K. Jung[1]

[1]Department of Urology, [2]Institute for Medical Informatics, Charité, Berlin, Germany

Subforms of free PSA (fPSA) have been proposed to enhance discrimination between prostate cancer (PCa) and benign prostate hyperplasia (BPH). The “benign” PSA (bPSA) form has been more closely associated with BPH and showed benefit in detecting PCa. The aim of this study was to combine the new automated Access BPHA (bPSA) Research Use Only (RUO) assay with a percent free PSA (%fPSA) based artificial neural network (ANN) model to enhance discrimination between patients with PCa and with "no evidence of malignancy" (NEM).

Methods: Sera from 287 PCa patients and 254 NEM patients were measured with the BPHA-RUO, total PSA (tPSA) and fPSA assays on Access immunoassay technology

within the 0-10 ng/mL tPSA range. Two ANN models with Bayesian regularization and leave-one-out validation by using the 4 input parameters age, prostate volume, tPSA and %fPSA, or the 4 parameters and additionally BPHA/tPSA, were constructed and compared by ROC-curve analysis.

Results: The ANN which includes BPHA/tPSA reached the highest significant AUC (0.81; p=0.0004 and 0.0024) and best specificities (53.9% and 44.5%) compared with the ANN without BPHA/tPSA (AUC 0.77; specificities 50% and 40.6%) and %fPSA (AUC 0.775; specificities 40.9% and 27.2%) at 90% and 95% sensitivity. The AUCs for tPSA (0.59), BPHA (0.57), BPHA/fPSA (0.51), prostate volume (0.69) and BPHA/tPSA (0.69) were significantly lower.

Conclusion: While bPSA, measured by the Access BPHA-RUO assay, as single marker or ratio to tPSA did not improve the diagnostic performance of %fPSA or tPSA, the incorporation of BPHA/tPSA into an ANN model increased the specificity compared with %fPSA by 13% and 17% at 90% and 95% sensitivity. Thus, the automated BPHA-RUO assay offers a new tool to improve PCa detection when incorporating this new marker into a %fPSA-based ANN.

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BIOCHEMICAL MARKERS IN PATIENTS WITH PROSTATE CANCER AND BENIGN PROSTATE HYPERPLASIA – A MULTIVARIATE ANALYSIS

F. Strittmatter[1], P. Stieber[2], D. Nagel[2], K. Hofmann[2], C. G. Stief[1], R. Waidelich[1]

[1]Department of Urology, [2]Institute of Clinical Chemistry, University Hospital, Campus Grosshadern, Munich, Germany

Prostate cancer is the most common malignancy in men and the leading cause of cancer related death due to urological tumours. Standard screening for prostate cancer includes a digital rectal examination and the measurement of prostate specific antigen (PSA). Since the diagnostic significance of these procedures is limited, there is still a large number of false diagnoses. Therefore the aim of the present study was to evaluate whether other common tumour markers are released in patients with prostate cancer and to evaluate their potential benefit to distinguish between benign lesions of the prostate (BPH) and prostate cancer (PC).

Methods: Frozen sera of 442 patients in total (131 with PC, 311 with BPH) were analysed for the presence of AFP, CA 125, CA 15-3, CA 19-9, CA 72-4, CEA, CYFRA 21-1, HCGB, NSE, fPSA, tPSA (all Roche, Elecsys), cPSA (Bayer, ADVIA Centaur), AP, CRP and LDH (Olympus, Olympus AU5400). In an univariate analysis we identified markers showing significant differences in the distribution among both groups. Those markers were combined with tPSA or cPSA in a multivariate analysis using logistic regression to evaluate whether a diagnostic benefit can be achieved. For multivariate analysis the collective was divided into subgroups accounting for tPSA-levels >10 ng/ml and <10 ng/ml.

Results: Differences in the distribution of the markers among both groups were seen as follows: tPSA, fPSA/tPSA, cPSA, cPSA/tPSA, AFP, CEA und NSE were significantly elevated in the PC group, whereas CA 125, CA 19-9, CYFRA 21-1, hCGß and CRP were higher in the BPH group. In the univariate analysis none of the non organ specific markers itself was superior to PSA measurement. Multivariate analysis revealed a diagnostic benefit for various combinations of tumour markers in both tPSA ranges expressed by a higher sensitivity at a 95% specificity, but only the combination of tPSA, fPSA, fPSA/tPSA, CA 125 and CEA showed a significant diagnostic improvement compared to tPSA alone at a tPSA-levels <10 ng/ml.

Conclusion: The measurement of single non-organ-specific tumour markers provides no clinical advantage for the detection of prostate-cancer because of their low sensitivity. However, a significant diagnostic improvement can be achieved by combination of several markers with tPSA and cPSA using multivariate analysis. Whether this model proves to be useful in clinical practice also with regard to cost-benefit ratio needs to be examined in further studies.

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VALUE OF PROCOLLAGEN TYPE 1 AMINO-TERMINAL PROPEPTIDE IN PATIENTS WITH RENAL CELL CARCINOMA

I. Jones[1], M. Klepzig[2], H. Sauer-Eppel[2], D. Jonas[1], G.M. Oremek[2]

[1]Department of Urology and [2]Department of Laboratory Medicine, Internal Medicine, Hospital of the Johann-Wolfgang-Goethe-University, Frankfurt, Germany

The aim of the study was to investigate whether the bone turnover marker procollagen type 1 amino-terminal propeptide (P1NP) could be useful for the early detection of bone metastases in patients with renal cell carcinoma (RCC) and if chemotherapy influences P1NP concentrations in patients with bone metastases.

Patients and Methods: Serum samples of 36 patients were analyzed using a specific immunoassay. Serum P1NP was measured with electrochemiluminescence immunoassay

ECLIA using Elecsys 2010 analyzer. This method is based on a sandwich principle, using streptavidin-coated microparticles, biotinylated monoclonal anti-P1NP antibodies and monoclonal anti-P1NP antibodies labeled with a ruthenium complex. Alkaline phosphatase (AP), another marker of bone formation, was also measured in order to compare its diagnostic effectiveness with P1NP. The AP analysis was performed with the Hitachi 917 analyzer. The patients were divided into three groups, 24 patients without metastatic spread, 6 patients with untreated bone metastases and 6 patients who had received Sorafenib.

Results: The P1NP cut-off was 60 ng/mL. The P1NP concentration was significantly higher ($p \leq 0.001$) in the patients with bone metastases (median: 396.10 ng/mL) than in the collective without bone involvement (median: 35.53 ng/mL). The patients treated with Sorafenib showed levels within the normal range (median: 28.96 ng/mL). In this study, the cut-off value for AP for male patients was 129 U/l and for female patients was 104 U/l. The AP was significantly higher in patients with untreated bone metastases (median: 358 U/l) than in the study group without malignant changes in bone metabolism (median: 66 U/l) ($p \leq 0.001$). The patients who had been treated with Sorafenib had median AP levels within the normal range (median: 103.0 U/l) thus being significantly lower than the AP concentrations in patients with untreated bone metastases ($p \leq 0.01$). A positive correlation was shown between the two markers of bone formation (correlation coefficient 0.474; $p \leq 0.01$).

Conclusion: P1NP is a significant diagnostic marker for the development of bone metastases in patients with RCC and could help to evaluate the progress of chemotherapy.

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PRIMARY LEIOMYOSARCOMA OF THE TESTIS: A CASE REPORT

A.P. Labanaris[1], V. Zugor[2], R. Smiszek[1], R. Nützel[1], R. Kühn[1]

[1]Department of Urology, Martha Maria Medical Center, Nurnberg, [2]Department of Urology, Salzgitter Medical Center, Germany

In 2006, a 73-year-old patient presented at the Department of Urology with a one month history of an enlarging lump in his right testicle causing mild discomfort. The patient had undergone a right hydrocelectomy 40 years before. He did not present with any other constitutional symptoms such as voiding complaints, weight loss, fatigue or fever. Physical examination did not reveal any superficial lymph node swelling but confirmed a hard, non-tender, right testicular mass. No ulceration of the overlying skin was evident. The digital rectal examination revealed a benign enlarged prostate. Scrotal ultrasonographic examination revealed a solid homogenous echo poor mass of the right testicle measuring 35 mm × 33 mm and consistent with the radiographic appearance of a testicular tumour. The left testicle and both epididymides were unremarkable and there was no evidence of hydrocele or paratesticular pathology. Chest, abdominal and pelvic computed tomography scans revealed no evidence of metastatic disease or lymphadenopathy. Liver function and tumour markers, including a fetoprotein, lactate dehydrogenase and beta human chorionic gonadotrophin assays, were all within normal ranges. Blood examination revealed only a white blood cell count of 10.9/ nL and the C-reactive protein was at 0.4 mg/dL.

Right inguinal radical orchiectomy with high ligation of the spermatic cord was performed without complication. On gross examination, a transverse opening of the testis showed a well defined mass, which measured 3.5×3.0×2.5 cm in the greatest dimension. The tumor was yellowish-white, solid, encapsulated, existed only in the testis and did not involve the spermatic cord, epididymis or tunica vaginalis. The surgical margins were negative.

Histologically, the tumour revealed a spindle cell neoplasm with moderate nuclear pleomorphism. Immunohistochemical stains, which included vimentin, desmin, smooth muscle actin, CD34, S100, CD-68 and HMB-45, were performed. Immunohistochemistry revealed positive staining for vimentin, smooth muscle actin and desmin while S100, CD34, CD-68 and HMB-45 were negative. The combined histologic and immunohistochemical findings were diagnostic of primary poorly differentiated leiomyosarcoma of the testis. The patient had an uneventful postoperative course and received no adjuvant therapy. The tumor follow-up, which included computed tomography scan of the abdomen, pelvis, bone and chest have remained normal and the patient has remained free of disease these last 28 months.

Primary leiomyosarcoma of the testis is an extremely rare disease entity of the genito-urinary tract. Diagnosis is achieved by combining histologic and immunohistochemical findings. Although the number of reported cases is not significant and the clinical and biological behaviour of these tumors is very hard to predict, radical orchidectomy followed by surveillance appears to be the treatment of choice. Retroperitoneal lymphadenectomy, radiotherapy and chemotherapy do not seem to have any place in its treatment.

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CONGENITAL JUVENILE GRANULOSA CELL TUMOR OF THE TESTIS IN NEWBORNS

V. Zugor[1], A.P. Labanaris[2], A. Seidler[3], K. Weingärtner[3], B. Wullich[4], G.E. Schott[4]

[1]Department of Urology, Salzgitter Medical Center, [2]Department of Urology, Martha Maria Medical Center, Nürnberg, [3]Department of Urology, Bamberg Medical Center, [4]Department of Urology, University Medical Center, Erlangen, Germany

Granulosa cell tumor of the testis is a rare intermediate stromal cell tumor that can be distinguished in the adult and juvenile type. The juvenile type is the most common reason of scrotal swelling in newborns under the age of six months old. Less than fifty cases of this disease entity have been reported in literature.

Patients and Methods: In the following article, two newborn patients with scrotal swelling and a histological confirmation of juvenile granulosa cell tumor of the testis will be presented.

Results: Case 1: A newborn patient presented with massive scrotal swelling. Sonography of the testicle exhibited a multiple septic and cystic enlargement of the testicle without distinction of the testicular parenchyma being possible. The laboratory findings demonstrated normal testosterone levels, β-HCG and Inhibin-B levels as well as an increased Alpha-Fetoprotein level of 35.350 ng/dL. Due to clinical and sonoraphic findings an inguinal exploration and later, due to the impossibility of distinction of the testicular parenchyma, an inguinal orchiectomy of the right testicle was performed. Case 2: Clinical and sonographic examination of a newborn patient demonstrated a suspicious process of the left testicle. Sonography exhibited an enlarged testicle with cystic formations with the distinction of the testicular parenchyma not being possible. The laboratory findings demonstrated normal testosterone levels, β-HCG and Inhibin-B levels as well as an increased Alpha-Fetoprotein level of 9.038 ng/dL and LDH of 768 U/I. An inguinal orchiectomy of the left testicle was performed. Histology: In both cases, a histological diagnosis of juvenile granulosa cell tumor of the testis was made.

Conclusion: These two aforementioned cases demonstrate that juvenile granulosa cell tumor of the testis is a benign disease encountered in the newborns, which exhibits an excellent prognosis. Inguinal orchiectomy is the therapy of choice. After surgical removal of the involved testicle is performed no further management is required.

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THE ROLE OF BCL2 AND CYCLIND1 EXPRESSION IN CORRELATION TO KNOWN PROGNOSTIC MARKERS AS TP53 AND KI67, IN INVASIVE BLADDER CANCER

J. Rokahr[1], J. Neumann[1], E. Herrmann[2], M. Bögemann[2], S. Bierer[2], Ch. Wülfing[2], E. Eltze[1]

[1]Institute of Pathology and [2]Department of Urology, University of Münster, Germany

The objective of the study was to examine the expression of BCl2, a suppressor of apoptosis initiation, and Cyclin D1, a commonly altered cell cycle regulator, and their prognostic potential in relation to other prognostic parameters like Ki67 and TP53 in invasive bladder cancer.

Methods: Immunhistochemical stainings were performed for Cyclin D1, BCl2, TP53 and Ki67 on TMA containing paraffin embedded tissues of 219 invasive bladder cancer patients who had undergone radical cystectomy. The results were correlated with clinicopathological parameters and overall and recurrence-free survival.

Results: Expression of the BCl2 was found in only 19 tumours, and only moderately in 6 of these. Overexpression of BCl2 correlated with a low proliferation rate (Ki67<10%, p=0.067) and a low grade (p=0.004). No correlation could be found to stage or TP53 expression. Cyclin D1 expression of more than 10% correlated significantly with pN1 (p=0.031). No correlations to tumour stage, grade, TP53 expression or proliferation rate could be detected. Kaplan Meyer analyses showed a significant shorter overall survival for patients with Cyclin D1 expressing tumors (p=0.022).

Conclusion: This is the first study showing a correlation between Cyclin D1 expression and pN stage and a correlation with a poor prognosis in invasive bladder cancer indicating a role in mediating invasion and metastasis of cancer cells.

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CLINICAL AND SURGICAL EXPERIENCE WITH WILMS' TUMOR: LONG-TERM RESULTS OF A SINGLE INSTITUTION

V. Zugor[1], G. E. Schott[3], R. Kühn[2], A. P. Labanaris[2]

[1]Department of Urology, Salzgitter, [2]Department of Urology, Martha Maria Medical Center, Nurnberg, [3]Department of Urology, University Medical Center, Erlangen, Germany

Although the NWTSG and SIOP studies have included the largest number of patients, several individual institutions have made likewise important contributions to the optimization of Wilms' tumor therapy. The purpose of this study is to present the experience obtained over the last 42 years by treating Wilms' tumor in childhood.

Patients and Methods: Throughout the period 1965-2006, 65 children with histological confirmation of Wilms' tumour were treated at the Department of Urology, University of Erlangen

Medical Centre. The records of all patients presenting to the institution with Wilms' tumour were examined.

Results: The results obtained by this study group are that prognosis in relation to age demonstrated no significance, but according to tumor size, lymph node involvement and distant metastasis there was a correlation. A 10 year follow-up revealed an 89.4% survival rate. Furthermore, the statistic evaluation performed in order to evaluate the significance of surgical complications following neoadjuvant therapy in comparison to non neo-adjuvant therapy exhibited a significant increase in complications in patients who were not treated with neoadjuvant therapy.

Discussion: Although the U.S.A. and Europe have different philosophies on preoperative chemotherapy, most patients with Wilms' tumor survive long term, regardless of the sequence of therapeutic interventions. In the absence of a clear choice between up-front nephrectomy and preoperative chemotherapy, it is reasonable to base the timing of resection on factors such as tumor size, the patient's clinical condition and the experience of the surgeon.

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TESTICULAR NON-HODGKIN'S LYMPHOMA

V. Zugor[1], A.P. Labanaris[2], S. Friedel[3], B. Wullich[4], G.E. Schott[4]

[1]Department of Urology, Salzgitter Medical Center, [2]Department of Urology, Martha Maria Medical Center, Nurenberg, [3]Department of Internal Medicine, Weissenburg, [4]Department of Urology, University Medical Center, Erlangen, Germany

The predisposing factors of testicular non-Hodgkin's lymphoma are not well documented in urologic literature. Few reports exist that discuss the clinical presentation as well as therapeutic options of this rare disease entity. In the following article, four adult patients with a histological confirmation of B-cell type lymphoma will be presented and the therapeutic options and prognostic factors will be discussed.

Patients and Methods: The median age of the patients at the time of diagnosis was 65.5 years old, with a minimum of 40 and maximum of 87 years old. The diagnostic parameters used included clinical examination, laboratory findings, tumor markers, sonography and computer tomography (CT) of the thorax, abdomen and pelvic region.

Results: In order to distinguish whether the disease was that of a primary testicular non-Hodgkin's lymphoma or a secondary testicular involvement of an advanced non-Hodgkin's disease, CT played the decisive role. All patients underwent an inguinal orchiectomy for diagnostic as well as therapeutic purposes. Postoperative, all patients underwent an adjuvant polychemotherapy of 6 cycles in an oncologic department. The polychemotherapy was decided according to the CHOP-Schema and included Cyclophosphamid, Doxorubicin, Vincristin and Prednison, with a combination of the monoclonal antibody Rituximab. A clinical follow up of 24 months reviled a stable condition in all patients.

Conclusion: Despite its high grade of malignity, patients exhibiting a primary testicular non-Hodgkin's lymphoma can be cured. Inguinal orchiectomy, chemotherapy according to the CHOP-Schema and the addition of specific monoclonal antibodies is the therapy of choice.

Therapy

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BRIVANIB, A NOVAL DUAL VEGF-R/BFGF-R INHIBITOR

W. Dempke

University Medical School Munich, Germany

The process of neo-vascularization from pre-existing blood vessels (angiogenesis) plays a critical role in both tumor growth and dissemination in multiple cancer types. Tumor angiogenesis appears therefore to be an attractive target for cancer treatment and the VEGF/VEGF-R and FGF/FGF-R systems have been identified as key factors for neo-angiogenesis. Several active compounds have been developed so far and some of them are already widely used in clinical protocols. However, currently only very few drugs are available to target the FGF receptor although the FGF/FGF-R system has been shown to act synergistically with VEGF. Brivanib (BMS-582664) is a novel orally available and selective receptor tyrosine kinase inhibitor that targets the key angiogenesis receptors VEGF-R-2 and FGF-R-2. The drug is currently under clinical evaluation (phase III) and has shown promising clinical activity and manageable side effets. Moreover, since VEGF-R2 is co-expressed with collagen IV and downregulated by VEGF-R2 antagonists, collagen IV may be a suitable biomarker (surrogate end point) for the anti-angiogenetic activity of brivanib in clinical trials. The potential of such biomarkers for brivanib is currently addressed in the ongoing clinical trials.

