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ABSTRACTS OF THE 16th INTERNATIONAL HAMBURG SYMPOSIUM ON TUMOR MARKERS

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APPLICATION OF NANOTECHNOLOGY FOR SPECIFIC CANCER TREATMENT – THE SEON-CONCEPT

S. Lyer[1], C. Janko[1], S. Dürr[1], R. Tietze[1], J. Zaloga[1], H. Unterweger[1], R.P. Friedrich[1], S. Odenbach[2], L. Trahms[3], C. Alexiou[1]

[1]Department of Otorhinolaryngology, Section of Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Foundation-Professorship, University Hospital Erlangen Germany;
[2]TU Dresden, Chair of Magnetofluiddynamics, Germany,
[3]PTB Berlin, Germany

The application of nanotechnology for cancer is an interdisciplinary area of research in science engineering and medicine with broad application for imaging, molecular diagnosis and targeted therapy. In particular superparamagnetic iron oxide nanoparticles (SPIONS) deserve attention as they can be used for diagnostic as well as for therapy ("theranostics"). For diagnosis *in vivo* SPION are already in use as contrast agents in magnetic resonance imaging, *in vitro* they are appointed for cell separation. From a drug delivery point of view, targeting of cancer is a most promising area concerning delivery of chemotherapeutics which can be combined with hyperthermia. A very promising approach in this field is Magnetic Drug Targeting (MDT), which enables a goal oriented local application of cancer therapeutics in the desired region (*i.e.* tumor). Very successful animal experiences have already been performed on this purpose. SEON (Section of Experimental Oncology and Nanomedicine) is aiming to translate this efficient therapeutic model into clinical trials. To gain this ambitious project, several requirements, such as detailed synthesis and characterization of the nanoparticles, nanotoxicological testings, *ex vivo* models to simulate *in vivo* conditions for appropriate adjustment for the necessary parameters and pre-clinical animal studies have to be addressed. These results are of pivotal importance to start with respective GMP production and approval, which is essential for clinical trials. Additionally interdisciplinary collaboration with physicists and engineers is necessary to reveal appropriate technical application modes and the possibility for quantitative analysis of particle distribution. SEON addresses these issues with a special focus on specific drug delivery using magnetic nanoparticles in cancer treatment and avoiding the negative side effect of conventional chemotherapy, which will be of medical and economic relevance concerning the increasing number of cancer patients.

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DETECTION AND CHARACTERIZATION OF VIABLE CIRCULATING TUMOR CELLS AS LIQUID BIOPSY FOR CANCER

C. Alix-Panabières

Laboratory of Rare Human Circulating Cells, Department of Cellular & Tissue Biopathology of Tumors, Institute of Regenerative Medicine & Biotherapy, Saint-Eloi Hospital, University Medical Centre of Montpellier, Montpellier, France

The enumeration and characterization of circulating tumor cells (CTCs) in the peripheral blood and disseminated tumor cells (DTCs) in bone marrow may provide important prognostic information and might help to monitor efficacy of therapy. Since current assays cannot distinguish between apoptotic and viable DTCs/CTCs, it is now possible to apply a novel ELISPOT assay (designated 'EPISPOT') that detects proteins secreted/released/shed from single epithelial cancer cells. Cells are cultured for a short time on a membrane coated with antibodies that capture the secreted/released/shed proteins that are subsequently detected by secondary antibodies labeled with fluorochromes. In breast cancer, we measured the release of cytokeratin-19 (CK19) and mucin-1 (MUC1) and demonstrated that many cancer patients harbored viable DTCs in their bone marrow, even patients with apparently localized tumors (stage M_0: 54%). Preliminary clinical data (n=57) showed that patients with DTC-releasing CK19 have an unfavorable outcome. We also studied CTCs or CK19-releasing cells (CK19-RC) in the peripheral blood of 194 M_1 breast cancer patients and showed that patients with CK19-RC had a worse clinical outcome. In prostate cancer patients (n=48), we used prostate-specific antigen (PSA) secretion as marker to detect PSA-secreting cells and observed that 83% and 42% of M_1 and M_0 cancer patients, respectively, had CTCs with a difference in the CTC median (29 for M_1 and 9 for M_0) and found that a significant fraction of CTCs also secreted fibroblast growth factor-2 (FGF-2), a known stem cell growth factor. More recently, in colon cancer, a considerable portion of viable CTCs detectable by the Epispot assay is trapped in the liver as the first filter organ in colon cancer patients. The enumeration of CK19-RC by the CK19-Epispot assay in 75 colorectal cancer patients revealed viable CTCs

in 65.9% and 55.4% (p=0.04) patients in mesenteric and peripheral blood, respectively, whereas CellSearch detected CTCs in 55.9% and 29.0% (p=0.0046) patients. In mesenteric blood, the number of CTC was significantly higher than in the peripheral blood. Our clinical data showed that localized colon cancer patients with a high level of CTCs have an unfavorableoutcome (n=60). In conclusion, the EPISPOT assay offers a new opportunity to detect and characterize viable DTCs/CTCs in cancer patients and it can be extended to a multi-parameter analysis revealing a CTC/DTC protein fingerprint.