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CETUXIMAB + PACLITAXEL/CARBOPLATINUM AS 2ND LINE CHEMOTHERAPY IN RECURRENT OR METASTATIC HEAD AND NECK CANCER

J. Büntzel[1], A. deVries[2], R. Mücke[3], A. Garayev[1], F. Bruns[3], O. Micke[4]

[1]Departement of Otolaryngology, Südharzkrankenhaus Nordhausen, [2]Departement of Radiotherapy, Landeskrankenhaus Feldkirch, [4]Departement of Radiotherapy, Franziskus Hospital Bielefeld, [3]AKTE, Germany

In patients with platinum-resistant recurrent head and neck cancer the Anti-EGF-receptor antibody cetuximab could be used as a treatment option. There are only limited data about results of this therapeutic option. The objective of this study was to evaluate the therapeutic benefit of this indication.

Methods: Thirty three patients with histologically confirmed recurrent head and neck cancer (30 male, 3 female, mean age 59±12 years) were included in this exploratory study. All recurrences had occurred after chemotherapy with platinum derivates. Thirty patients received radiation therapy during primary treatment. No surgical or radiotherapeutic option in recurrent disease was possible. Fifteen patients were suffering from local or locoregional recurrences. Eighteen patients had distant metastasis (17 pulmonary and 1 cerebral). The second line therapy consisted of carboplatinum (200 mg/m^2) + paclitaxel (200 mg/m^2) every three weeks (week 1, 4 and 7) and additionally cetuximab, which was given at 400 mg/m^2.

Results: A significant tumor response was observed in 19/33 patients (56%), 13 partial, 5 minor and one complete remission were registered. The median survival time was 7 months (range 1-14), 10 patients are still alive. Median time to progression was 5 months (range 2-8). Side effects were rash (21/33), fever (12/33) and typical chemotherapy induced toxicities as neuropathy (10/33) and cytopenia (7/33). All side-effects were moderate and easy to handle.

Conclusion: The described combined chemoimmune therapy with cetuximab and paclitaxel + carboplatinum seems to offer new strategies in second and third line chemotherapy for patients with platinum-resistant head and neck cancer, potentially overcoming primary platinum resistance.

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INSULIN-LIKE GROWTH FACTOR-1-RECEPTOR (IGF-1R) IN PRIMARY AND METASTATIC UNDIFFERENTIATED CARCINOMAS OF THE HEAD AND NECK: A POSSIBLE TARGET OF IMMUNOTHERAPY

R.E. Friedrich[1], C. Hagel[2], S. Bartel-Friedrich[3]

[1]Maxillofacial Surgery, [2]Neuropathology, Eppendorf University Hospital, Hamburg, [3]Otorhinolaryngology, Martin-Luther University, Halle-Wittenberg, Halle a.d.S., Germany

Squamous cell carcinoma (SCC) of the head and neck (HN) account for more than 90% of all malignant tumors of the oral cavity and pharynx. This entity is one of the six most frequent cancers worldwide. Although tobacco and alcohol abuse are considered as the primary risk factors, it is obvious that further factors contribute to the genesis of this cancer, *e.g.* viruses acting as oncogenes. Insulin-like growth-factor 1-receptor (IGF-1R) plays a key role in the development of cancer. Earlier studies suggested a correlation between the serum levels of IGF-1 and IGF-binding proteins and prognosis in head and neck carcinogenesis. IGF-1R is a phylogenetically conserved receptor tyrosine kinase, ubiquitously expressed in tissues. IGF-1R plays a role in the regulation of tissue growth, predominantly *via* the growth hormone (GH). Targeting the IGF-1R is a current topic of cancer research. Investigations on IGF-1R for head and neck neoplasms were restricted to SCC. Undifferentiated carcinomas of the nasopharynx (NPC) are different from SCC of the oropharyngeal mucosa in terms of theories of tumor development, in particular with regards to the role of herpes virus in oncogenesis. These tumors might have a different pathogenesis. Oncogenic viruses like human papilloma virus and Epstein-Barr virus are associated with poorly differentiated oropharyngeal and nasopharyngeal carcinoma. The aim of this study was to detect the IGF-1 receptor in samples of undifferentiated carcinomas of the oropharyngeal and nasopharyngeal region. Currently there are no data available for undifferentiated carcinomas of the head and neck and IGF-1R expression.

Materials and Methods: Twelve routinely formalin-fixed and paraffin-embedded specimens of 10 patients with undifferentiated carcinoma were investigated. IGF-1R was detected immunohistochemically. Control of the antibody reaction in tissue was performed with slices incubated and processed according to the protocol but omitting the primary antibody. In some cases the infection status with EBV (DNA) or HPV (RNA) was known.

Results and Conclusion: IGF-1R was detected in all tumors, regardless of differentiation or proof for EBV or HPV integration to the genome. This study reveals a broad expression of IGF-1R in undifferentiated carcinomas of the oropharyngeal and nasopharyngeal region, both in primaries and regional lymph nodes. The results suggest that IGF-1R expression in these tumors is capable of transmitting mitogenic signals to the neoplastic cells. IGF-1R signalling is known to play a crucial role in the development and progression of cancer. This receptor is involved in the regulation of cell proliferation, anti-apoptosis, differentiation and cell motility. It was recently shown that IGF-1R signalling in HNSCC cells induced VEGF signaling (Slomiany *et al*. 2007). The presence of

IGF-1R in undifferentiated carcinomas suggests that these tumors can respond to IGF. Targeting this receptor seems to be a promising tool for the treatment of advanced stage NPC.

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ADHERENCE TO ENDOCRINE THERAPY IN POSTMENOPAUSAL WOMEN WITH BREAST CANCER

T. Bauer, M. Ziller, W. Holzhauer, V. Ziller, U. Wagner, P. Hadji

Universitätsklinikum Gießen und Marburg GmbH, Klinik für Gynäkologie, Gyn. Endokrinologie und Onkologie Schwerpunkt Gyn. Endokrinologie, Reproduktionsmedizin und Osteologie, Marburg, Germany

The level of compliance for various pharmacological therapies in chronic diseases varies but is predominantly low. With tamoxifen (TAM), 23% and 50% of non-compliance after 1 and 4 years have been reported. The aim of this study was to evaluate the compliance rate of a randomly selected sample of women with breast cancer, who have been assigned to an adjuvant endocrine treatment with tamoxifene (TAM) or anastrozole (ANA).

Materials and Methods: A random sample of 100 postmenopausal women with breast cancer (50 TAM and 50 ANA) who had been operated at hospital in 2004/2005 and were thereafter assigned to an adjuvant endocrine treatment were studied. The compliance rate was evaluated with a validated, detailed questionnaire and additionally a prescription control of the hospital chart and by recall of the local physicians was performed.

Results: Baseline characteristics comprised a significantly different mean age of 65 (±3) and 72 (±3) in women on ANA *vs*. TAM ($p<0.001$). The median duration of treatment was 13.6 (±8.8) months for TAM and 16.6 (±5.4) months for ANA. All women on TAM and ANA reported to be 100% compliant by self report. After the controlling for prescriptions, only 40 (80%) and 27 (69%) of the women on TAM and ANA were still classified compliant ($p<0.0027$ and $p<0.00055$). No significant correlation of compliance was found in accordance to baseline characteristics and side-effects in a log regression model.

Conclusion: The results indicated the suboptimal compliance of women with breast cancer on adjuvant TAM treatment. Additionally, an equivalent low compliance with ANA has been evaluated for the first time. More prospective studies are needed to increase the understanding of the reasons for non-compliance in women with breast cancer.

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SIMULATION OF SCATTERING EFFECTS OF IRRADIATION ON SURROUNDINGS USING THE EXAMPLE OF TITANIUM DENTAL IMPLANTS: A MONTE CARLO APPROACH

R.E. Friedrich[1], M. Todorovic[2], A. Krüll[2]

[1]Maxillofacial Surgery, [2]Radiotherapy (Ambulanzzentrum), Eppendorf Medical Center, University of Hamburg, Germany

Irradiation of the head and neck region as an adjunct to ablative surgery or applied in curative intention is one of the most important therapies for head and neck cancer. Occasionally head and neck cancer patients treated with high-energy X-rays and gamma rays have titanium metal dental implants in their jaws. The resulting effect of the bone-metal interface on the radiation dose is of interest for therapists and patients. This study was carried out to calculate alterations of the irradiated bone caused by a foreign body, representing in size and physical qualities a titanium implant, in a stochastic model termed Monte Carlo simulation.

Methods: A two-step Monte Carlo simulation was used. A clinical linear accelerator was simulated using BEAM/EGS4. A 2×2 cm^2 6 MV photon beam of a Siemens Mevatron was modelled in a distance of 100 cm from the target. Initially 109 electrons with energy of 5.58 MeV impinged on the target.

Results: The calculations showed that the presence of an implant results in differences of the dose distribution all around the implant, not only behind and in front of it. Compared to the dose in the plain water phantom, the dose right next to the implant is almost 8% higher. Again this is a result of scattering effects of the photons and electrons. Dental implants made of titanium in the field of irradiation are capable of causing significant scattering of irradiation. The risk for dose enhancement is notably important in the bone in direct contact to the foreign body.

Conclusion: Therapists involved in the irradiation planning are well advised to consider this impact of dental implants on the irradiation beam as a putative cause of osteoradionecrosis.

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PREDICTING RESISTANCE TO CHEMOTHERAPY WITH THE ATP TUMOR CHEMOSENSITIVITY ASSAY IN PRIMARY OVARIAN CANCER

M. Zwirner, M. Stefanova, C. Meisner, E. Solomayer, D. Wallwiener, T. Fehm, H. Neubauer

Department of Obstetrics and Gynecology, Institute for Medical Information Processing, University of Tuebingen, Germany

The standard therapy in ovarian cancer is surgery followed by platinum-containing chemotherapy. Most patients with primary epithelial ovarian cancer (PEOC) treated with carboplatin/ paclitaxel will relapse between one to two years. The goal was to investigate the predictive value for resistence to platinum-containing chemotherapy using the *in vitro* "non-clonogenic" ATP Tumor Chemosensitivity assay (ATP-TCA) and to define the optimal predicting calculation method applied to PEOC treated with platinum-containing chemotherapy.

Materials and Methods: ATP-TCA results from 80 PEOC specimens were analyzed by applying 50% inhibition concentration, sensitivity index (IndexSUM) and area under curve by testing multiple cut-off levels. Correlation between *in vitro* results and clinical outcome was performed for 61 (76%) patients by univariate and multivariate analysis. Tumor recurrence 6 months after chemotherapy was classified as platinum-resistance.

Results: The IndexSUM set at >250 had the highest test sensitivity, specificity, positive and negative predictive values of 90%, 43%, 62% and 81%, respectively. Patients whose tumors were shown to be resistant by ATP-TCA had a higher risk for recurrence (RR) compared to those who tested as sensitive ($p<0.003$, RR=3.3, 95% CI=1.2-9.4). This result was confirmed after adjustment for FIGO stage by logistic regression ($p<0.004$, Odds ratio=8.3, 95%CI=1.9-35.5). In multivariate analysis ATP-TCA and the FIGO-stage were independent predictive factors of early recurrence.

Conclusion: Compared to other known clinical factors the ATP-TCA results analysed with IndexSUM >250 are most useful for predicting the clinical outcome after platinum-containing chemotherapy in PEOC. The combination with the FIGO stage can improve its predicting power.

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EFFECT OF ZOLEDRONATE ON PERSISTING ISOLATED TUMOR CELLS IN THE BONE MARROW OF PATIENTS WITHOUT RECURRENCE OF EARLY BREAST CANCER

B. Rack[1], J. Jückstock[1], E.-M. Genss[1], A. Schoberth[2], C. Schindlbeck[1], B. Strobl[1], M. Heinrigs[1], G. Rammel[1], T. Zwingers[3]; H. Sommer[1], K. Friese[1], W. Janni[1]

[1]I. Frauenklinik, Ludwig-Maximilians-Universität, [2]Laboratory Drs. Tiller and Partners, Muenchen, [3]Thomas Zwingers, estimate GmbH, Augsburg, Germany

Recently, the highest level of evidence was reached for the prognostic impact of isolated tumor cells (ITC) in bone marrow of breast cancer patients both at primary diagnosis and during recurrence-free follow-up. Adjuvant chemotherapy, however, seems to have only a limited effect on ITC in dormant state. The goal of the present study is to investigate the therapeutic efficacy of zoledronate treatment on the persistence of ITC in the bone marrow of breast cancer patients after completion of primary therapy.

Method: One hundred and seventy two primary breast cancer patients without evidence for distant recurrence but detection of ITC in bone marrow were followed. As part of an interventional pilot study, zoledronate was applied at 4 mg q4w x 6 months (loading dose 8 mg) to 31 patients who had completed surgery, leading to R0 resection of their tumor, and adjuvant chemotherapy for at least 6 months. In a matched pair analysis, these patients were compared to those 141 patients who were treated according to standard guidelines but did not receive additional zoledronate treatment. The bone marrow was re-examined after a median of 7.9 months (std 0.89) in the treatment group and 11.5 months (std 12.41; $p=0.11$) in the control group. ITC were detected by immunocytochemical staining using the monoclonal pan-cytokeratin antibody A45-B/B3 and the APAAP technique. Patients were followed prospectively for a median of 39 months following the first aspiration.

Results: Primary tumor characteristics, *i.e.* tumor size ($p=0.09$), axillary nodal status ($p=0.85$), hormone receptor status ($p=0.67$) and histopathological grading ($p=0.35$), as well as surgical ($p=0.22$), adjuvant systemic ($p=0.18$) and irradiation treatment ($p=0.51$) were well balanced between both patient groups. While ITC were detected in all 172 patients at the time of first bone marrow aspiration, four patients (13%) showed ITC following 6 months of zoledronate therapy. In contrast, persisting ITC were detected in 38 patients (27%) of the control group without zoledronate treatment ($p=0.099$). The reduction in cell numbers between first and second aspiration reached statistical significance in the zoledronate group ($p=0.02$) in contrast to control patients ($p=0.14$). Persistent ITC at the follow-up aspiration were associated with reduced recurrence-free survival ($p=0.05$). Among 12 patients without detection of ITC in bone marrow after treatment who underwent additional aspirations, 10 patients showed a persistently negative bone marrow status after a median of 19 months (range 4.7-38.7 months) following treatment. Zoledronate treatment was well tolerated with mild bone pain as the most common side effect in 45% of patients (n=14).

Conclusion: These results indicate a potential antineoplastic effect of the cell-cycle independent agent zoledronate on persisting ITC in dormant state. The data provide a hypothesis generating basis to investigate the therapeutic efficacy of zoledronate on ITC in the secondary adjuvant setting by prospectively randomized trials.

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INHIBITORY EFFECTS OF THE FLAXROOT ON A HORMONE-DEPENDENT HUMAN MAMMA CARCINOMA CELL LINE IN COMPARISON TO OTHER COMMON PHYTOESTROGENS – AN *IN VITRO* STUDY

C. Theil[1], V. Briese[1], S. Abarzua[2], A. Schlichting[3], D.-U. Richter[1]

[1]Department of Obstetrics and Gynaecology, Faculty of Medicine, [2]Institute of Biological Sciences, Faculty of Natural Sciences, University, Rostock, [3]STC Soil Biotechnology, Groß Lüsewitz, Germany

In several studies, extracts from flaxseeds have been proved to reduce tumour growth of hormone-dependent gynaecological cancers. In this study, the anticancer effect of flaxroots was tested on a hormone-dependent human mamma carcinoma cell line (MCF7). The objective of this work is to show the cytotoxic and antiproliferative potential of the flaxroot as a whole in comparison to several single Lignans and Flavonoids: Genistin, Genistein, Daidzin, Daidzein and Secoisolariciresinol, which are self components of the flaxroot.

Methods: The extract was made out of roots from Linum usitatissimum by a method of Luyengi *et al*. (1996) and afterwards dissolved in Ethanol. The molecular-chemical composition of the extract was analysed by pyrolysis-field ionisation mass spectrometry. The MCF7 cell line was cultivated by using DMEM as culture medium supplemented with antibiotics and fungicides. The receptor status of the cells (Estrogen-, Progesterone-, and Androgen-Receptors) was tested by immunhistochemical procedures. The influence of the flaxroot extract and the phytoestrogens on the tumour cells was determined by using three different tests. To quantify cytotoxicity the activity of lactate dehydrogenase was detected (LDH-test). For the determination of cell proliferation, two different methods (MTT-test and BrdU-test) were used. Each of the three tests is a colometric assay and the results were measured by photometric detection with an ELISA Reader. The influence of the flaxroot extract was tested at 8 different concentrations: 0.01, 0.1, 1, 10, 50, 100, 500 and 1000 µg/mL. The single phytoestrogens, also dissolved in ethanol, were respectively used in concentrations of 1, 5, 10, 50 µg/mL. In each test procedure 17β-estradiol and tamoxifen were used as controls, respectively in concentrations of 1, 5, 10, 50 µg/mL.

Results: In mass spectrometry the flaxroot extract was shown to contain low amounts of lignans and flavonoids. The immunhistochemistry demonstrated that all above named receptors were positive. The flaxroot extract showed significant cytotoxic and antiproliferative effects of up to 80% at higher concentrations in all three test methods. Among the single phytoestrogens, genistein induced cytotoxic and antiproliferative effects at higher concentrations in the LDH- and BrdU-test. Secoisolariciresinol showed only in its highest concentration an antiproliferative effect and only in the BrdU-test. The experiments have also demonstrated that the aglycone form genistein has significantly more effect than the glucoside form genistin, which could be seen mainly in the LDH-test.

Conclusion: Thus, substances extracted from flaxroots might have beneficial effects for the prevention and therapy of hormone-dependent mamma tumours. Which specific substance of the roots is responsible for the inhibitory effects needs to be further investigated.

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THE INFLUENCE OF FLAX ROOT EXTRACTS FROM DIFFERENT STAGES OF MATURITY ON THE PROLIFERATION AND CYTOTOXICITY OF ESTOGEN RECEPTOR POSITIVE BREAST CANCER CELLS

N. Strater[1], A. Schlichting[2], S. Abarzua[3], V. Briese[1], D.-U. Richter[1]

[1]Department of Obstetrics and Gynaecology, Faculty of Medicine, University, Rostock, [2]STC Soil Biotechnology, Groß Lüsewitz, [3]Institute of Biological Sciences, Faculty of Natural Sciences, University, Rostock, Germany

Based on the fact that flax contains hormone - like contents (Lignans), the influence of flax root extracts of different stages of maturity were analysed *in vitro* on the proliferation/cytotoxicity of estrogen receptor positive breast cancer cells (MCF7). The stage of maturity was divided into 3 different grades: Grade I: 9 week old roots, Grade II: 6 week old roots, and Grade III: 3 week old roots.

Materials and Methods: The flax root extracts were compounded by the method of lignan extraction from Luyengi *et al*. and analysed by the pyrolysis field ionisation mass spectrometry (Py-FIMS). The extracts were diluted to different concentrations. In order to test the extracts, the commercially acquired breast cancer cell line MCF7 was used. The MCF7's hormone receptor status was verified immunohistochemically. To detect cytotoxicity and grade of proliferation, LDH-Test, BrdU and MTT assays were used, respectively. Estradiol was used as positive control in all analyses and tamoxifen as negative control.

Results: By comparing the different extract grades a cytotoxic effect was discovered. This effect was detected by using high concentrations of extracts grade I and grade III. At concentrations of 1000 µg/mL the result was significant (54% cells were destroyed). The BrdU test showed a

corresponding outcome: the proliferation of the cells was reduced to 40% (Grade I; 1000 μg/mL).

Conclusion: In summary, it can be established that the results suggest more test series with the flax root extracts of grade I and III and with the high concentration levels should be performed to ensure the inhibitory effect of the proliferation of estrogen receptor positive breast cancer cells.

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SEQUENTIAL X-IRRADIATION INDUCED ACQUIRED RESISTANCE TO OXALIPLATIN BUT INCREASED SENSITIVITY TO CISPLATIN IN TWO HUMAN TERATOMA CELL LINES *IN VITRO*

S. Eisenmann, W. Voigt, T. Mueller, W. Dempke

Department of Medical Oncology and Hematology, Martin-Luther-University, Halle/Saale, Germany

Cisplatin (CDDP) and oxaliplatin (OXA-P) are potential therapeutic drugs in the treatment of tes-ticular cancer. However, the emergence of drug resistance has been documented not only in pa-tients after chemotherapy but also subsequently to fractionated X-irradiation. Specific radiation-induced biochemical alterations may play a role in the observed resistance. Since irradiation influ-ences the cellular responses to chemotherapy we investigated changes in the expression of key proteins in the regulation of DNA repair and apoptosis subsequently to sequential irradiation.

Methods: Logarithmically growing human teratocarcinoma cell lines 2101 EP and H 12.1 were subsequently irradiated (10 fractions of 4 Gy *in vitro*) to establish the sublines 2101 EP/DXR-10 and H 12.1/DXR-10. Radiosensitivity was assayed using the clonogenic survival assay. Drug re-sponse was assayed using the sulforhodamine B assay. Expression of p53, PARP, hMSH2 and Fas was detected by Western blotting.

Results: Both DXR-10 sublines showed a significant increase in sensitivity towards CDDP as com-pared to their parental cell line, however, there was a concomitant increase in resistance against OXA-P. No significant changes in radiosensitivity between parental and DXR-10 cell lines were observed. In addition, there was an upregulation of PARP, p53, hMSH2 and Fas in the DXR-10 sublines implicating induced damage tolerance and repair mechanisms following irradiation.

Conclusion: The results suggest that radiation preceeding chemotherapy might induce resistance to subsequent chemotherapy with oxaliplatin but not to cisplatin. This is a novel observation and, if confirmed, particulary in other tumor types, may have clinical implications.

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RECORDING OF RADIATION-INDUCED LATE EFFECTS IN THE EYE - PROPOSAL FOR A NEW CLASSIFICATION

K.M. Sell[1], F. Bruns[2], N. Willich[3], O. Micke[3,4]

[1]Department of Ophthalmology, Ev. Krankenhaus Mülheim, [2]Department of Radiotherapy and Special Oncology, Hannover University Hospital, [3]Department of Radiotherapy and Radiation On-cology, Münster University Hospital, [4]Department of Radiotherapy and Radiation Oncology, Fran-ziskus Hospital Bielefeld, Germany

Radiotherapy in the ocular or orbital region is a less frequent indication. Nevertheless there is a need for a reliable and exact grading of the radiation late effects in this organ system. First steps in this direction are the grading system of the EORTC/RTOG and the LENT-SOMA-Score. Our aim was to develop a score, which allows a fine grading of ocular late effects, involving subjective as well as objective findings adopted from LENT-SOMA and the private health insurance

Table I. *Complication*.

Conjunctivitis	0	1/30	3/30	5/30	7/30	10/30	13/30	15/30	17/30	20/30	22/30	25/30	28/30	30/30
Dry Eye Syndrome	0	1/30	3/30	5/30	7/30	10/30	13/30	15/30	17/30	20/30	22/30	25/30	28/30	30/30
Corneal Ulceration	3/30	5/30	7/30	10/30	13/30	15/30	17/30	20/30	22/30	25/30	28/30	30/30	30/30	30/30
Secondary Cataract	5/30	7/30	10/30	13/30	15/30	17/30	20/30	22/30	25/30	28/30	30/30	30/30	30/30	30/30
Retinopathy	7/30	10/30	13/30	15/30	17/30	20/30	22/30	25/30	28/30	30/30	30/30	30/30	30/30	30/30
Proliferative Retinopathy	10/30	13/30	15/30	17/30	20/30	22/30	25/30	28/30	30/30	30/30	30/30	30/30	30/30	30/30
Secondary Glaucoma	15/30	17/30	20/30	22/30	25/30	28/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30
Optical Nerve Atrophy	17/30	20/30	22/30	25/30	28/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30
Visual Acuity	20/20	20/24	20/32	20/40	20/48	20/60	20/80	20/100	20/120	20/200	20/240	20/400	20/1000	0.0

assessment of diminution of ocular function. This score is based on the service ability of the individual suffering from the late effects. The side-effects are assessed at 1/30 taking the visual and functional loss of the affected eye into account as well as the reversibility of the pathologic process (Table I).

Discussion: This new classification is not only related to the visual acuity caused by the various complications, but also takes into consideration the severity and even prognosis of the complica-tions. Further advantage of this classification is the easy use. The alpha-numeric grading allows an easy and reliable comparison of the side-effects and it is possible to add values of different items to a total.

Conclusion: With the described score it is possible to grade ocular late effects. It was tested in a group of 312 patients with age-related macular degeneration (treated with a total dose of 12 Gy) as well as in a group of 34 patients treated for various ocular or orbital malignancies with higher irra-diation doses (30 to 60 Gy). These first results showed that this grading system was applicable and easy to use and has a high clinical relevance.

Hematology/Melanoma/Bone Metastasis

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25 YEARS OF MULTIPLE MYELOMA: A HISTORICAL UPDATE IN DIAGNOSIS, THERAPY, TRENDS AND PERSPECTIVES

R. Lamerz

Medical Department II, LMU-Klinikum Campus-Grosshadern, Munich, Germany

Multiple myeloma (MM) is a malignant plasma cell disorder with an incicence of about 4.3/100,000 in an age-adjusted US or Western population accounting for 10% of all hematologic cancers. It originates from an asymptomatic premalignant stage of clonal plasma cell proliferation (monoclonal gammopathy of undetermined significance, MGUS) in more than 3% of the population above the age of 50 years progressing to myeloma or related malignancy at a rate of 1% per year apart from an intermittent more advanced asymptomatic stage (smoldering myeloma, SMM) in some patients. A historical overview on myeloma disease is presented comprising the pathogenesis, diagnosis and therapy at initial diagnosis (induction therapy, maintenance) and in relapsing/refractory disease in patients with common or high risk myeloma up to the actual state with further trends and perspectives. Although myeloma is still regarded an incurable disease, early detection and control by well developed diagnostic and staging procedures have significantly improved the chances of appropriate start and choice of medication and longer survival with better quality of life. Besides less often applied radiotherapy and supportive care by critical use of bisphosphonates and vertebro- and kyphoplasty, ongoing progress by the development of "small molecules" inhibitors and search for and treatment of malignant plasma stem cells is still a great challenge for further improvement and hopefully also cure of MM.

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EXTRAMEDULLARY PLASMOCYTOMA: A RARE CASE WITH BIFOCAL MANIFESTATION AT UNCOMMON SITES

F. Bruns[1], S. Janssen[1], F. Laenger[2], C. Dobbelstein[3], C. Reuter[3]

[1]Department of Radiotherapy, [2]Department of Pathology and [3]Department of Hematology, Hemostaseology and Oncology, Medical School, Hannover, Germany

Most patients with plasma cell neoplasia show generalized disease at the time of diagnosis (multiple myeloma). However, about 5% of patients present with either a single bone lesion or, even less commonly, with an extramedullary plasmocytoma (SEP), both without signs of systemic spread. As these tumours are rare and can present without a typical clinical picture, correct diagnosis is difficult to confirm, particularly when the lesions occur at uncommon sites.

Case Report: This is the case report of a 63 year old patient who initially presented with an isolated tumour of the left knee. The patient had been treated for arthrosis of the knees for two years prior to a lump on his left knee being detected and further diagnosed *via* X-ray, CT-scan and fine needle aspiration. The anaplastic tumour was initially diagnosed as rhabdomyosarcoma (RMS) localized at the knee. An interdisciplinary approach with neoadjuvant radiotherapy (RT) was initiated, but shortly before beginning the RT the patient suffered a stroke with hemiplegia. CT and MRI of the head revealed a single cerebral lesion on the left side. Assuming a solitary brain metastasis of the previously diagnosed RMS a surgical resection of this cerebral lesion was performed. After a comprehensive review of this specimen and the previous biopsy including clonality analysis the diagnosis was changed to anaplastic extramedullary plasmocytoma. A generalized disease was excluded by imaging, laboratory tests and bone marrow aspiration. The patient was treated with definitive RT of the left knee with a total dose of 50 Gy and with additional postoperative RT of the cerebral metastasis with a total dose of 35 Gy. Surgery of the knee tumour was not performed after RT.

Conclusion: Correct diagnosis of extramedullary plasmocytomas may be difficult, particularly as this disease is rare and can present with an atypical clinical picture and an atypical immunophenotype. Review by a histopathologist with a special interest in either bone tumours or lymphoproliferative disorders is strongly recommended. In this case two separate extramedullary lesions occurred both in uncommon sites, which posed additional difficulties.

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HAEMOGLOBIN CONCENTRATION AS A PROGNOSTIC PARAMETER OF TREATMENT RESPONSE IN HEAD AND NECK CANCER PATIENTS

J. Büntzel[1], U. Schäfer[2], O. Micke[2], K. Kisters[2], F. Bruns[2], M. Glatzel[3], R. Mücke[2]

[1]Department of Otolaryngology, Suedharzkrankenhaus Nordhausen, [3]Department of Radiotherapy, Zentralklinikum Suhl, Germany, [2]AKTE

Objective: Pre-therapeutic anaemia is a well known independent prognostic parameter in head and neck cancer patients. On the other hand there is only limited data on the importance of treatment induced anaemia during radio-chemotherapy for these patients.

Materials and Methods: A total of 149 patients with head neck cancer were included in this study. Fifty patients received radiotherapy alone (RT) and 99 patients got a carboplatinum-based radio-chemotherapy (RCT). Both groups were comparable regarding tumour localization, age and tumour stage. The pre-therapeutic haemoglobin concentration (HB) was measured as well as the lowest HB during the RT/RCT course. The survival time was evaluated according to the individual out-patients-department file. The median follow up time was 46 months (range 18-96).

Results: Before treatment a clinically significant anaemia was observed in only 7/149 patients (5%). During therapy 4/50 RT (8%) and 65/99 RCT (66%) patients had developed anaemia according to the WHO criteria. If the HB nadir is lower than 6.9 mmol/L, the overall 5-years-survival of RCT patients decreased from 60% to 39% (p=0.0236). No influence was seen for the therapy-related HB decrease during RT alone.

Conclusion: The results suggest that treatment-induced anaemia is a possible marker for the outcome in patients treated with platinum based RCT for head and neck cancer. Further studies are necessary to investigate the influence of other RCT combinations.

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TREATMENT RESULTS AND PROGNOSTIC FACTORS FOR RADIATION THERAPY FOR GIANT CELL TUMORS OF BONE: LONG-TERM RESULTS OF A MULTICENTER STUDY IN GERMANY

O. Micke[1], F. Bruns[2], H.T. Eich[3], R. Muecke[4], J. Buentzel[5], N. Willich[6], M.H. Seegenschmiedt[7], German Cooperative Group on Radiotherapy for Benign Diseases

[1]Department of Radiotherapy, Franzikus Hospital Bielefeld, [2]Department of Radiotherapy and Special Oncology, University Hospital Hannover, [3]Department of Radiotherapy, University Hospital Cologne, [4]Department of Radiotherapy, St. Josef Hospital, Wiesbaden, [5]ENT Department, Südharz Hospital Nordhausen, [6]Department of Radiotherapy, University Hospital Münster, [7]Department of Radiation Oncology, Therapeutic Radiology and Nuclear Medicine, Alfried Krupp Hospital, Essen, Germany

Giant cell tumors (GCT) of the bone are rare tumors accounting for about 5% of all primary bone tumors. Although histologically benign, their clinical behavior is unpredictable and they may grow locally aggressive and potentially metastasize. Thus they have been characterized as semi-malignant. Surgical resection is the primary treatment option in GCT. Historically, radiotherapy (RT) has been avoided because GCT was considered to be radioresistant and RT was thought to increase the risk of sarcomatous transformation. Nowadays RT is still used in unresectable, recurrent or incompletely resected cases. Since the place of RT in the multimodal treatment of GCT is not well defined and prognostic factors for radiation response not well described, the German Cooperative Group on Radiotherapy for Benign Diseases (GCG-BD) performed a national cohort study.

Methods: Six cooperating German institutions collected the clinical features, treatment concepts and outcome data of all patients with GCT referred to local RT during the last 30 years; the updated outcome was determined from case history notes, tumor registry correspondence or by individual telephone interview. Study end points were pain relief after RT (complete, partial or no pain relief), symptomatic and radiological response, recurrent disease activity and treatment-related side-effects. Median follow-up was 65 (6-358) months. Uni- and multivariate survival analyses were performed to identify factors to predict radiation response and tumor control. For comparison a comprehensive literature review with 34 published studies (1960-2007) representing 243 patients was used.

Results: From 1975 to 2005 a total of 26 patients with 34 lesions were irradiated for GCT. There were 14 female and 11

male patients (gender ratio: 1.3:1). Median age was 30 (12-71) years. All tumors were pathologically confirmed. The sites of involvement were as follows: cervical spine (n=3; 11.5%), thoracic and lumbar (n=10; 38.6%), sacrum (n=9; 34.7%), temporal bone (n=1; 3.8%), humerus (n=1; 3.8%), femur (n=1; 3.8%) and hand (n=1; 3.8%). Fifteen patients (57.7%) had undergone RT for primary or recurrent gross tumor disease and the remaining 11 (42.3%) had been treated with RT after incomplete or intralesional resection. Radiotherapy of the involved bony and soft tissue structures was performed with a median total dose of 42 (35-60) Gy, median single dose 2 (1.6-3) Gy. Twenty one of 26 tumors (80.8%) were controlled locally. All of the local recurrences occurred within the irradiated field. Three patients (11.5%) developed distant metastasis. The actuarial 5-year overall and disease-free survival rates (Kaplan-Meier method) were 88% and 56%, respectively, and the actuarial 5-year local control and distant metastasis-free survival rates were 60% and 89%, respectively. Univariate analysis of overall, disease-free survival and local control showed no influence of gender, age or previous treatment on the treatment outcome. On the other hand prior surgical resection, no gross tumor disease, tumor size less than 10 cm and a RT dose of more than 42 Gy were favorable prognostic factors. No acute and late radiogenic side-effects >Grade 2 (RTOG/EORTC) were observed, and particularly no secondary malignancies. In comparison, the literature review revealed a similar local control rate with a mean of 77%.

Conclusion: This study comprises one of the largest data bases of cases reported for RT in GCT. RT is an easy, safe and effective method of treatment in GCT. Total doses of at least 42 Gy result in better tumor control. RT is effective as an adjuvant measure, even in unresectable cases RT gives satisfactory treatment results. The study may serve as a starting point for a pattern of care study on RT for GCT. An international registry for rare benign diseases is recommended to include this benign disorder.

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PROGNOSTIC VALUE OF BONE MARKERS IN PATIENTS WITH CARCINOMA

A. Oertl[1], M. Speda[2], H. Sauer-Eppel[2], D. Jonas[1], G.M. Oremek[2]

[1]Department of Urology, [2]Department Laboratory Medicine, Internal Medicine, Hospital of the Johann-Wolfgang-Goethe-University, Frankfurt, Germany

The usefulness of bone markers alkaline phosphatase (AP) and tartrate resistant acid phosphatase 5b (TRACP 5b) for diagnosis, treatment and monitoring of patients with carcinoma of different origin was investigated. AP is a marker of bone formation, while TRACP 5b is a marker of bone resorption. The isoform 5b of the enzyme TRACP is expressed by osteoclasts and can be measured in blood. The aim of this study was to evaluate the prognostic value of the bone markers AP and TRACP 5b to detect bone metastasis and pathological bone metabolism.

Materials and Methods: The study comprised 101 patients with positive tumor markers. Sera of these patients were collected and the bone markers AP and TRACP 5b were determined. TRACP 5b was measured by a colorimetric test which determines the TRACP 5b by using the phosphatase activity of this enzyme by dephosphorylation of p-Nitrophenyl-phosphate (pNPP). The test is a two site immunoassay. The AP analysis was performed with the Hitachi 917 analyzer.

Results: The sensitivity and specificity for AP in this study were 52.9% and 53.9%, respectively. TRACP 5b shows a sensitivity of 64.7% and a specifity of 70.9%. An elevated TRACP 5b activity is significantly associated with bone metastasis (p=0.01). In patients with chronically elevated levels of liver enzymes a significant elevation of TRACP 5b (p=0.005) was observed. The mean activity of AP in the group without malignancy has been 171.37 U/l, in patients with malignant disease but without bone metastasis 262.80 U/l and in patients with bone metastasis 495.41 U/l. The mean serum levels of TRACP 5b activity in patients without malignancy was 4.22 U/l, the highest activity was found in patients with malignancy and additional bone metastasis with 6.75 U/l and patients with malignancy had a mean activity of 5.04 U/l. A significant difference between the patients without malignant disease, those with malignancy and the patients with additional bone metastasis could be shown for AP (p=0.009) and TRACP 5b (p=0.01).

Conclusion: TRACP 5b is more sensitive and specific in the detection of bone metastasis and bone turnover than the AP. In patients with multimorbidity the origin of AP is not clear due to its multiorganic appearance. The levels of TRACP 5b are elevated in patients with bone metastasis and in patients with chronical dysfunction of the liver. TRACP 5b might be helpful in the diagnostic procedure of tumor patients to detect bone metastasis. Moreover TRACP 5b seems to be helpful to indicate oncological patients with early dysfunctions in bone metabolism and to provide early treatment to these patients.