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BIOMARKERS FOR MELANOMA – TREATMENT RESPONSE ASSESSMENT AND METASTASIS PREDICTION

V. Barak, S. Frenkel, S. Merims, V. Leibovici, M. Lotem, T. Peretz, J. Pe'er

Immunology Lab for Tumor Diagnosis, Oncology, Dermatology and Ophthalmology Departments, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Background: Cutaneous Malignant Melanoma (CMM) patients have a poor survival in spite of new drug developments. Uveal Melanoma (UM) metastasizes mainly to the liver. Therefore, it is essential to detect early liver metastasis formation and improve survival. Biomarkers assessing both patients' response to new therapies and prognosis, are therefore important.

Aims: To evaluate a panel of Biomarkers: Osteopontin (OPN), S-100b, Melanoma-inhibitory activity (MIA), Tissue Polypeptide-Specific Antigen (TPS), TK, VEGF, in metastatic CMM and UM *vs*. Disease-Free (DF) patients (pts), aiming to study which biomarker will best predict and detect early liver metastasis, thus enabling effective treatment with impact on patient survival.

Methods and Patients: Levels of serum biomarkers were analyzed for 129 DF, 88 metastatic UM and 68 CMM, and compared to 100 healthy Controls. A matched-pair analysis was used to compare pre- and post-metastasis, and post treatment marker levels. Differences in biomarker levels were analyzed for each treatment group and correlated to survival and metastasis-free status.

Results: UM pts underwent brachytherapy and part of them were enucleated. A significant decrease in S-100 levels in the enucleated pts (0.09 to 0.04 μg/l, p=0.0492) after 1 m and in the brachytherapy pts (0.11 to 0.05 μg /l, p=0.0251) after 9 m, were demonstrated. Significantly increased levels (5-25 fold) (p=0.002) in the BioMarkers OPN, MIA, VEGF, TK, TPS and S -100b were predictive of liver metastases, as shown later by CT. In the CMM pts, increases in all markers indicated unresponsiveness to treatment and recurrence, while significant decreases were correlated to PR to the new biological therapies.

Conclusion: Treatments of UM resulted in significant decreases in Biomarker (S-100,OPN, MIA, TK,VEGF, TPS) levels. Significant increases in all markers were detected prior to CT, predicting the development of liver metastases. We introduced S100 as a routine biomarker for CMM and UM pts follow up, to verify response to new biological therapies and reduce the number of CT tests.

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DIAGNOSTIC AND PROGNOSTIC TUMOR MARKERS FOR HEAD & NECK CANCER

V. Barak, M. Wigoda, A. Meirovich, V. Leibovici, K. Rachmut, T. Peretz, R. Eliashar, M. Gross

Immunology Lab for Tumor Diagnosis, Depts of Oncology, ENT, Head &Neck Surgery, Dermatology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Aims: To evaluate SCC, CEA, TPS and CYFRA 21-1 as Tumor Markers for assessing treatment response or prognosis in head & neck cancer patients (pts).

Methods: We have assessed 72 Larynx Ca pts.- pre and 38 post- surgery, 46 Oral Cavity Ca pts. pre and 29 post-surgery. In addition, 35 healthy age-and sex-matched controls were included. Correlations of marker levels (median) to stage, lymph node involvement, grade and pathology, were performed. All 4 markers were evaluated by ELISA assays.