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NOVEL MARKERS OF BONE METASTATIC PROCESS – USE OF MULTIPLEX ASSAY: A PILOT STUDY

J. Vrzalova, M. Prazakova, O. Topolcan, L. Holubec Jr.

University Hospital Pilsen, Czech Republic

The rapidly developing multiplex analytical technology opens the doors for multimarker blood monitoring of cancer processes, which can further help in bone metastasis detection. One of these tools can be the multiplex Human Bone Panel introduced in 2007. The aim of this study is to monitor the usefulness of multiplex bone metabolism panel for tumour induced bone disease (bone metastases) detection by serum test and to set up the normal serum levels for parameters included in multiplex panel.

Methods: The following patient cohorts were studied: Group 0: control group - 20 healthy blood donors – (8 men, 12 women); Group 1: 13 cancer patients with bone metastases; Group 2: 11 cancer patients without bone metastases. Serum levels of osteoprotegerin, osteopontin, osteocalcin, parathormon and leptin were measured by multiplex xMAP technology with the use of Human Bone Panel A. Routinely used serum bone markers: PINP, PIIINP, ostase, ICTP and 25-hydroxyvitamin D were assessed for groups 1 and 2. Blood marker levels were compared between groups by Wilcoxon test. Normal values for multiplex markers were set as a 95 percentile of group 0. A scoring system was created for better discrimination of group 1 and group 2 – each value above normal level is scored by 1 point, points for osteoprotegerin, osteopontin, P3NP, ICTP and ostase were counted up.

Results: Significantly higher levels of osteoprotegerin and osteopontin in both cancer groups compared to the control group were found. Significantly higher levels of PIIINP and ostase were found in group 1 compared to group 2. Three of 4 patients with multiple bone metastases had values above the set normal values both for osteoprotegerin and osteopontin in comparison to all other cancer patients, where only one of these markers was positive. A positive score of 3 or higher was counted for 46% of patients in group 1 (6/13) in comparison to 9% in group 2 (1/11), and score 2 or higher for 61.5% patients (8/13) in group 1 in comparison to 36% (4/11) in group 2.

Conclusion: It has been shown that multiplex immunoanalysis can be used for oncology, however the most promising have turned out to be osteoprotegerin and osteopontin, which agree with literature sources. It seems that the incorporation of other bone markers such as PIIINP or ostase into the multiplex panel would be very useful for oncology, nevertheless, an investigation on a larger cohort is necessary. *This study was supported by research project VZ MSM 0021620819.*

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EXPRESSION OF PROTEASES IN GIANT CELL LESIONS OF THE JAWS, TENDON SHEATH AND SALIVARY GLANDS

R.E. Friedrich[1], J. Eisenmann[2], K. Röser[2], T. Löning[2,3]

[1]Maxillofacial Surgery, [2]Department of Oral Pathology, Institute of Pathology, Eppendorf University Hospital, [3]Institute of Pathology, Albertinen-Krankenhaus, Hamburg, Germany

The giant cell lesion of the jaw (*epulis gigantocellularis*) is a soft tissue tumor situated on the gingiva being of hemispheric shape. The lesion is poorly defined and shows tumor-like qualities. The bone adjacent to this lesion often shows extensive lytic areas. These investigations were carried out to better define the cellular compartments of the lesion and to compare the proteases-expression profile in giant cell lesions of the jaws to giant cell lesions of other sites.

Materials and Methods: This immunocytochemical study comprises 54 giant cell lesions, 30 of the jaws, 22 of tendon sheaths and 2 of salivary glands. The micro-array technique was applied to the immunohistochemical study of osteoclast-specific or osteoclast-like features in giant cell lesions of different sites (CD 68, CD 51, RANK, M-CSF). Proteases were immunohistochemically identified (cathepsin K, L, S and matrix metalloproteinase 9 (MMP 9)).

Results: The giant cells of all lesions were identified by the antibodies identifying CD 68 (macrophages/monocytes) and CD 51 (vitronectin-receptor, osteoclast-specific marker). Factors indicating the differentiation and activation of osteoclasts were detected in all lesions (RANK, M-CSF). The expression profile of M-CSF in giant cells and stroma cells was of a medium grade in cases with no apparent osteolysis of bone, whereas RANK was expressed only weakly in mono- or polynuclear CD-68 positive cells. Cathepsin K was detected in all giant cell lesions. Despite an intense staining in peripheral giant cell granuloma (epulis gigantocellularis) an osteolysis of jaws could not be revealed. MMP 9 was revealed in giant cell lesions of all types. Cathepsin S expression was restricted to mononuclear CD-68 positive cells. However, cathepsin L was detected in both mono- and polynuclear giant cells.

Conclusion: The results of this study reveal an identical cellular composition of all lesions irrespective of site. Giant cells contain the same osteolytic proteases and express cytokines that are effective in bone metabolism. Therefore the giant cells of all lesions possess osteoclast-like characteristics and fulfill the morphological pre-requisite to disintegrate bone matrix. The reason for the absent osteolysis in some “epulis” cases might be due to the topography of the lesion. Several studies have shown that only osteoclasts express “mature” cathepsin K that are in close attachment to the bone. Furthermore, the differentiation of mononuclear cells to mature osteoclasts requires the interaction of ligand generation by stimulation of M-CSF. Possibly the reduced number of binding sites, revealed by the low expression profile of RANK, might be responsible for an absent or only superficial osteolysis in these cases, despite evidence of M-CSF.

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DYSPLASIA OF THE ORBIT AND ADJACENT BONE ASSOCIATED WITH PLEXIFORM NEUROFIBROMA AND OCULAR DISEASE IN NF1

R.E. Friedrich[1], C. Hagel[2], H. A. Scheuer[3]

[1]Maxillofacial Surgery, [2]Neuropathology, [3]Orthodontics, University Hospital Hamburg, Germany

Background and Perspective: Neurofibromatosis type 1 (NF1) is an autosomal-dominant inherited disease. Neurofibromas are the hallmark of phakomatosis. Interestingly, generalized and localized interference or dysfunction of bone is also a key element of NF1 phenotype, *e.g.* short stature or pseudarthrosis. In the skull, NF1-associated orbital dysplasia often results in a severe disfigurement of affected individuals. However, the underlying pathology of orbital dysplasia is a complex phenomenon and up to now poorly understood. Earlier reports focussed on the dysplastic sphenoid bone as the cause of orbit deformity. They argued for a primary dysplasia of bone, resulting in temporal lobe displacement into the orbit and consecutive extension of orbital walls. This phenotype is eventually associated with unilateral pulsating exophthalmos. Current reports point to the fact that plexiform neurofibroma (PNF), arising in the branches of the trigeminal nerve, might also contribute to orbital dysplasia (Jacquiemin *et al*. AJNR 2003). Therefore, orbital dysplasia visible on plain radiographs, computed tomograms (CT) or magnetic resonance images (MRI) could be an indicator of a PNF. This study was performed to describe the orbit in NF1 patients with a PNF of the orbital region.

Materials and Methods: This study comprises physical, radiological and histological findings of 42 patients. All patients were diagnosed as being affected by NF1 according to the updated NIH criteria (Gutmann *et al*. JAMA 1997). Inclusion criteria to the study were a visible affection of the orbital region by a histologically proven, diffuse or invasive plexiform neurofibroma (Friedrich *et al*., Anticancer Res. 2003) of the eyelids or orbit. The extension of the orbital or eyelid PNF to adjacent regions (lids, orbit, brow, oral cavity, cheek, nose, temporal region) was determined and functional defects evaluated. Tumor extension and evidence for optic nerve glioma were determined on magnetic resonance images and computed tomograms. Correlative statistics were carried out to provide evidence for association of lesions (Kendell Tau B).

Results: The tumors were plexiform-diffuse neurofibroma in all but one patient with no further specification this tumor. A dysplastic orbit on the affected side was diagnosed in 80.9%. Age at time of NF1-diagnosis varied (1 to 5 years (ys): 21 (50%), 6 to 19 ys: 4 (9.52%), 11 to 15 ys: 2 (4.76), 16 to 20 ys: 4 (9.52%), 21 to 30 ys: 5 (11.9%), >30ys: 0). Orbital tumor extension to adjacent regions revealed a significant correlation of orbit and temporal region (0.33, $p<0.034$), cheek and oral cavity (0.4, $p>0.011$), oral cavity and nose (0.35, $p<0.026$), and temporal region and cheek (0.46, $p<0.003$). Alterations of the optic nerve and adjacent structures were identified on MRI or CT in 14 patients (thickening of the nerve: 5, optic nerve atrophy: 2, enlargement of optic canal: 4, irregular borders of the optic canal: 1, glioma: 3, glioma including the chiasma opticum: 3). On plain radiographs only the sphenoid wing dysplasia and ipsilateral orbital enlargement were significantly correlated (0.528, $p<0.01$). Enlarged orbit was associated with PNF and/or optic glioma. However, a diminished orbit was also found in 3 cases (PNF of the temporal fossa only; shrunken orbit following exenteration). Concerning physical findings and excluding the visual acuity of patients, ptosis and ectropion of the lower lid were the only functional deficits with a statistically significant correlation (0.41, $p<0.009$). The vision of the eye of the affected side was reduced in more than 50% of patients (0.0-0.5, n=18; 0.6-0.9, n=6). The prevalence of optic glioma (14.3%) is in the expected range concerning this entity as far as imaging modalities like CT and MRI were available for evaluation. The prevalence of optic glioma is higher than currently published if patients with radiologic findings typically found in optic glioma are additionally considered, that were evaluable on plain radiographs only (30.9%).

Conclusion: Up to now the current hypothesis that PNF act as main factor of orbital dysplasia is based on radiological assumptions and lacks the histological evidence. This study reveals PNF as the main component of soft tissue affecting eye lids and orbit in those cases, which show a soft tissue mass in the affected orbital region. The invasive growth of PNF into the orbit is likely to result in orbital disfigurement, in particular in patients with growth spurts of the tumor in early phases of life. The oval-shaped orbital rim, typically seen on plain skull radiographs in sagittal projections, seems to be strongly associated with the extension of a PNF and independent from sphenoid wing dysplasia. This oval shape is frequently associated with facial scoliosis. The optic pathway glioma is a further independent finding and a main diagnostic feature of NF1. The study shows that the co-incidence of PNF with an optic glioma is likely to occur more frequently than previously expected. Several factors constitute the individual orbital dysplasia, including the growth of the invasive PNF.

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ROLE OF SERUM FREE LIGHT CHAIN MEASUREMENTS IN DIAGNOSIS AND MONITORING OF MULTIPLE MYELOMA AND OTHER MONOCLONAL GAMMOPATHIES

J. Wierecky

Praxis für Innere Medizin, Hämatologie und Internistische Onkologie, Hamburg, Germany

Multiple Myeloma is a malignant disease characterised by uncontrolled growth of plasma cells in the bone marrow. The majority of patients with myeloma produce an excess of free light chains. Until now, detection of monoclonal gammopathies has relied on serum and urine electrophoretic assays. These are, at best, semi-quantitative, insensitive and time-consuming. Moreover, urine assays include complications and difficulties of urine collection and testing and can also be adversely affected by renal function. The free light chain assay allows accurate measurement of serum free light chains. Clinical application of serum free light chain assay is well documented in the diagnosis and monitoring of patients with AL Amyloidosis, Nonsecretory Multiple Myeloma, Light Chain Multiple Myeloma and Intact Immunoglobulin Multiple Myeloma. Its high sensitivity enables to identify patients missed by electrophoretic assays alone when screening for monoclonal gammopathies. Due to their short half-life, free light chains in serum can be used confidently as a rapid indicator of response to treatment. Moreover, the free light chain assay can help identify patients with a high risk of progression and poor prognosis. It enables risk stratification of MGUS patients and identifies those at high risk of progression to monoclonal disease. The free light chain assay has improved detection and monitoring of monoclonal gammopathies.

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THE DYNAMICS OF SERUM TUMOR MARKERS AND THEIR ROLE IN PREDICTING METASTATIC UVEAL MELANOMA

V. Barak, I. Kaiserman, S. Frenkel, K. Hendler, I. Kalickman, R. Folberg, J. Peter

Hadassah - Hebrew University Medical Center, Jerusalem, Israel; The University of Illinois at Chicago, USA

The Tumor Markers Osteopontin (OPN), S-100β, Melanoma-Inhibitory Activity (MIA) and Tissue Polypeptide-Specific Antigen (TPS) were found to be elevated in patients (pts) with Metastatic Uveal Melanoma, as compared to Disease Free (DF) pts. The aims of this study were to examine the kinetic increases in the Tumor Marker levels and to evaluate their potential and Lead Time to predict Liver Metastases.

Methods: The study included 3 groups: 64 Uveal Melanoma pts who remained disease-free (DF) for at least 10y; 37 pts with Liver Metastases, in 29/37 we had documented pre- and post-metastasis levels; and 53 controls. The mean levels of OPN, S-100β, MIA and TPS were calculated at 6, 12, 18, 24, and >24m prior to confirmation of Metastases by Liver US, C.T. and biopsy .All levels were compared to those in >24 m time window, using Student's *t*-test.

Results: A statistically significant difference between high OPN (p=0.0037), MIA (p=0.0005), S-100β (p=0.0111) and TPS (p=0.001) levels in Metastatic Uveal Melanoma pts and low levels in DF pts or Controls, was demonstrated. Significant differences were found between all 4 marker levels in the Metastatic stage as compared to their pre-Metastatic stage (p<0.05). ROC analysis was performed for Metastatic *vs*. DF pts and revealed AUC for single tests: MIA -88%, S100β -77%, OPN -73%, TPS- 71% and for combinations: OPN+S100β-85%, OPN+MIA-82%, OPN+TPS-82% and for all 4 markers -91%. The Lead-Time was 6-12 m for OPN (p=0.006), 6-9 m for S-100β (p=0.03) and MIA (p=0.06). The increase in TPS levels was not consistent.

Conclusion: A significant increase in the Tumor Markers OPN, MIA, S-100β and TPS levels, prior (Lead Time of 6-12 m) to CT diagnosis, predict the development of liver metastases in Uveal Melanoma and may enable earlier and more effective therapeutic intervention to prolong survival.

Baltic Sea Session

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THYMIDINE KINASE AS A MARKER IN MALIGNANCY

I. Jaunalksne[1], T. Romanova[1], V. Priedite[1], S. Donina[2], J. Basko[3]

[1]Clinical Immunology Center, [2]VSIA Eastern Hospital, [3]Thoracal Surgery Center,VSIA Paula Stradina KUS, Riga, Latvia

Thymidine kinase (TK) is an enzyme which takes part in in cell devision processes. In normal cell development serum TK is produced in low levels. In oncological diseases TK from the dying cells is released, and further tumor cells produce TK allowing its detection in serum. TK is a differentiation marker for slow and rapid growing tumors. Nowadays new methods for TK detection are coming into routine practice. The aim of this study is to observe TK detectection by enzyme linked immunosorbent assay (ELISA) routinely for oncological patients.

Materials and Methods: One hundred and twenty patients were divided into three groups: group 1 - gastrointestinal

cancer patients; group 2 - patients with lung cancer; group 3 - control group. TK was detected by Elisa in patients serum. Results above 5 U/l were interpreteated as positive.

Results: TK level in the control group was 4.4±1.8 U/l, gastrointestinal cancer patients had 2.8±1.7 U/l in average and 10.4±14.1U/l 10 days after surgery, lung cancer patients had 7.6±17.5 U/l, and patients with benign disease had 3.5±1.8U/l.

Conclusion: Lung cancer patients had elevated TK levels, but gastrointestinal cancer patients showed an elevation after surgery suggesting an inflammatory component after surgery or higher release from tumor cells. TK monitoring could be used as an additional marker for cell proliferation indication.

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THE RELATION BETWEEN HER-2 AND HER-3 IN TISSUE AND THE LEVEL OF HER-2 IN SERUM IN TUMOR EFFUSIONS OF PATIENTS WITH OVARIAN CARCINOMA

E. Sobańska[1], A. Harłozińska[1], P. Sedlaczek[1], J. Rabczyński[2]

[1]Department of Clinical Immunology and
[2]Department of Pathomorphology,
Medical University, Wroclaw, Poland

It is known that HER-2 and HER-3 are tyrosine kinase receptors associated with signal transduction pathway and that HER-2/HER-3 heterodimers deliver the most potent and long-lasting signal among the possible combinations of the four members of HER family receptors. The possibility of applying herceptin and an antibody directed against HER-3 in the therapy of ovarian carcinoma patients is discussed, however interactions between HER receptors in these carcinomas remain unclear. Similarly, the association between HER-2 expression in tissue and its concentration in sera of patients are still controversial. The aim of the study was the immunohistochemical evaluation of the expression of HER-2 and HER-3 oncoproteins in tumor tissue sections.

Methods: Tumor sections of 63 histologically verified ovarian carcinomas were investigated, taking into account the conventional pathological variables. The mutual relations between studied markers were also assessed. Moreover, in 28 patients it was possible to estimate the level of circulating HER-2 and compare the expression of this marker in tissue, serum and cyst or ascitic fliud of individual patients.

Results: Significant inter- and intra- tumoral heterogeneity of staining for both parameters was observed. The expression of HER-2 was detected in 37% and HER-3 in 62% of carcinomas. The analysis of mutual relations between both proteins revealed that simultaneous expression of these receptors was detected in 29% of cases. A weak correlation between HER-2 and HER-3 was observed in ovarian carcinomas (r=0.26, p=0.039). No significant correlation between HER-2 and HER-3 and histopathological subtypes, grade of diffrentiation and stage of disease was revealed, however, a trend towards a higher HER-2 expression in III/IV FIGO stages was observed. The higher values of HER-2 in tumor effusions than in corresponding patients sera were detected. Contrary to the sera, the concentration of HER-2 in tumor effusions appeared to be more associated with its expression in tumor tissue.

Conclusion: Preliminary results indicate the existence of subgroups of patients with different expression of HER-2 and HER-3 receptors probably determining their sensitivity for therapy with defined antibodies. In therapy of patients with phenotype HER-2+/HER-3+ application of two types of antibodies could be taken into account. Moreover, HER-2 evaluation *in situ* for circulation seems to be more helpful in planning of therapy blocking HER-2 receptor in ovarian carcinoma patients.

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INSULINE LIKE GROWTH FACTOR-1 (IGF-1) CEA AND CA15-3 IN BREAST CANCER (BC) PATIENTS AT THE TIME OF DIAGNOSIS

S. Donina, I. Nuke, L. Engele

Laboratory of Immunology, East Clinical University Hospital, Oncology Center, Riga, Latvia

It has been suggested that risk of cancer is higher among people with raised concentration of IGF-1 with some studies supporting the role of IGF-1 as a breast cancer risk biomarker. The aim of this study was to determine the sensitivity of IGF-1 in premenopausal and postmenopausal breast cancer patients and compare these findings with sensitivity of well known tumor markers for breast cancer CA15-3 and CEA.