Results: Serum levels (median) of all 4 markers were higher before surgery and decreased thereafter in patients with Larynx Ca. (SCC- 3.64±0.3 to 0.7±0.08, p=0.02; CEA-17.2+1.56 to 3.8+0.2, TPS-98.5±8.1 to 43.7±6.1 ns; CYFRA 21-1- 3.24±0.3 to 1.11±0.17, p=0.09). In Oral Cavity Ca. pts, SCC levels decreased significantly (3.6±0.2 to 0.95±0.19, p=0.05). Higher levels of all 4 markers were found in T3, T4 pts as compared to T1, T2 pts (SCC-2.97±0.9 to 0.88±0.17, p=0.07; CEA- 15.3+1.78 to 5.9+0.8, TPS-77.3±9.9 to 61.7±6.7, p=0.012; CYFRA 21-1-2.3±0.2 to 0.89±0.2, ns).Significantly higher levels of CYFRA 21-1 were found in Larynx Ca pts than in Oral Cavity Ca pts. Node positive pts had significantly higher TPS levels as compared to node negative pts (79±0.91 *vs*. 41±1.17, p=0.02). All parameters were placed in a multivariate analysis. TPS and Cyfra 21-1 were found as independent prognosticators.

Conclusion: TPS, CYFRA 21-1, CEA and SCC serum levels can all serve as markers of Head & Neck Cancer. Of all 4 markers, TPS proved to be the most sensitive predictor of advanced disease and poor prognosis, correlating to stage

and nodes. Overall survival was significantly correlated to high levels of TPS-57% pts alive after 2 y and 46 % after 5y. *vs*. 90% pts alive after 5y, for those having low TPS levels, pre surgery.

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CLINICAL UTILITY OF CYTOKINE BIOMARKERS IN A LUNG CANCER PATIENT TREATED WITH TOCILUZUMAB

H. Nechushtan, L. Appelbaum, I. Kalichman, T. Peretz, V. Barak

Immunology Lab. for Tumor Diagnosis, Oncology Department, Hadassah - Hebrew University Medical Center, Jerusalem, Israel

Introduction: Current understanding of drug resistance in cancer patients focuses on the appearance of new mutations. The possible causal role of cytokines in such resistance is less studied.

Aims: To evaluate the clinical utility of cytokine biomarkers in a patient receiving a cytokine based new therapy.

Methods: A 49 year old patient with metastatic lung cancer and a Ret translocated tumor, was treated for over three years with various treatments. Initially, he had a complete response to chemotherapy, but following resection of a single metastasis, multiple liver metastases appeared, as his only disease site. He was treated with intraarterial SIRTEX- Ytrium labeled microsphers. Later he had lung and abdominal metastases, but liver metastases appeared stable. He had a major gastric ulcer bleeding (Hgb 3.6) following radiation and treatment with Sunitinib. Following this bleeding there was a rapid growth of multiple lung nodules and severe life threatening dyspnea.

Results: The rapid progression of multiple metastases following a traumatic event of massive bleeding, suggested that perhaps high expression and accumulation of cytokines may be the reason for tumor progression. Several serum cytokines and growth factors levels were assessed (IL-6, TNFa, IL-2R, TPS, VEGF). Very high IL-6 levels were demonstrated (1717-2967 pg/ml), more than 10 times the normal upper limit. The drug resistance was accompanied with massive increases in all those biomarker levels. He was therefore treated with monthly doses of Tociluzumab - Anti-IL-6 Receptor Antibody.Within two days of the first treatment he had a marked improvement in his dyspnea. Three weeks later his dyspnea recurred and again responded to this therapy. As his disease continued, his IL-6 and other cytokines levels increased significantly, following the Tociluzumab (4 mg/Kg) treatments.

Conclusion: Treatments consisting mainly of Anti IL-6R Antibody caused a rapid relief of severe dyspnea. Cytokine markers determined response or unresponsiveness to this new therapy very accurately.

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NEW APPROACHES IN SYSTEMIC THERAPY OF PANCREATIC CANCER

A. Block

University of Hamburg, University Hospital Hamburg - Eppendorf, Department of Oncology and Hematology, Hamburg, Germany

Pancreatic cancer remains one of the most challenging diseases in oncology with surgical resection as the only curative option in very early stages and a 5-year survival rate of only 3-15%. With gemcitabine as the standard chemotherapy for more than a decade, the outcome remained dismal. Recent regimens combining gemcitabine and nab-paclitaxel or FOLFIRINOX significantly improved response rates in patients with metastatic pancreatic cancer. Moreover, many clinical trials with new drugs directed against novel targets are in advanced stages offering the possibility for the development of new therapeutics in the near future. These new therapeutics include immune mediated therapies, tumor stroma disrupting agents, PARP inhibitors, as well as new cytotoxic substances. The new challenge will be choosing the best sequence of therapies adjusted to the expected outcome in the individual patient. Thus, new approaches will improve response and overall survival, but certainly demand new biomarkers to better predict response and survival in the individual case.