Methods: Sixty four premenopausal and 117 postmenopausal BC patients were examined for CEA, CA15-3 and IGF-1 before any treatment. Fourty eight premenopausal and 82 postmenopausal healthy women were used as a control for IGF-1. Tumor markers and IGF-1 were detected in patients serum by chemiluminiscence using commercial kits. Blood samples were collected and examined in the same day.

Results: IGF-1 levels in premenopausal and postmenopausal control group were 162.5±58.3 ng/mL and 122.5±38.7 ng/mL, respectively, and these levels were accepted as the cut-off value for each group. Two out of 64 (3.1%) premenopausal BC patients had elevated CEA level at the time of diagnosis. 2/64 (18.8%) patients had elevated CA15-3 level above cut-off and

18/64 (28.1%) – IGF-1 level above 162.5 ng/mL. Twenty one out of 117 (17.9%) postmenopausal BC patients had pretreatment CEA level above 5 ng/mL. Thirty four out of 117 (29.0%) showed CA15-3 concentration in serum above 37 U/mL and 42/117 (35.9%) had IGF-1 serum level above the accepted cut-off in the postmenopausal women group.

Conclusion: IGF-1 showed higher sensitivity in postmenopausal BC patients at the time of diagnosis and could be used as additional serological tumor marker in this group of patients.

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SITE SPECIFIC MONOCLONAL ANTIBODIES AGAINST THYMIDINE KINASE 1 AND THEIR USE IN TUMOR MARKER STUDIES

S. Eriksson[1,2], E. He[3], E. Sjuvarsson[2], S. Skog[3], P. Matsson[1], A.-C. Aronsson[1]

[1]AroCell AB, [2]Swedish University of Agricultural Sciences, BMC, Uppsala, Sweden, [3]SSTK Inc., Shenzhen, China

Thymidine kinase 1 (TK1) is a key enzyme in DNA synthesis and TK1 is expressed only in proliferating cells, including tumor cells. Increased activity and TK1 protein levels have been found in sera from patients with malignancies. So far no defined monoclonal antibodies against human TK1 are available. The design, production and characteristics of two site specific TK1 monoclonal antibodies is presented as well as their use in tumor marker studies.

Methods: Peptides corresponding to the highly conserved thymidine binding region of TK1 (XPA161) as well as to the variable C-terminal region (XPA210), involved in cell cycle regulation, were conjugated to a carrier protein and used for immunization of Balb/C mice. Hybridoma cells were produced and cloned by serial dilutions using the peptide conjugates for initial and secondary screenings. Two sets of monoclonal antibodies were identified, produced and characterized i.e. anti-XPA161-528-2 and anti-XPA210-57M.

Results and Conclusion: Both types of monoclonal antibodies reacted with high affinity with recombinant and native TK1 in western blotting and in immunohistochemistry studies. Proliferating but not resting or differentiated cells in normal tissues such as skin and lymph nodes were strongly stained with both type of antibodies as were different type of tumor tissues *e.g.* from breast and lung carcinomas. Sera from patients with breast and head and neck carcinomas were analysed in the dot blot ECL procedure, showing highly increased levels of TK1 as compared to healthy controls with anti-XPA161-528-2 or anti-XPA210-57M alone. When combined the number of positive sera increased significantly, indicating a higher sensitivity of the assay. Preliminary results with a prototype ELISA as well as results from dot blot ECL assays will be presented.

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RELEVANCE OF BIOMARKER MODELS FOR PREDICTION OF THERAPY RESPONSE AND ESTIMATION OF PROGNOSIS IN ADVANCED LUNG CANCER PATIENTS

S. Holdenrieder[1], D. Nagel[1], V. Heinemann[1], J. von Pawel[2], H. Raith[2], K. Feldmann[2], U.-H. Stenman[3], P. Stieber[1]

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

Pre-therapeutical parameters are used to estimate the probable therapy response (prediction) and overall survival (prognosis) to stratify cancer patients for systemic therapies while intra-therapeutical markers indicate the effective therapy response.

Methods: In a prospective study on 300 patients with newly diagnosed advanced NSCLC undergoing first-line chemotherapy, 60 clinical, "classical" laboratory and oncological biomarkers were investigated pre- and intra-therapeutically to test whether i) predictive and prognostic markers are identical, ii) biomarkers have additive prognostic impact to clinical factors, iii) intratherapeutical biomarkers improve prognostic models, and iv) biomarkers are useful for early estimation of therapy response. Univariate evaluations were done by Wilcoxon and Logrank tests; for multivariate analyses Cox regressions were used.

Results: Thirty percent of patients had progression after 2 cycles of chemotherapy and 57% deceased during observation time (1-29 months). Concerning pre-therapeutic parameters, strong predictors of therapy response were also highly relevant for prognosis, such as performance score (PS), metastases other than lung (MOL), chemotherapy, WBC, CRP, albumin, CYFRA 21-1, nucleosomes, CA125, CA15-3 and CA72-4; while LDH, AP, GGT and cholesterol had only prognostic relevance. In multivariate analysis, PS, MOL, chemotherapy, CRP and CYFRA 21-1 were independent prognostic markers. When intra-therapeutic parameters joined multivariate analysis, baseline values of CYFRA 21-1 and nucleosomes before 2nd cycle of chemotherapy (BV2) and therapy response were strong independent prognostic markers along with PS, MOL, CRP and chemotherapy, and improved the strength of the prognostic model. Further, the combination of nucleosomes on day 8 and CYFRA 21-1 (BV2) already enabled the correct detection of insufficient therapy efficacy

after one application of chemotherapy in 29% of progressive patients with 100% specificity. At 90% specificity, sensitivity rose to 55%.

Conclusion: In advanced lung cancer, highly predictive pre-therapeutical biomarkers had high prognostic relevance. Biomarkers determined during the first cycle of chemotherapy improved the prognostic model and enabled the early estimation of therapy response.

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IL-6 AND VEGF IN SMALL CELL LUNG CANCER PATIENTS

J.K. Kulpa, E. Wojcik, P. Skotnicki, J. Tarapacz

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

Angiogenesis is one of the processes recognized as essential for tumor growth and metastases. Vascular endothelial growth factor (VEGF) plays secreted by tumor cells plays an important role in tumor angiogenesis. Several studies have suggested that inflammatory cytokines can enhance the tumorigenic process by up-regulating important mediators of angiogenesis, such as VEGF. The aim of the presented study was the assessment of VEGF and IL-6 in comparison with NSE and ProGRP levels in respect of prognosis of small cell lung cancer patients.

Materials and Methods: Determinations of NSE, ProGRP, VEGF and IL-6 concentrations were carried out before treatment in 72 patients with SCLC (40 with limited and 32 with extensive disease). The same set of parameters were assessed in the reference group consisting of 36 healthy persons.

Results: In small cell lung cancer patients as compared to the reference group, significantly higher levels of all parameters under study were found. Elevated levels of VEGF, IL-6, NSE and ProGRP in SCLC patients were 45.8%, 51.4%, 72.2% and 73.6%, respectively. Significant correlations were found between IL-6 *vs*. NSE and IL-6 *vs*. VEGF. Univariate analysis revealed significant relationship between overall survival of patients and stage of disease, as well as VEGF, IL-6, NSE and ProGRP. The multivariate analysis has shown that apart from stage of disease, IL-6 is an independent prognostic factor in the studied group of SCLC patients. Moreover, analysis of prognostic value complementary to IL-6 determinations of VEGF, NSE and ProGRP revealed that relative risk of death was more than 2 times lower in patients with limited disease and with IL-6 lower than 6.0 ng/mL as well as with those having ProGRP lower than 670 pg/mL.

Conclusion: Apart of stage of disease, in SCLC patients impact of survival is also observed for IL-6 and ProGRP.

New Methods

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EVALUATION OF TWO COMMERCIALIZED *IN SITU* HYBRIDISATION ASSAYS FOR DETECTING HPV DNA IN PARAFFIN-FIXED TISSUE

M. Montag, T. J. F. Blankenstein, S. Schulze, C. Kuhn, A. Brüning, U. Jeschke, K. Friese, I. Mylonas

First Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University, München, Germany

The role of HPV in the pathogenesis of cervical carcinoma is now unquestionable. However, HPV detection in formalin-fixed and paraffin-embedded tissues is still controversially discussed. Therefore, the aim of this study was to evaluate morphological changes with a potential HPV-DNA detection directly in tissue specimens through HPV *in situ* hybridisation.

Materials and Methods: Samples from patients with cervical carcinoma were analysed using the GenPoint HPV DNA Probe Cocktail and the ZytoFast HPV Screening ISH-Kit. Three cervical carcinoma cell lines with a well-defined HPV copy number per cell (SiHa, HeLa, CaSki) served as a positive control, while two HPV-negative cell lines (AC-1M32, MCF-7) were used as negative controls. Ten different cervical carcinoma tissue samples were analysed, while brain tissue samples formed the negative histological control. Moreover, to assess the validity of the *in situ* hybridisation, the expression of HPV-16-DNA was demonstrated by HPV-16-E6-specific PCR in cell lines.

Results: Both HPV-screening assays showed strong signals of episomal and integrated HPV-DNA at a HPV copy number of more than 50 copies per cell. All cervical carcinoma samples were positive in the Dako assay, which identifies 13 high-risk HPV genotypes (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), whereas HPV-DNA could be detected in 9/10 cervical carcinoma samples with the Zytomed assay, identifying HPV 16, 18, 31, 33 and 35. No positive HPV finding could be reported in tissue samples of the negative controls.

Conclusion: HPV *in situ* hybridisation is an easy and powerful tool in demonstating HPV-DNA in formalin-fixed and paraffin-embedded tissue samples. Therefore, this technique allows for adequate analysis of a potential HPV infection in classical pathological slides.

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CHROMOA: ELISA ASSAY OF THE CHROMOGRANIN A PROTEIN – ANALYTICAL RESULTS

C. Mombrial, M. Amoravain, M. Bétard, L. Burty, J. Fauque, D. Pons, V. Cartalas

Cisbio Bioassays, Bagnols sur Cèze, France

Chromogranin A (CGA) is a hydrophilic protein of 49 kD, particularly sensitive to proteolysis. CGA is a member of the granin family, it is present in the chromaffin granules of the neuroendocrine cells and is released into the circulating blood after cellular death. Circulating CGA is present in healthy individuals, and the values obtained are independent of age and sex. CGA is a general marker for neuroendocrine tumors, and is also present in neuroendocrine differentiated prostatic carcinomas. The expression of circulating CGA is linked to a tumor mass.

Materials and Methods: The Chromoa kit is an ELISA-type immunoassay for the quantitative measurement of Chromogranin A in serum or plasma. A first monoclonal antibody, coated on a microplate, captures the CGA protein contained in the samples. After washing steps, the second antibody coupled to HRP binds to the antigen. After washing and revealing, the OD is read at 450 nm. The sample volume is 20 µl, and the range is from 0 to 1200 ng/ml.

Results: The intra- and inter-assay repeatability are respectively lower than 6 and 10%. The analytical detection limit has been assessed as being 2.6 ng/ml, while the functional detection limit is at 11 ng/ml. No hook effect has been observed up to 200,000 ng/ml. The dilution and recovery tests give results between 90 and 110%. A study of 114 supposedly normal samples (Occupational Health Service) showed that 95% of the values obtained were lower than 94 ng/ml, with a median of 44 ng/ml. The antibodies used in the Chromoa kit are identical to those of the CGA RIACT, a kit also developed by Cis bio and available on the market since 1998. The two kits are perfectly correlated (r^2=0.99), and the normal values are superimposable. The assay's interpretation is therefore identical to that of CGA-RIACT

Conclusion: Be it at the analytical or clinical level, the Chromoa assay developed by CISBIO meets the requirements imposed on a commercial CGA assay for use in the framework of the diagnosis and follow-up of neuroendocrine tumours, and also as a prognostic factor in cases of neuroendocrine differentiated prostatic cancer.

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STRATEGIES FOR MARKER DISCOVERY IN ONCOLOGY

M. Roeßler, M.-L. Hagmann, I. Lindner, J. Klöckner, J. Karl, B. Risse, M. Tacke, G. Pestlin, P. Heiss

Roche Professional Diagnostics, Penzberg, Germany

Cancer is a major health problem in developed countries all over the world and currently one in four deaths in the Western population is cancer-related. Generally, the probability of curing cancer is highest when diagnosed at early stages and early detection is therefore highly promising for limiting cancer morbidity and mortality in the future. However, many of the existing screening tests suffer from problems with sensitivity, specificity, costs, and/or compliance. For many other cancer types, tests for early detection are not available at all. To fill this diagnostic gap, accurate, non-invasive diagnostic markers in body fluids indicating early stage disease would fulfill a high medical need. We are actively seeking such markers combining several technologies. With the advent of highly efficient mass spectrometry (MS) instruments proteomics technologies offer the possibility to identify a large number of proteins from a given sample. However, in order to achieve a "proteome" as comprehensive as possible, it is necessary to combine several prefractionation methods with different MS-technologies. Here we present our approach to identify new marker candidates from cancer tissue samples. This approach includes conventional two-dimensional gel electrophoresis (2-DE) and identification of proteins by MALDI-MS as well as liquid chromatography coupled to ESI-MS. After marker discovery programs applying proteomics technologies it is of utmost importance to validate the findings with independent methods. We apply immunoblot analyses as well as immunohistochemistry to scrutinize the value of the identified marker candidates. Using these technologies it is possible to assess the potential of the identified marker candidates in a larger panel of cancer tissue samples as well as in other malignancies. For the most promising candidates, highly sensitive immunoassays are developed to analyze panels of well characterized clinical blood samples.

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CLINICAL EVALUATION OF THE ARCHITECT® PROGRP ASSAY

P. Stieber[1], B. Dowell[2], R. Doss[2], T. Yoshimura[3], F. Blankenburg[1], K. Hofmann[1], C. Scheuer[1], A. Högg[1], R. Hatz[4]

[1]Institute of Clinical Chemistry, University of Munich, Germany, [2]Abbott Laboratories, Lake County, United States, [3]Abbott Japan, Matsudo, Japan, [4]Surgical Department, University of Munich, München, Germany

ProGRP (Pro Gastrin Releasing Peptide) is known to be a biomarker with high diagnostic capacity for small cell lung cancer (SCLC). Recently the first automated assay for the determination of ProGRP became available (ARCHITECT®, Abbott Diagnostics). We evaluated the clinical capacity of this new assay as compared to an established ELISA (ALSI, Japan).

Methods: We measured ProGRP with both assays in all samples under the same conditions. We investigated retrospectively the sera (stored at –80˚C) of 466 patients with histologically proven lung carcinomas (123 SCLC, 343 non small cell lung cancer (NSCLC)). Sera of 183 patients with benign lung diseases and sera of 100 normals served as reference groups.

Results and Conclusion: For Architect® ProGRP median of normals was 21.7 pg/ml (ALSI 20.9), 95th percentile 37.7 pg/ml (32.9). The median of benign lung diseases was 18.8 pg/ml (16), 95th percentile 48 pg/ml (42.9). The medians of squamous cell, adeno- and large cell carcinomas were 15.2, 15.7, 16.2 pg/ml (12.8, 13.5, 14.8), 95th percentile 36.7, 40.4, 50.2 pg/ml (31.4, 40.3, 50.2), highest value in NSCLC 77.4 pg/ml (74.3). In SCLC the median was 247 pg/ml (249), 95th percentile 11130 pg/ml (11476), highest value 250390 pg/ml (253600). At 95% specificity for benign lung diseases Architect ProGRP reached a sensitivity of 70% for SCLC (AUC 86%) (ALSI 72% (AUC 86%)). At 100% specificity 63% SCLC patients were true positive.

In summary the ARCHITECT® ProGRP assay represents an effective diagnostic tool for the detection of SCLC.

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DIKKOPF-1 (DKK1): A RELEVANT BIOMARKER FOR LUNG CANCER?

P. Stieber[1], F. Blankenburg[1], C. Gruber[1], J. Reinmiedl[2], K. Hofmann[1], M. Wick[1], R. Hatz[3]

[1]Institute of Clinical Chemistry, University, Campus Großhadern, Munich, [2]Asklepios Lungenfachkliniken, Gauting, [3]Surgical Department, University, Campus Großhadern, Munich, Germany

DKK1 (Dikkopf-1) was recently described to be a relevant biomarker in diagnosis of lung cancer patients. Following the guidelines of the EGTM (European Group on Tumor Markers) we evaluated the clinical capacity of this new marker as compared to established biomarkers like carcinoembryonic antigen (CEA), cytokeratin 19 fragments (CYFRA 21-1), squamous cell cancer antigen (SCCA), neuron-specific enolase (NSE) and Pro Gastrin Releasing Peptide (ProGRP).

Methods: We investigated retrospectively DKK1 by ELISA (Assay Designs, Michigan USA) using sera (stored at –80˚C) of 190 patients with histologically proven lung carcinomas (56 squamous cell (SC), 49 adenocarcinomas (AC), 37 large cell (LC), 48 small cell lung carcinomas (SCLC)). Sera of 94 patients with benign lung diseases and sera of 48 normals as well as 49 patients with various other benign diseases served as reference groups. We compared the results with CEA (Abbott, AxSYM), CYFRA 21-1 and NSE (Roche, Elecsys), SCCA (Abbott, IMx) and ProGRP (ALSI, Japan).

Results: DKK1 median of normals was 14257 pg/ml (range 7425-45018). The median of benign lung diseases was 21210 pg/ml (range 4216-82938). The median of lung cancer was 18495 pg/ml (squamous cell: 14599, adeno: 18180, large cell: 24473, small cell: 19004). The highest value in lung cancer was 44219 pg/ml, the overall highest values were reached in benign lung disease and hepatitis (97206 pg/ml). At 95% specificity for benign lung diseases DKK1 reached a sensitivity of 14% for lung cancer in general (AUC 39%), 2% for SC (31%), LC (52%) and SCLC (40%) each, 4% for AC (38%), at 100% specificity overall sensitivity of DKK1 was 0%. The overall leading marker for lung cancer versus benign lung disease was CYFRA 21-1 (AUC 80%), followed by CEA (68%). In SC CYFRA 21-1 (AUC 80%) and SCCA (71%) were leading, in AC CYFRA 21-1 (77%) and CEA (72%), in LC CYFRA 21-1 (83%) and CEA (73%), in SCLC ProGRP (83%) and NSE (84%), ProGRP and/or NSE lead to an AUC of 92% and 65% sensitivity at 100% specificity.

Conclusion: In summary we could not observe a relevant release of DKK1 in lung cancer irrespective of the histological subtype. In addition we could not observe an additional diagnostic capacity of DKK1 as compared to CEA, CYFRA 21-1, SCCA, NSE or ProGRP.

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MONITORING CANCER PATIENTS: CLINICAL PERFORMANCE OF THE ACCESS® BR MONITOR (CA15-3 ANTIGEN), GI MONITOR (CA19-9 ANTIGEN) AND OV MONITOR (CA 125 ANTIGEN) ASSAYS ON BECKMAN COULTER'S UNICEL® DXI 800 IMMUNOASSAY SYSTEM: A EUROPEAN MULTICENTER STUDY

P. Stieber[1], R. Molina[2], M. Gion[3], A. Gressner[4], F. Troalen[5], J. M. Auge[2], S. Holdenrieder[1], M. Zancan[3], M. Wycislo[4], D. Nage[1], V. Jarrige[6]

[1]Institut für Klinische Chemie, Klinikum der Universität Campus Grosshadern, Munich, Germany; [2]Laboratory for Clinical Biochemistry, Hospital Clinic, Barcelona, Spain; [3]ABO Association c/o the Center for the Study of Biological Markers of Malignancy, General Regional Hospital, Venice, Italy; [4]Institut für Klinische Chemie,

Universitätsklinikum der RWTH, Aachen, Germany; [5]Departement de Biologie Clinique, Institut Gustave Roussy, Villejuif, France; [6]Beckman Coulter Eurocenter, Nyon, Switzerland

The 3 Beckman Coulter immunoassays BR-, GI- and OV Monitor are based upon different antibodies as compared to the original Centocor antibodies, thus it is of special interest to compare their diagnostic capacity not only at the time of primary diagnosis but also during follow up care of cancer patients.

Materials and Methods: The Access BR Monitor assay uses Ma 552 as monoclonal tracer antibody, which recognizes the same epitope within the MUC1 protein core as the Centocor DF3 antibody, and Ma 695 as monoclonal capture antibody, which detects a carbohydrate epitop of MUC 1 similar to the one recognized by the Centocor 115-D8 monoclonal antibody. The Access GI Monitor assay uses the monoclonal antibody C192 as tracer and capture antibody, which recognizes the same epitope (same specificity) as the Centocor 1116-NS-19-9 monoclonal antibody. The Access OV Monitor assay uses OVK 95 as monoclonal tracer antibody, recognizing the same epitope as the Centocor OC 125 antibody, and OV 185 as monoclonal capture antibody, which detects a similar epitop as the Centocor M11 antibody. All results were compared with an established reference method for CA 15-3, CA 19-9 and CA 125 (Elecsys, Roche Diagnostics, Germany). Using BR-Monitor we investigated serial samples of 19 patients suffering from breast cancer, using OV-Monitor serial samples of 33 ovarian cancer patients and using GI-Monitor serial samples of 43 pancreatic cancer patients.

Results: The serial values were summarized for each marker and the corresponding reference method in 3 groups: 1) increase of marker >25%, 2) decrease of marker >25% and 3) stable marker for increase <25% and decrease <25%. Concerning the correlations of these groups between the 2 assays we observed for BR Monitor and CA 15-3 in 67%, for GI Monitor and CA 19-9 in 84% and for OV Monitor and CA 125 in 92% a complete coincidence of the 3 groups. For OV Monitor and CA 125 as well as for GI Monitor and CA 19-9 there was no case with increase (group 1) of one assay and decrease (group 2) of the other assay. For BR Monitor and CA 15-3 this situation occurred in one case (2 serial samples within 128 samples).

Conclusion: The comparability of the serial measurements of the new assays BR Monitor, GI Monitor and OV Monitor using different monoclonal antibodies with the reference assays using the original Centocor antibodies revealed good results. Concerning absolute value levels the assays are not comparable but must be based upon the reference data for each method and must be measured in parallel in case of change of assay during follow up.

Varia

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PROLIFERATION BEHAVIOUR OF TUMOUR AND NORMAL CELL LINES AGAINST SUTURE MATERIALS

D.-U. Richter[1], B. Gerber[2], L. Jonas[2], V. Briese[1]

[1]Department of Obstetrics and Gynaecology,
[2]Department of Pathology, University, Rostock, Germany

Suture materials are increasingly tested *in vitro* on compatibility with cell cultures. This study should resolve the question of differences of adherence between tumour and normal cells to diverse suture materials. Furthermore, the possibility of a correlation between the adherence and the surface structure is suggested. In the present study the influence of suture materials on tumour proliferation is being investigated as well as the question of whether the deposition of resorbable textile implants for local onco-therapy is justified.

Materials and Methods: For the present survey normal fibroblast cells (both commercially acquired and primary cells from sub-epithelial tissues) and five different commercial tumour cell lines (2x mamma carcinoma cells, 2x endometrial carcinoma cells and 1x chorionic carcinoma cells) were used. For the immunohistochemical characterisation of the primary cells the fibroblast marker vimentin was utilised and the verification of the purity of the primary fibroblasts culture succeeded *via* CD 68 marker. The study includes a total of 10 different surgical suture materials (laminated/unlaminated, non-/absorbable yarns). The abovementioned cell cultures were cultivated in an incubator (37°C, 5 % CO_2, with water vapour saturated atmosphere) for 10 days. The adherence of the fibroblasts could be obtained and analysed with light- and electron microscopes. Simultaneously the vitality determination was specified by trypane blue staining (cell count). After the end of the cultivating time the cytoxicity was determined by use of the LDH- test whereas the proliferation was appraised by use of the MTT- test. For all suture materials an EDX-analysis (energy dispersive X-ray spectroscopy) was realised.

Results: Cell adherence could be noticed for all suture materials. A correlation between the different stitches and the quantity of cells in the space of the same cell lines could not be observed. During the period of cultivation a 10-fold increase in the cell concentration was noticed. The vitality amounted regularly ≥98% and no significant differences were observed by using cytoxicity- and proliferation tests. Finally three stitches of the numerous series of tests were selected and presented here with light- and electron microscopical imaging. During the cultivation of the *SUPRAMED* stitch with the cell line of mamma carcinoma cells MCF 7 there was a good

adherence determined. All the other stitches presented just a minimal adherence. Otherwise good growth behaviour of the *Vicryl plus* stitch (*Ethicon*) with chorionic carcinoma- and fibroblast cell lines was observed. The most qualified for inhibition of cell growth is the *SERAFIT* stitch which is approved by biochemical tests and microscopic analysis.

Discussion: The *in vitro* examination of the tissue compatibility of biomaterials is an accepted and valid method. Living cells react very sensitively to foreign materials. Thereby giving necessary information about direct effects of the biomaterials on the cell metabolism and the growth behaviour. More tests on the relationship of cells with suture materials should be performed. The development of suture materials that can inhibit tumor growth in situ is essential for onco-surgery.

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PROGNOSTIC FACTOR FOR LONG-TERM SURVIVAL AFTER GLIOBLASTOMA MULTIFORME TREATED BY CONCURRENT TEMOZOLOMIDE (TMZ) AND RADIOTHERAPY

O. Micke[1,2], U. Schäfer[2,3], F. Bruns[4], J. Büntzel[5], N. Willich[2]

[1]Klinik für Strahlentherapie und Radioonkologie, Franziskus Hospital Bielefeld, [2]Klinik und Poliklinik für Strahlentherapie und Radioonkologie, Universitätsklinikum Münster, [3]Klinik für Strahlentherapie, Klinikum Lippe, [4]Klinik für Strahlentherapie und spezielle Onkologie, Medizinische Hochschule Hannover, [5]Klinik für Hals-Nasen-Ohren-Heilkunde, Südharzkrankenhaus Nordhausen, Germany

Despite recent innovations in neurosurgery, neurooncology and radiotherapy, the prognosis of glioblastoma multiforme (GBM) is still very poor. The majority of GBM patients decease within the first year after diagnosis. Long-term survival generally is rare. Nevertheless, new approaches in combining radiotherapy with prolonged application of temozolomide (TMZ) showed a slowly increasing number of survivors. For long-term application TMZ is very cost intensive and has considerable chronic bone marrow toxicity. The retrospective analysis of a patient collective, which was treated with continuous application of TMZ after (chemo-) radiotherapy, for long-term survivors and for prognostic factors which may predict long-term survival is presented.

Materials and Methods: Since January 2000 a total of 84 patients with histologically proven glioblastoma (+/- surgery) were treated with continuous application of oral TMZ after (chemo-)radiotherapy. Fourty five patients (Group A) treated with primary or postoperative radiotherapy received TMZ (75 mg/m^2/day) concurrent with 60 Gy of conventional radiotherapy (5×2 Gy/week). The patients continued TMZ therapy (5 days; 200 mg/m^2/d) every 28 days until progression. Thirty nine patients (Group B) received postoperative radiotherapy (60 Gy) alone, followed by TMZ therapy until progression. Long-term survivors were defined as patients surviving more than 36 months after the beginning of treatment. A historical control collective of patients (Group C; n=100) was treated with radiotherapy alone (median dose: 60 Gy). Uni- and multivariate survival analyses were performed to identify factors to predict tumor prognosis and the possibility of long-term survival.

Results: In group A the median actuarial overall survival (OS) was calculated to 19.4 months, the progression-free survival (PFS) was 12.3 months. In group B OS was 16.2 months, the progression-free survival (PFS) was 10.7 months. In group C OS was 12.1 months, the progression-free survival (PFS) was 8.8 months. Overall there were 17 long-term (>36 months) surviving patients (20.2%) observed. Group A: 10 (22.2%); group B: 6 (15.3%); and group C: 1 (1%). The difference gained statistical signgificance (p=0.04, chi2-test). Toxicity was limited and well manageable. Uni- and multivariate survival analyses revealed age, Karnofsky performance status (KPS), frontal lobe location, resection status and long-term application TMZ as prognostic factors for long-term survival.

Conclusion: The results suggest that TMZ is able to improve long-term survival rates compared to conventional radiotherapy with acceptable toxicity. Long-term survival is generally possible and the rate of long-term survivors is significantly increased after concurrent TMZ application to radiotherapy. Age, KPS, frontal lobe location, resection status and long-term application TMZ were identified as prognostic factors for long-term survival in GBM.

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VISUALIZATION OF TUMOR REGRESSION AFTER LOCAL CHEMOTHERAPY WITH MAGNETIC NANOPARTICLES

St. Lyer[1], R. Tietze[1], R. Jurgons[1], T. Struffert[2], T. Engelhorn[2], E. Schreiber[1], A. Dörfler[2], C. Alexiou[1]

[1]Department of Oto-Rhino-Laryngology, Head and Neck Surgery, [2]Department of Neuroradiology Friedrich-Alexander-University, Erlangen-Nürnberg, Germany

Aim: One disadvantage of conventional systemic application of chemotherapy in cancer treatment is the application-to-tumor-dose-ratio. This implies an often insufficient drug dose in the tumor, associated with severe side-effects for the patient. Therefore the aim of Magnetic Drug Targeting

(MDT) is to increase the dose in the tumor and simultaneously reduce the overall dose due to the application of chemotherapeutic bound magnetic nanoparticles, which are focused by an external magnetic field to the tumor. An important factor for this kind of therapy is the vascularisation of the targeted tumor. In this pilot study the vascularisation and size of the tumor before and after MDT was investigated in an experimental *in vivo* tumor model.

Methods: A VX-2 tumor was implanted at the left hind limb of five rabbits. Mitoxantrone was bound to superparamagnetic Fe_3O_4-nanoparticles (size measured by light scattering 11 nm) in an aqueous solution (=ferrofluid). This drug loaded ferrofluid was applied through the femoral artery close to the tumor. The magnetic nanoparticles were attracted to the tumors by a focused external magnetic field during the application. The visualization of vascularisation and tumor size before and after MDT was done using a biplane angiographic system with rotational flat-panel CT (DYNA-CT, Siemens Axiom Artis dBA). The contrast agent was a standard agent with an iodine concentration of 300 mg/mL.

Results: The tumors and the supplying vessels could be displayed clearly by DYNA-CT. The tumors were then treated with an equivalent of 10% of the systemic dose of mitoxantrone with MDT. After the treatment the tumor size reduction was monitored by DYNA-CT again. Ten to 15 weeks after MDT the tumors had disappeared entirely and the angiography showed a regular vascularisation without any remaining tumor vessels.

Conclusion: Magnetic Drug Targeting lead to complete tumor remission after one cycle of chemotherapy with a reduced dose. In this study DYNA-CT offered an excellent possibility to monitor the vascularisation and the size of the tumors before and after MDT.

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OSTEOPONTIN (OPN) EXPRESSION IN THYROID CARCINOMA

J. Briese[1,2,3], S. Cheng[1], S. Ezzat[4], W. Liu[1], D. Winer[1], C. Wagener[5], A.-M. Bamberger[6], S.L. Asa[1]

[1]Department of Laboratory Medicine and Pathobiology, University, Department of Pathology, University Health Network, Ontario Cancer Institute, Toronto, Ontario, Canada, [2]Institute of Molecular Medicine, University of Leeds, United Kingdom, [3]Department of Gynaecology and Obstetrics, University Clinic Greifswald, Germany, [4]Department of Medicine, University of Toronto, Mount Sinai Hospital, Cancer Institute, Toronto, Ontario, Canada, [5]Department of Clinical Chemistry, [6]Laboratory of Endocrinology and Metabolism of Ageing, University Medical Center, Hamburg, Germany

Osteopontin (OPN) and its interacting partner CEA-cell adhesion molecule (CEACAM1) mediate similar biological functions and have been expressed in several types of cancer. Here the role and prognostic significance of OPN in thyroid tumours is investigated and correlated with the expression of CEACAM1.

Methods and Results: Two hundred and ninety seven human thyroid samples (including normal, benign and malignant tumours) were collected in a tissue microarray and as fresh frozen samples to perform immunohistochemistry and western blotting for OPN and to compare these data with previously published data on CEACAM1 expression. Nearly all normal samples were negative for OPN, correlating with CEACAM1 ($p<0.001$). Fifty one percent of thyroid adenomas were weakly OPN positive whereas 100% were negative for CEACAM1 ($p<0.001$). OPN was strongly expressed in 81% of papillary carcinomas, in 75% of medullary carcinomas and in all anaplastic carcinomas ($p<0.001$ *vs.* normal tissue). In biopsies of lymph nodes, 25/35 of papillary, all medullary and all anaplastic carcinoma metastases were positive for OPN. In contrast to CEACAM1, which was preferentially expressed in metastatic papillary carcinomas, no associations were found between OPN expression and patient age, gender, tumour size or the presence of lymphatic or distant metastases.

Conclusion: The data indicate that OPN is not expressed in normal thyroid tissue but is expressed in thyroid neoplasms with increasing expression in malignant tumours. Unlike CEACAM1, this marker is not predictive of metastastic potential. The relationship between these two proteins remains of relevance to the understanding of the process of carcinogenesis, and these results may have future implications for the diagnosis and management of patients with thyroid cancers.

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MALFORMATIONS, DEFORMITIES AND OSTEOLYSIS OF THE FACIAL SKELETON IN NF1 PATIENTS ASSOCIATED WITH NEUROFIBROMA

R.E. Friedrich[1], M. Giese[3], H.A. Scheuer[2]

[1]Oral and Maxillofacial Surgery, [2]Orthodontics, Eppendorf University Hospital, [3]Oral and Maxillofacial Surgery, Bundeswehrkrankenhaus, Hamburg, Germany

Aim: Neurofibromatosis type 1 (NF1) is an autosomal hereditary disorder prone to undergo malignant transformation, particularly from plexiform neurofibroma (pNF). Bone malformations are an independent finding in NF1. On the other hand, NF1 affecting the facial region can be associated with severe skeletal disfigurement and radiotranslucencies that

appear similar to bone destructions caused by malignant tumors. The aim of this study was to analyse jaw malformations in patients with neurofibromatosis type 1 (NF1) and to analyse the soft tissue adjacent to these malformations.

Materials and Methods: Reports on jaw and teeth malformations in neurofibromatosis affected individuals during the last 120 years were evaluated and published. Any detail regarding skeletal findings and the presence and distribution pattern of neurofibroma were recorded. Forty-eight patients treated in a single institution were included in the study (male/female 24 each). All fulfilled the current NIH diagnostic criteria for NF1. The age range was 2.5 to 66 years. The type of neurofibroma (NF) was histologically proven in surgically treated patients. Patients with disseminated cutaneous NF (DCNF) and those with the plexiform type (facial plexiform NF: FPNF) were distinguished. The analysis included physical investigations and photographs, panoramic radiographs and dental casts. The minimum follow-up period after surgery was 5 years.

Results: One hundred and eighty three cases of maxillofacial neurofibromatosis were found in the literature. This is the largest analysis so far presented on this subject. From the literature analysis certain patterns of skeletal malformation in NF1 were revealed. No subtyping of neurofibroma with skeletal malformation was yet noted. With emphasis on alterations of tooth position, deformities of the adjacent bones and malocclusion, the vast majority of these patients were obviously affected by pNF. The skeletal findings and the pNF of the trigeminal nerve were always unilateral in pNF. In patients with DCNF, malformations of the alveolar ridge and jaws were absent and individual oral symptoms were rarely found, mild and not impairing in all cases. Numerical aberrations and retention of molars were associated with a trigeminal nerve giving rise to a FPNF. Unilateral aplasia of the second inferior molar was recognized in four of these FPNF patients.

Conclusion: It is widely accepted that malformations of the facial skeleton are genetical in origin. However, in this study these malformations were strongly associated with pNF originating from the trigeminal nerve. Thus, in addition to presently unknown genetic factors, the pattern of skeletal malformation can be caused by tumor invasion and local destruction, *e.g.* the neuromuscular unit, in the early years of life. Another way of action could be prenatal development of the PNF, *e.g.* in the inferior alveolar nerve, causing mandibular dysmorphy. Epidemiologic studies on the incidence and severity of NF1 in the oral and maxillofacial region have to distinguish between patients with or without pNF, when analysing alterations and deformities of the jaws, teeth and malocclusion. The malformation pattern of jaws in NF1 is indicative for the plexiform type of neurofibroma, in particular the diffusely invasive type. (Supported in part by a grant-in-aid of the Deutsche Krebshilfe, Bonn, Germany).

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ASSOCIATION OF IL-6, HYPOTHALAMUS-PITUITARY-ADRENAL (HPA) AXIS FUNCTION AND DEPRESSION IN CANCER PATIENTS

C.F. Jehn[1], D. Kuehnhardt[1], A. Bartholomae[2], S. Pfeiffer[3], P. Schmid[1], B.C. Flath[1], K. Possinger[1], D. Lüftner[1]

[1]Department of Oncology and Hematology, [2]Department of Psychiatry, [3]Department of Immunology, University Hospital Charite, Berlin, Germany

Evidence suggests cytokines (IL-6) and alteration of the Hypothalamic-Pituitary-Adrenal (HPA) axis play a crucial role in the ethiology of depression. Patients (pts) with cancer show elevated prevalence rates for depression. The objective of this study was to investigate if cancer pts with depression exhibit these neuroimmune system abnormalities.