7
CHALLENGES OF CONDUCTING CLINICAL TRIALS IN ONCOLOGY

A. Block

University of Hamburg, University Hospital Hamburg - Eppendorf, Department of Oncology, Clinical Trials Center, Hamburg, Germany

A significant improvement in the diagnosis and treatment of malignant disorders has been achieved over the past few decades. Understanding how to conduct clinical trials warrants medical progress without putting the patient at unnecessary risk. Recent discovery of several new groups of medication, the molecular understanding of malignancies with highly personalized therapeutic approaches and on increasingly tight

legal framework have posed a major challenge for clinical researchers. Furthermore, a thorough understanding of economical aspects is required in conducting clinical trials. The presentation will focus on innovative instruments to successfully conduct clinical trials despite the demanding legal, scientific, ethical and economical requirements without compromising on the patient's interest.

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BORDERLINE RESECTABLE PANCREATIC CANCER – A CONSENSUS STATEMENT BY THE INTERNATIONAL STUDY GROUP OF PANCREATIC SURGERY (ISGPS)

M. Bockhorn, F.G. Uzunoglu and J.R. Izbicki

For the International Study Group of Pancreatic Surgery, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Background: This position statement was developed in order to expedite a consensus on definition and treatment for borderline resectable pancreatic ductal adenocarcinoma that would have worldwide acceptability.

Methods: An international panel of pancreatic surgeons from well-established high-volume centers collaborated on a literature review and development of consensus on issues related to borderline resectable pancreatic cancer.

Results: The ISGPS supports the National Comprehensive Cancer Network (NCCN) criteria for borderline resectability. Current evidence supports operative exploration and resection in the case of involvement of the mesenteric-portalvenous axis; in addition, a new classification of venous resections is proposed by the ISGPS. Suspicion of arterial involvement should lead to exploration to confirm the imaging-based findings. Formal arterial resections are not recommended. However, in exceptional circumstances, individual therapeutic approaches may be evaluated under experimental protocols. The ISGPS endorses the recommendations for specimen examination and the definition of an R1 resection used by the British Royal College of Pathologists (RCPath). Standard preoperative diagnostics for borderline resectable patients may include serum levels of CA19-9 as these have been shown to predict survival in large retrospective series, and also the modified Glasgow Prognostic Score (mGPS) and the neutrophil/lymphocyte ratio (NLR), because of the prognostic relevance of the systemic inflammatory response. Various regimens of neoadjuvant therapy are recommended only in the setting of prospective trials at high-volume centers.

Conclusion: Current evidence justifies only porto-mesenteric venous resection in patients with borderline resectable pancreatic cancer. Basic definitions were identified, that are currently lacking but that are needed to obtain further evidence and improvement for this important patient subgroup. A consensus for each topic is given.

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CIRCULATING TUMOR CELLS (CTC) AS BIOMARKERS IN RECURRENT AND CASTRATION RESISTANT PROSTATE CANCER

M. Bögemann

Klinik und Poliklinik für Urologie, Uroonkologie, Universitätsklinikum Münster, Münster, Germany

PSA is the most commonly used tumor marker in prostate cancer. It is broadly accepted as a marker in recurrent disease after definitive therapy. It is also commonly accepted as a response indicator within initial androgen deprivation therapy. Dynamic PSA-changes like PSA doubling time or PSA-velocity are correlated with predicting recurrence and development of metastases. In castration resistant prostate cancer, PSA can rise early under modern antitumor therapy before eventually declining, thus challenging physicians in assessing therapy response. In bone metastatic prostate cancer bone-flare can mimic progression in early imaging during therapy leading to a treatment-dilemma in asymptomatic patients, with the option for biomarkers in helping to decide on continuation of therapy. The role of PSA and its isoforms, CTCs and ALP in the given situations is being reviewed considering current guidelines and the relevance for clinical trials and everyday practice.

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PREVENTION OF SKIN CANCER

N. Booken, V. Steinkraus

Dermatologikum, Hamburg, Germany

Skin cancer, including melanoma and non-melanoma skin cancer (NMSC) are the most frequent types of cancer in white population worldwide. Both tumor entities show an increasing incidence rate but a stable or decreasing mortality rate. Changes in outdoor activities and exposure to sunlight during the past 50 years are an important factor for the increasing incidence. While NMSC can lead to considerable morbidity, the prognosis of MM is highly dependent on stage at diagnosis. If detected at an early stage, 5-year survival rates are over 90%. In contrast, if MM is diagnosed at stage IV, only about 15-20% of patients are still alive 5 years after diagnosis. The rising incidence rates of skin cancer are probably caused by a