Methods: Plasma concentrations of IL-6 and cortisol were measured in cancer pts with (n=39) and without depression (n=61). The relative diurnal variation of cortisol (cortisol VAR), expressed in percent, was calculated.

Results: Mean age and Karnofsky-index did not differ between pts with depression and pts without depression (62.7 *vs*. 59.4 years (p=0.073) and 62.1 *vs*. 65.2% (p=0.385), respectively). There was a significant difference in median plasma concentration of IL-6 between the pts with depression and pts without depression (18.7 *vs*. 2.7 pg/mL; p<0.001). Relative cortisol VAR was decreased in depressed pts compared to pts without depression (11.72 *vs*. 60.6%, p=0.037). A positive correlation between the HADS-D score and IL-6 concentrations was found (r=0.469, p<0.001). Negative correlations were found between cortisol VAR *vs*. HADS-D and cortisol VAR *vs*. IL-6 (r=–0.6, p<0.001 and r=–0.52, p<0.001).

Conclusion: Depression in cancer is associated with increased plasma IL-6 concentrations and dysfunction of the HPA axis.

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ASSOCIATION OF BORNA DISEASE VIRUS INFECTION WITH DEPRESSION IN CANCER PATIENTS

C.F. Jehn, L. Bode, A. Dieing, S. Lehenbauer-Dehm, P. Schmid, K. Possinger, C.B. Flath

Medizinische Klinik mit Schwerpunkt Onkologie und Hämatologie, CCM, Charité, Universitätsmedizin, Berlin, Germany

Background: Borna disease virus (BDV) is an RNA virus which can persistently infect neurons of the limbic system.

Several seroepidemiologic data suggest an association of BDV with neuropsychiatric disorders, however inconsistent detectability has weakened a possible linkage. The objective of this cross-sectional study was to investigate if an association exists between BDV infection and depression in patients with advanced cancer receiving chemotherapy.

Methods: Fifty five patients (pts) with metastatic cancer (Stage IV) were assessed by the Hospital Anxiety and Depression Scale (HADS) for depressive symptoms and diagnoses of major depression (MD) was established according to the DSM-IV criteria. IL-6, BDV-specific circulating immune complexes (CIC), antibodies and plasma antigens were determined by enzyme immunoassays (EIAs). In the statistical analysis the Mann-Whitney test and Spearman-Rho correlations were applied.

Results: Fifty five pts (age: 59.9 years; SD 10,2) had a mean Karnofsky-index (KI) of 66.5% (SD:12,1). Twenty six pts had MD. Pts with MD showed a significant increase in BDV-specific antigens (p=0.050) and antibodies (p=0.045) and IL-6 (p<0.001), compared to patients without MD. CIC were not increased in MD (p=0.53). Depressive symptoms were more closely correlated with level of BDV antibodies (r=–0.296; p=0.028) and IL-6 (r=5.6; p<0.001) than symptoms of anxiety. Symptoms of anxiety showed a significant correlation to increased age(r=–0.28; p=0.042), whereas depressive symptoms correlated more closely with a decreased KI (r=–0.35; p=0.011). No correlations were found for level of symptoms *vs*. BDV-antigen or CIC.

Conclusion: In pts with metastatic cancer, MD is associated with increased levels of BDV-specific antigen and antibody. Symptoms of depression and anxiety are only correlated with increased levels of BDV-antibody. Symptoms of anxiety seem to be related to age, whereas symptoms of depression are related to decreased KI.

Complementary Medicine – Hyperthermia

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COMPLEMENTARY THERAPY AND HYPERTHERMIA IN THE CASE OF GASTROINTESTINAL TUMORS

H. Wehner

Gisunt-Klinik, Zetel, Germany

The results for the survival time and recidivism- and metastasis free intervals are not satisfactory and therefore, justify considerations about the improvement of prognosis within cancer therapy. When trying to influence the survival time, aspects of the quality of life should be in the focus of attention. An open-minded search and an accurate evaluation can offer control approved options in studies. Hyperthermia proved to be a prime example for an integrative approach, initially being a complementary therapy. Meanwhile hyperthermia has demonstrated its significance in several trials. The benefits of the treatment were shown in cases of advanced colorectal cancer as well as pancreatic cancer. Other complementary possibilities, particularly vitamin C infusions and mistletoe therapy, could prove their positive influence on the quality of life and in order to enable timely chemotherapy.

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DENDRITIC CELL BASED IMMUNOTHERAPY; INFLUENCE OF THE MICROENVIRONMENT ON THE IMMUNE RESPONSE

T. Neßelhut

Institut für Tumortherapie, Duderstadt, Germany

Especially in advanced stages of cancer disease the common therapy strategies show insufficient results accompanied by a reduced quality of life due to partly strong side-effects. Today, one of the most promising innovative therapy approaches in treatment of various types of human cancer is a specific immunotherapy with dendritic cells. Meanwhile, several groups have shown that immunotherapy with monocyte-derived dendritic cells (MoDC) loaded either with tumor-cell-lysate, known tumor specific peptides or with specific RNA or DNA can induce a clinical antitumor response in patients with various types of cancer accompanied by less side-effects and good tolerability. Nevertheless, most of the treated patients fail to respond to the therapy. The non effective clinical anti-tumor response to a dendritic cell based therapy may be partly due to an inflammatory tumor microenvironment. During the last years several investigations show an increasing evidence of a strong association between chronically infection, inflammation and cancer development as well as in tumor progression. Many of the same inflammatory mediators that are secreted by wounds are found in the tumor microenvironment. Meanwhile it is known that also the tumor by itself can change the immunological balance into an inflammatory microenvironment leading not only to promotion of the tumor growth but also to inhibition of an efficient immune response and thus declares a non-successful immunotherapy based on dendritic cells. Beside several other cytokines which promote an immune suppressive microenvironment, such as for example IL-10, regulatory T-cells (T-reg) play an important role in regulation of an immune

response. An increase of those regulatory T-cells is often found in cancer patients and may limit the antigen specific immune response. To reduce these T-reg cells to a normal range a metronomic chemotherapy should be considered as pretreatment prior to an immune therapy with dendritic cells, which may enhance the antigen specific CD-8-T-cell response. Moreover, an anti-inflammatory treatment may result in a non-inflammatory microenvironment, which can promote DC activation and can enhance tumor immunity as well as the clinical anti-tumor response. An efficient induction of a clinical antitumor response requires, besides a change of the immune suppressive tumor associated microenvironment, a polarization of MoDC in a TH1 direction. However, less IL-12 and high IL-10 production by the MoDC favoring a TH2 immune response rather than a TH1 response is often found in cancer patients. Culture conditions with supplement of certain TLR-ligands can signal the presence of infection ("danger signal") and may overcome the possible defective MoDC of cancer patients. Using culture conditions with sequential supplementation of a synthetic lipopeptide and LPS, we are able to change an IL-12/IL-10 production profile <1 to an IL12/IL-10 production profile >1, which favours a TH-1 response. Furthermore we show that activation of the Toll-like receptor 3 by adding (poly I:C), to the cultures as well as NDV can lead to DC, which are able to produce IL 12 p70 at a higher level than IL-10. The IL-12/1L10 ratio seems to be correlated to the clinical response. Thus, addition of TLR-agonists can effectively improve the maturation status as well as the TH1-polarisation of MoDC. Taken together, a successful dendritic cell based immunotherapy requires more information about the cancer characteristics to define the appropriate patient.

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INDIVIDUALIZED CHEMOTHERAPY – IMPORTANT FOR THE PATIENT, OPTIONS FOR THE PHYSICIAN

Ch. Sartori

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

An individualized, patient-oriented chemotherapy of cancer diseases, based on test results, does – in contrast to the treatment standard for infectious diseases – generally not exist to date. While the number of cytostatic drugs available for chemotherapy increases and although new approaches, *e.g.* neoadjuvant treatment of breast carcinomas, require successful chemotherapy as an obligatory condition for subsequent surgery, the variety of drugs actually used in treatment of organ diseases increases very slowly. Fundamental cause of this situation is the currently used concept to transfer results of clinical studies – gained from collectives of patients – onto the individual patient. As a consequence, the heterogeneity, *i.e.* different patients' responses upon treatment with a given cytostatic drug, is neglected. As an example, 9 out of 10 patients suffering from ovarian carcinoma, receive chemotherapy as a combination of platinum and Paclitaxel while only about 5 of these patients are actually sensitive for this (German) standard regimen. Consequently, many patients recur early and progress under therapy is frequently observed; afterwards, as per definition, the female patient is palliatively treated without curative approach. Compared to this relatively favorable situation, response rates after chemotherapy of other tumor entities are dramatically lower: For example, conventional chemotherapy – according to best clinician's choice of malignant melanomas is characterized by low response rates (ca. 9%) and quick progress. However, a recent German phase 2 study on this entity (Ugurel *et al*., Clinical Cancer Research, 2006) describes the successful approach to identify for the individual patient the most effective single drugs or drug combinations, based on results of the ATP Tumor Chemosensitivity Assay (ATP-TCA). The assay identified chemosensitive patients whose median overall survival (OAS) significantly improved from 7 to 15 months following treatment according to results of the ATP-TCA. In October 2008, recruitment into a DeCOG randomized prospective study commenced of up to 400 patients suffering from metastasized malignant melanoma. Also pancreatic carcinoma, routinely treated with a very limited choice of chemotherapeutic drugs, offers opportunities to improve success of chemotherapy in terms of clinical response, extended progression free survival (PFS) and OAS (Michalski *et al*., Br J Cancer, 2008). The ATP-TCA serves to determine patient-individual responses to chemotherapeutic drugs onto tumor cell growth. Being selective for tumor cells, to date this assay was evaluated in nearly 80 publications which include phase 1 and 2 studies. Based on its excellent positive and negative predictive values (~90%), the ATP-TCA is ideally suited to eliminate ineffective drugs prior to making a decision to treat and to identify those drugs or drug combinations which will be most effective for the individual patient. As a consequence of treatment according to test result, response, PFS, and OAS, resp. will be improved. In addition, undesirable side-effects will obviously be reduced, and even costs can be minimized by choosing *e.g.* the less expensive one of two drugs being equally effective.

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EVIDENCE-BASED COMPLEMENTARY ONCOLOGY - INNOVATIVE APPROACHES TO OPTIMIZE STANDARD THERAPY STRATEGIES

J. Beuth

University of Cologne, Institute for Naturopathy, Cologne, Germany

Cancer diseases demand diagnostic and therapeutic measures with proven quality, safety and efficacy. Basis for evaluation are clinical studies representing levels I or II (randomized controlled trials/RCT or epidemiological cohort studies) in accordance to recommendations of the Centre for Evidence-Based Medicine, University of Oxford, UK. Regarding these claims, surgery, chemo-, radio- and hormone therapy have emerged as gold standards in the treatment of cancers. These therapies only have proven their cancer destructive potencies and their curative feasibility, dependent on cancer entity and stage. Complementary therapies are recommended to support and optimize the scientifically-based cancer standard treatment. Complementary medicine is currently widely debated by the oncology community, because the required scientific proof of safety and effectiveness for most of the therapeutic approaches has not yet been met with definitive results. In the past years, basic research and clinical evaluation of defined complementary therapeutic concepts in oncology have been intensified in an attempt to integrate these procedures into evidence-based medicine. According to definition, scientifically-based therapies of complementary medicine cannot replace the well studied conventional cancer-destructive therapies such as operation, chemo-, radio- or hormone therapy. Accordingly, they are by no means “alternative therapies”. Complementary approaches in oncology that are recommended as addition to standard cancer destructive therapies claim to optimize this therapy. A great body of data emerging from scientifically sound clinical trials prove that defined complementary procedures are beneficial for the patients. Complementary medicine should primarily be regarded as an optimization of current standard treatment options in oncology. It is to be differentiated from “alternative medicine”, which postulates to have replacements for conventional toxic approaches. Although complementary and alternative medicines are grouped together in the popular acronym “CAM”, they are in fact quite different in their aims. Since many alternative treatments are still poorly documented, equating the two could lead to a misguided and undeserved rejection of all complementary medicine. That complementary recommendations concerning balanced nutrition, physical activity, psychooncologic support as well as defined medications, *e.g.* proteolytic enzymes, sodium-selenite or defined trace elements and vitamins or indication-based immunotherapy with standardized mistletoe extract can optimize standard treatment has been proved in clinical studies that have shown an increase in quality of life as well as in overall survival.

Parental Nutrition

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PORT SYSTEMS – INDICATION, CARE, COMPLICATIONS

M. L. Brenzinger, R. Klapdor

Internal Unit, ZeTDT GmbH, Hamburg, Germany

For long term intravenous nutrition or long term application of cytostatics and pain therapy, clinical or home parenteral nutrition so-called central venous catheters are preferred or needed in contrast to venous systems inserted into peripheral veins or central catheters inserted *via* a peripheral vein. In our unit, we mainly use subcutaneously positioned Port-A–Cath-Systems allowing repeated access to the vessel system for repeated parenteral delivery of medications like cytostatics or pain drugs, nutritional solutions for short or long term nutrition as well as for repeated sampling of blood samples for controlling and continuation of chemotherapy in patients with unsuitable peripheral veins. We will discuss our experience on various kinds of port systems, implantation prodedures and puncture techniques, as well as potential contraindications. In a second part the potential benefit of a port system for the patients allowing nearly painless punctures, improvement of quality of life and increase or high flexibility and activity even in advanced tumor disease states. In a third part we will discuss the ways of system access in clinical routine mainly focussing on special needle equipment required, to disinfection, puncture techniques and fixation as well as withdrawal of the puncture needles and maintenance of the patency of the system by flushing the port system with saline or heparinized saline solutions periodically. Last not least potential complications will be demonstrated like local infections, extravasations, thrombosis, pinching problems, medical recanalization, *e.g.* by urokinase stop technique, problems due to insufficient fixation of the port chamber as well as potential dysfunction of the infusion sets and pumps will be discussed.

Summarizing the Port-A-Cath-System seems to be a safe and very valuable systems for treatment of patients needing a central venous access, specially trained nurses, caretakers and assistants and in the case of the possibility of immediate diagnosis and intervention in the case of potential complications.

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PARENTERALE HEIMERNÄHRUNG: ORGANISATION UND DURCHFÜHRUNG

V. Sörensen

Compuond and Care Pharma GmbH, Hamburg, Germany

Die parenterale Heimernährung hat sich in den letzten Jahren einen festen Platz in der Therapie und Betreuung onkologisch erkrankter Menschen erworben. Es ist heute nicht mehr nötig, für eine parenterale Ernährung in eine Klinik aufgenommen zu werden. Eine komplette parenterale Ernährung kann heute auch ambulant durchgeführt werden. Voraussetzung ist die Schaffung eines Zugangs zur kontinuierlichen Applikation der Nährlösungen, die Möglichkeit der Berechnung des individuellen Bedarfes und der Nahrungszusammensetzung sowie die Gewähr und Sicherstellung der ambulanten Versorgung. Es gibt heute zahlreiche Möglichkeiten der Zubereitung der Nährlösungen. Es gibt unterschiedliche Pumpsysteme zur Gewährleistung einer kontinuierlichen Nährstoffapplikation. Es gibt feststehende, aber auch mobile Pumpen, die u. a. auch in einem Rucksack mitgeführt werden können, so dass die Mobilität der Patienten nicht eingeschränkt zu sein braucht. Benötigt werden weiter Hilfsmittel zur Versorgung des Patienten vor Ort, wie Infusionsständer, Verbandmaterial, Abdecktücher, Kochsalzlösungen zum Spülen der Systeme bzw. des Ports, Mullkompressen, Abdeckpflaster und spezielle Punktionsnadeln für den Port. Wichtig ist auch eine genaue Dokumentation aller Vorgänge und der Infusionen. Die Therapieziele müssen im Voraus festgelegt und verfolgt werden. Voraussetzung sind weiter regelmäßige und sorgfältige Schulungen der Pflegedienst-Mitarbeiter und der Aufsichtspersonen. Auch Erfahrung muss großgeschrieben werden. Die Mitarbeiter/Betreuer müssen Veränderungen des Patienten oder evtl. Komplikationen schnell erkennen und gut beobachten und entweder selbst oder über den Arzt sofort reagieren. Organisation und Durchführung der parenteralen Ernährung werden an verschiedenen Beispielen demonstriert, ebenso unser Vorschlag für die Dokumentation und für den Ablauf der Bestellung der Nährstofflösungen und der Hilfsmittel.

Selbsthilfegruppen

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PROSTATAKREBS

R. Stratmann

Bundesverband Prostatakrebs Selbsthilfe, Hamburg, Germany

Der Bundesverband Prostatakrebs Selbsthilfe wurde im Jahr 2000 als Zusammenschluss von 18 Selbsthilfegruppen gegründet. Mittlerweile gehören ihm bereits über 180 Prostatakrebs-Selbsthilfegruppen an (Stand: August 2007). Der BPS ist damit europaweit die größte und weltweit die zweitgrößte Organisation von und für Prostatakrebspatienten. Der BPS ist ein gemeinnütziger Verein. Er ist Mitglied im Deutschen Paritätischen Wohlfahrtsverband, in der europäischen Prostatakrebsvereinigung "Europa UOMO" und steht unter der Schirmherrschaft der Deutschen Krebshilfe. Das Anliegen des BPS ist es, die Öffentlichkeit - vor allem Betroffene und ihre Angehörigen - über die medizinischen, psychologischen und sozialen Aspekte einer Prostatakrebserkrankung aufzuklären und sowohl online als auch im Rahmen von Vortragsveranstaltungen und Patiententagen ein Forum für den Informations- und Erfahrungsaustausch zu bieten. Das Initiieren und Fördern von prostatakrebsbezogenen Selbsthilfeaktivitäten bildet einen weiteren Schwerpunkt unserer Arbeit. Als Interessenvertreter aller von Prostatakrebs betroffenen Männer in Deutschland sind wir schließlich auch auf gesundheitspolitischer Ebene aktiv. Als akkreditierte Patientenvertreter im Gemeinsamen Bundesausschuss fordern wir zum Beispiel:

- Eine verbesserte, beitragsfinanzierte Früherkennung (z. B. PSA-Test)
- Eine bessere Auswertung und schnellere Umsetzung neuer
- Diagnoseverfahren und Behandlungsmethoden
- Das Stärken von Patientenrechten

Der aktuelle Flyer mit Informationen über den BPS (im PDF-Format) kann heruntergeladen werden unter: www.prostatakrebs-bps.de.

Die rechtlichen Grundlagen unserer Verbandstätigkeit sind:

- Die Satzung
- Die Finanzordnung
- Die Selbstverpflichtung des BPS hinsichtlich der Zusammenarbeit mit Wirtschaftsunternehmen im Gesundheitswesen, insbesondere mit Unternehmen der pharmazeutischen Industrie
- Die Geschäfts- und Wahlordnung der Mitgliederversammlung
- Die Schiedsordnung
- Die Ehrenordnung

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DIE FRAUENSELBSTHILFE NACH KREBS

H. Schulte

Frauenselbsthilfe nach Krebs, Bundesgeschäftsstelle, Bonn, Germany

Die Gründung der Frauenselbsthilfe nach Krebs erfolgte bereits im Jahre 1976. Seit dieser Zeit ist der Wandel das einzig Beständige. Denn in all den Jahren hat es zahlreiche gesellschaftliche, politische und medizinische Veränderungen gegeben, denen wir uns gestellt und die wir als Chance zur Weiterentwicklung genutzt haben. So wurde aus den 15 brustamputierten Frauen in Mannheim eine bundesweit tätige Selbsthilfeorganisation mit einem Bundesverband, zwölf Landesverbänden und 430 Gruppen, in denen etwa 50 000 krebskranke Menschen Rat und Hilfe finden.

Als Bindeglied und einheitliche Richtschnur galt von Anfang an ein 5-Punkte-Programm, das unsere Arbeit bis zum heutigen Tage prägt und im Jahre 2001 um einen 6. Punkt, um die gesundheitspolitische Interessenvertretung, ergänzt wurde. Im Wesentlichen lässt sich das Programm in dem Motto "Auffangen, Informieren, Begleiten" zusammenfassen:

Programm:

- Auffangen - nach dem Schock der Diagnose
- Informieren - über Hilfen zur Krankheitsbewältigung
- Begleiten - in ein Leben mit oder nach Krebs

Unser spezielles Anliegen ist die Stärkung der Patientenkompetenz, damit krebskranke Menschen als informierte, mündige Patienten und selbstbewusste Partner gegenüber den professionellen Helfern auftreten können sowie Strukturen und Chancen unseres Gesundheitssystems in dem für sie notwendigen Maße zu nutzen wissen.

Die Inhalte unserer Arbeit werden allein von den Interessen krebskranker Menschen bestimmt. Das Bemerkenswerte ist, dass alle Mitglieder an Krebs erkrankt und aktiv tätig sind. Sie arbeiten alle ehrenamtlich, alle Ämter/Funktionen werden durch demokratische Wahlen mit Laien besetzt, alle Ebenen des Verbandes sind neutral und unabhängig und jederzeit offen für neue krebskranke Menschen.

Dank der Schirmherrschaft und finanziellen Förderung der Deutschen Krebshilfe können wir unsere Basisarbeit mit den wesentlichen Aufgaben und Zielen uneingeschränkt verfolgen. Wir arbeiten mit den gesetzlichen Krankenkassen im Rahmen der kassenübergreifenden und kassenindividuellen Selbsthilfeförderung zusammen. Wir sind Mitglied in der Bundesarbeitsgemeinschaft (BAG) Selbsthilfe, in der Deutschen Arbeitsgemeinschaft (DAG) Selbsthilfegruppen und im Paritätischen Wohlfahrtsverband. In diesen Gremien führen wir einen regen Erfahrungsaustausch mit anderen Selbsthilfeorganisationen und Arbeitsgemeinschaften.

Bei der Erstellung von medizinischen Leitlinien für die Behandlung von Krebserkrankungen vertreten wir Patienteninteressen genauso wie in Untergliederungen des Gemeinsamen Bundesausschuss. Wir arbeiten mit in Arbeitsgruppen des BMG zur Umsetzung des Nationalen Krebsplanes, im Patientenbeirat der Deutschen Krebshilfe, im Patientenforum der Bundesärztekammer, im Kooperationsverbund Qualitätssicherung durch klinische Krebsregister (KoQK), in Zertifizierungskommissionen für Organzentren und haben mehr als 100 Kooperationsverträge mit Brustzentren abgeschlossen.

Im Jahre 2006 sind wir von Mannheim nach Bonn umgezogen. Im Haus der Krebsselbsthilfe in Bonn wohnen wir mit weiteren sechs bundesweit tätigen Krebsselbsthilfeorganisationen, um Synergieeffekte und stärkere gesundheitspolitische Durchsetzungskraft zu erzielen. Im gleichen Jahr haben wir ein Positionspapier zur Versorgung krebskranker Menschen in Deutschland herausgegeben. Unsere beiden Fachausschüsse Gesundheitspolitik und Qualität sorgen mit dem Blick von außen dafür, dass wir innen besser werden.

Damit die Mitglieder auf der regionalen Ebene den veränderten Anforderungen weiterhin gerecht werden können, haben wir ein Qualifizierungskonzept mit sieben Blöcken entwickelt, das wir seit 2005 erfolgreich in allen Landesverbänden schulen. Dabei geht es um die Sicherung der Beratungsqualität, um die Abgrenzung zur professionellen/fachlichen Beratung, um die Klärung des Selbstverständnisses, um die Stärkung der gemeinsamen Identität, um das Miteinander im Gruppenalltag, um Gesundheitspolitik und über die künftige strategische Ausrichtung.

Eine weitere Qualifizierungsmaßnahme ist die Schulung unserer Mitglieder in den Landesvorständen. Hier vermitteln wir Kompetenzen aus den Bereichen Organisationsentwicklung und Führungsverständnis, Mitarbeiterführung, Teamentwicklung, Konfliktmanagement, Öffentlichkeitsarbeit, Selbstkompetenz und Nachfolgesicherung.

Wir geben zahlreiche Broschüren, zwei DVDs und das viermal jährlich erscheinende Magazin *perspektive* heraus, ein Buch ist in Zusammenarbeit mit uns erschienen.

In Leitfäden haben wir das über viele Jahre gesammelte und gebündelte Wissen zur Führung eines bundesweit, ehrenamtlich getragenen Verbandes in schriftlicher Form festgehalten. Die Veröffentlichung soll zum Austausch unter Selbsthilfeverbänden anregen und die professionellen Kooperationspartner einladen, einmal hinter die Kulissen der Frauenselbsthilfe nach Krebs zu schauen und zu erkennen, dass wir nicht nur Qualität im Gesundheitssystem fordern, sondern Qualität auch selbst bieten.

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SELBSTHILFE LUNGENKREBS

B. Baysal

Berlin, Germany

Die Selbsthilfe Lungenkrebs Berlin wurde im April 2003 gegründet und wurde bis Februar 2006 von Frau Karin Leuschner geleitet. Da sich Frau Leuschner anderen ehrenamtlichen Aufgaben widmete, übernahmen wir, Barbara Baysal, Gründungsmitglied, und Heidi Jäger, Mitglied seit

2004, die Gruppe, die sich bis dahin einmal monatlich in den Räumen des Onkologischen Patientenseminars in der Berliner Charité, Campus Virchow traf. Seit 2005 bestehen auch ein Internetforum sowie eine Homepage.

Etwa zeitgleich mit unserer Gruppe wurde eine Gruppe in Hamburg durch Frau Brigitte Drabinski gegründet und geleitet. Sie arbeitet regional unter dem Dach der Frauenselbsthilfe nach Krebs.

Für den Bereich Lungenkrebs gab es bis zum Jahre 2003 keine Selbsthilfegruppe. Die Betroffenen fanden sich in allgemeinen Krebsgruppen wieder, die jedoch auf die spezifischen Probleme der Lungenkrebserkrankten nicht eingehen konnten. Für uns als Betroffene war es wichtig, auf ebenfalls Betroffene zu treffen, die Möglichkeit des Austauschs zu haben, unsere Erfahrungen mitzuteilen und zu sehen, wie andere mit der Erkrankung umgehen und damit leben. Wir erleben Selbsthilfe als sinnvolle und notwendige Ergänzung, die den Umgang mit der Erkrankung erleichtern kann. Selbsthilfe stärkt die Kompetenz des Patienten, hilft Ängste abzubauen und Behandlungen besser zu verstehen. Hier treffen Patienten und Angehörige auf Menschen, die das gleiche Schicksal erleben; vieles muss nicht erklärt werden, man wird verstanden! Auch Fragen hinsichtlich Schwerbehinderung, Rente, Reha, Pflegedienste usw. können durch die Erfahrungen der anderen beantwortet werden. Sozialdienste der Kliniken sowie Ärzte und ein Psychologe stehen unserer Selbsthilfegruppe beratend zur Verfügung.

Seit Übernahme der Berliner Gruppe arbeiten wir auch kontinuierlich am Aufbau von weiteren Gruppen innerhalb Berlins, was dazu geführt hat, dass es mittlerweile in Berlin drei Gruppentreffen gibt, und zwar in der Charité, Campus Virchow, in der Evangelischen Lungenklinik Berlin sowie in der Klinik Heckeshorn im Emil von Behring, Zehlendorf. Wir sind offen für Betroffene und Angehörige, da wir der Meinung sind, dass Angehörige nicht weniger betroffen sind wie die Erkrankten selbst.

Ferner haben wir uns es zur Aufgabe gemacht, bundesweit Gruppen zu initiieren und Hilfe zur Gründung zu geben. Hierbei werden wir u. a. durch die Aktion Der Zweite Atem unterstützt, welche bundesweit Informationsveranstaltungen für Betroffene, Angehörige und Interessierte durchführt. Wir nehmen gern an diesen Veranstaltungen teil, da diese neutral sind, keine Medikamentenwerbung beinhalten und es hier um reine Informationen zum Lungenkrebs mit Experten aus den jeweiligen Regionen geht. Gleichzeitig haben wir die Möglichkeit, für die Gründung neuer Selbsthilfegruppen zu werben und auch hier Unterstützung durch die Veranstalter zu erfahren.

Mittlerweile besteht auch eine Gruppe in Stuttgart (seit Juli 2008). In Gründung sind Gruppen in Bochum, Halle an der Saale, Straussberg bei Berlin und Würzburg.

Wir stehen beratend telefonisch bundesweit für Betroffene und Angehörige zur Verfügung und versenden Informationspakete, so dass sich Betroffene über ihre Erkrankung informieren können. Dies erfolgt leider durch die behandelnden Ärzte und Kliniken oft noch nicht in dem Sinne, wie wir es uns wünschen und es für erforderlich halten. Sogar aus der Schweiz und Österreich werden wir angerufen und um Rat und Hilfe gebeten.

Mittlerweile haben wir als Selbsthilfe Lungenkrebs an der S3-Leitlinie zur Behandlung des Lungenkrebses mitgearbeitet, arbeiten mit in der Berliner Projektgruppe Lungenkarzinom im Turmorzentrum Berlin, haben am Berliner Qualitätshandbuch zur Behandlung des Lungentumors mitgewirkt und vertreten die Selbsthilfe Lungenkrebs auf Kongressen und Veranstaltungen.

Finanziert wird die Berliner Gruppe durch Fördermittel der Krankenkassen gem. § 20 ____G, teilweise durch Portoerstattungen, unser "Spendenschweinchen" und viel viel Eigeninitiative.

Unser Ziel ist es auch, die Selbsthilfe im Bereich Lungenkrebs öffentlich machen, uns von dem Makel zu befreien, ggf. selbst an der Erkrankung schuld zu sein und dadurch nicht über die Erkrankung reden zu dürfen.

Warum dies alles? Ich bin selbst Betroffene und bereits "Wiederholungstäter" und war auf der Suche. Ich hatte viele Fragen, jedoch keine Antworten! Dies wollen wir Neuerkrankten ersparen und ihnen die Möglichkeit bieten, sich zu informieren, denn wir sind "Experten" im Erleben und Leben mit der Erkrankung!

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DIE DEUTSCHE ILCO - LEBEN MIT DARMKREBS - OB MIT ODER OHNE STOMA

E. Hartkopf

Region Hamburg, Bonn, Germany der Deutschen ILCO e. V.

Rund 70000 Menschen erkranken pro Jahr neu an Darmkrebs. Operation und oftmals weitere The-rapien sind die Folge. Je nach betroffenem Darmteil muss manchmal ein künstlicher Darmausgang angelegt werden, häufig allerdings nur vorübergehend. Ein Stoma (= künstlicher Ausgang) kann zwar auch wegen anderer Grunderkrankungen nötig werden. Darmkrebs ist allerdings die häufig-ste Ursache. Die Deutsche ILCO ist die Solidargemeinschaft von Stomaträgern und von Menschen mit Darmkrebs auch ohne Stoma sowie deren Angehörigen. Etwa 60% der ILCO-Mitglieder sind an Darmkrebs erkrankt.

Die Diagnose "Sie haben Darmkrebs" ist auch heute noch für die meisten Betroffenen ein Schock. Und wenn der Arzt am Ende gründlicher Untersuchungen oder als Lösung eines langen Leidens eröffnet: "Wir müssen einen künstlichen Ausgang, ein Stoma, anlegen", bricht für viele erst einmal eine

Welt zusammen. Nur wenige können sich vorstellen, dass man heute auch mit Stoma ein Leben führen kann, das sich kaum vom Leben "davor" unterscheiden muss. Ungefähr 100 000 Menschen leben in Deutschland mit einem Stoma.

Durch spezielle Operationsmethoden kann heute in den meisten Fällen die Anlage eines dauerhaf-ten Stomas vermieden werden. Häufig kann sogar bei einem nahe am Afterschließmuskel gelege-nen Rektumkarzinom der Schließmuskel erhalten werden, sodass ein zum Schutz der Darmnaht vorübergehend angelegtes Stoma später wieder zurückverlegt werden kann. Die Anliegen vieler Ratsuchender in der Deutschen ILCO zeigen allerdings, dass der Alltag danach nicht immer unge-trübt ist. Verändertes Stuhlverhalten (häufigere Stuhlgänge, Durchfälle) oder ungünstige Stuhlkonsistenz können Folgen sein, welche die Betroffenen über einen kürzeren oder längeren Zeitraum, manchmal sogar dauerhaft sehr belasten. Die Deutsche ILCO sammelt seit einiger Zeit die Erfah-rungen darmkrebsbetroffener Menschen mittels eines Fragebogens. Sie erhält dadurch einen bes-seren Überblick über Belastungen und Bedürfnisse und kann so ihre Unterstützungsangebote wei-ter entwickeln.

Zur Unterstützung von Betroffenen bietet die Deutsche ILCO:

- Informationen rund um das Stoma und den Darmkrebs, unter anderem in Veranstaltungen, Infoschriften, Broschüren und der vierteljährlich erscheinenden Mitgliederzeitschrift ILCO-PRAXIS
- Gespräche mit Gleichbetroffenen und Beratung zu Fragen des täglichen Lebens mit einem Stoma sowie der Darmkrebserkrankung, auf Wunsch schon im Krankenhaus
- Unabhängige gesundheits- und sozialpolitische Interessenvertretung bei Stoma und bei darmkrebsbezogenen Anliegen
- Die Vermittlung zur Fachberatung

Für Verbesserungen in der Versorgung von Darmkrebsbetroffenen und von Stomaträgern sucht die Deutsche ILCO eine enge Zusammenarbeit mit den beteiligten Fachgruppen z. B. aus Medizin, Krankenpflege, Sozialarbeit, Psychosozialer Unterstützung, Stomaversorgung.

Die Deutsche ILCO hat etwa 8800 Mitglieder und über 300 Gruppen in ganz Deutschland. Selbst-hilfe, Ehrenamt sowie inhaltliche und finanzielle Unabhängigkeit sind die wesentlichen Arbeitsprin-zipien. Etwa 800 ausnahmslos ehrenamtliche Mitarbeiter sowie die in der Bundesgeschäftsstelle tätigen hauptamtlichen Mitarbeiter unterstützen - über den Kreis der Mitglieder hinaus - mehr als 20000 Betroffene im Jahr.

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DER ARBEITSKREIS DER PANKREATEKTOMIERTEN e.V. (AdP e.V.)

J. Kleeberg

Bonn, Germany

Die schwierigen und vielfältigen Situationen nach einer Operation an der Bauchspeicheldrüse brachten Betroffene 1976 in Heidelberg auf die Idee, unter dem Motto "Hilfe durch Selbsthilfe" einen Arbeitskreis der Pankreatektomierten e.V. zu gründen. Von Anfang an erklärten sich Ärzte und Diätassistentinnen zur Mitarbeit bereit. Zweck des AdP ist die Förderung der Gesundheit und Rehabilitation von partiell und total Pankreasoperierten sowie der Menschen, die an anderen Erkrankungen der Bauchspeicheldrüse leiden.

Seit 1979 ist der AdP ein eingetragener Verein. Im Laufe der vergangenen Jahre hat der AdP mit der Hilfe von Ärzten verschiedener Disziplinen, Ernährungswissenschaftlern, Psychologen und Sozialexperten ein gut funktionierendes System der Hilfe entwickelt, das allseitig Anerkennung findet.

Der AdP ist Mitglied im Patientenbeirat der Deutschen Krebshilfe e.V., im Deutschen Diabetikerbund, im Paritätischen Wohlfahrtsverband und in der Gesellschaft für Rehabilitation bei Verdauungs- und Stoffwechselerkrankungen e.V. (GVRS). Er wird von der Deutschen Krebshilfe umfassend finanziell gefördert.

Ein wissenschaftlicher Beirat des AdP unterstützt den Vorstand des AdP und die über 40 Regionalgruppen in ganz Deutschland mit wichtigen, aktuellen medizinischen Informationen. Augenblicklich sind 1125 Mitglieder Im AdP organisiert

Aus unserem Programm:

- Jährliche, bundesweite Informationstreffen mit Vorträgen, Diskussionsgruppen und Einzelberatungen
- Regionaltreffen in Zusammenarbeit mit medizinischen Zentren für Pankreaserkrankungen
- Ein Handbuch des AdP für jedes Mitglied als Loseblattsammlung mit jährlich zweimaliger Aktualisierung
- Z. Z. 43 Regionalgruppen in den einzelnen Bundesländern als Ansprechpartner vor Ort
- Experten für sozial-rechtliche Fragen stehen zur Verfügung
- Mitglieder des wiss. Beirates stehen für individuelle Beratungen zur Verfügung
- Beratungen für chronisch an der Bauschspeicheldrüse erkrankte Menschen werden angeboten
- Der AdP übermittelt aktuelle Informationen über seine Internetseite
- Eine Bundesgeschäftstelle in Bonn ist Ansprechpartner für alle Interessenten

Zu folgenden Problemen werden wir hauptsächlich angesprochen:

- Ernährungsfragen nach einer OP und bei anderen Erkrankungen der Drüse-Überwindung des Gewichtsverlustes
- Zur Einnahme von Pankreasenzympräparaten
- Zur Nachsorge-Chemo-REHA-alternative Behandlungen
- Informationen über Ärzte, Kliniken, REHA-Einrichtungen
- Der Wunsch, mit Gleichbetroffenen in Kontakt zu kommen.

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