combination of increased exposure to UV-radiation, increased outdoor activities, changes in clothing style, increased longevity, ozone depletion, genetics and in some cases, immune suppression. This is supported by the International Agency for Research on Cancer (IARC) that recently defined UV radiation as a carcinogen. To reduce the burden of skin cancer primary and secondary preventive activities are important. Primary preventive activities are predominantly aimed at changing people's behavior. This needs, that the public is informed by simple and balanced messages about the possible harms and benefits of UV-exposure. For this purpose information and recommendations for the public must be age- and target-group specific to cover all periods of life and to reach all sub-groups of a population. This is especially important due to the ongoing Vitamin D debate, where possible positive effects of UV have to be balanced with the well known skin cancer risk of UV. Secondary preventive activities are mainly aimed at early detection of skin cancer. This includes the regular self examination of the skin and the routine skin cancer screening by a dermatologist.

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SPORTS IN THE REHABILITATION OF PATIENTS AFTER TOTAL LARYNGECTOMY

J. Büntzel, H. Büntzel, R. Mücke, A. Besser, O. Micke

Klinik für HNO-Erkrankungen, Kopf-Hals-Chirurgie, Interdisziplinäre Palliativstation, Südharz Klinikum Nordhausen GmbH, Nordhausen, Germany

Objective: Laryngectomy due to cancer leads to decreased physical activity of the patients. Rehabilitation programs are focussing on the improvement of voice and swallowing. Which role could rehabilitation sports play for those cancer patients?

Materials and Methods: We have interviewed all 38 patients (5 women, 33 men, median age 56 years) of our patient's advocacy group. All were laryngectomized because of cancer in the past. We asked for reporting their sportive activities and summarized the descriptions.

Results: 12 patients were members of our swimming group and had performed aqua gymnastics and swimming training in order to stabilize or improve the muscle structure of the neck and backbone. Further 4 patients have only taken part in swimming training. The training frequency was twice per month. If patients were introduced to the program they increased the frequency to 4-5/month. Three patients have started bicycle tours with mountain bikes of distances between 30 and 50 km again. Further two patients were able to follow our bicycle tours by e-bikes. 20 patients reported about weekly walking tours between 10 and 16 km. All patients summarized the positive impact of sports on their personal well being.

Conclusion: Swimming, aqua-fitness, biking and (nordic) walking are favourable sport disciplines for cancer patients without larynx. Special training programs should be developed and included to rehabilitation procedures after multimodal therapy of laryngeal cancer.

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REDUCED LATE TOXICITIES IN HEAD AND NECK CANCER PATIENTS AFTER INTENSITY MODULATING RADIOTHERAPY

J. Büntzel[1], R. Böhme[2], H. Büntzel[1], B. Hentschel[2], W. Oehler[2]

[1]Department of Otolaryngology, Südharz Klinikum Nordhausen;
[2]Department of Radiotherapy, Südharz Klinikum Nordhausen, Germany

Objective: Intensity modulating radiotherapy was established in the multimodal therapy of head and neck cancer during the last decade. Improved survival rates and reduced acute toxicities were reported. We know less about late toxicities.

Materials and Methods: Between 1999 and 2014 we performed 1056 semi-structured interviews with former head and neck cancer patients. All patients were treated with primary surgery plus radiotherapy or radiotherapy alone. We were asking the late toxicities during the time after successful therapy of head and neck cancer. The analysis was focused on the nutrition-associated toxicities which were classified according to the RTOG scale. *Results:* We have analysed 851 patients (58.1±9.8 years, 903±827 days) after conventional radiotherapy and 206 patients (59.3±12.4 years, 1248±1084 days) after IMRT radiotherapy. The following incidences were observed:

	Conventional RT (n=851)		IMRT (n=206)		
	Yes	No	Yes	No	*p*-Value
Dry mouth	86 (10%)	765 (90%)	39 (19%)	167 (81%)	<0.001
Dysphagia	181 (21%)	670 (79%)	56 (27%)	150 (73%)	0.063
Esophageal stenosis	621 (73%)	230 (27%)	163 (79%)	43 (21%)	0.070
Loss of taste	532 (63%)	319 (37%)	119 (58%)	87 (42%)	0.209
No appetite	751 (88%)	100 (12%)	191 (93%)	15 (7%)	0.065

Conclusion: Functional orientated surgery and intensity modulating radiotherapy have improved nutritional situation after successful finished therapy of head and neck cancer.

13
SERUM MAGNESIUM LEVEL AND POST-OPERATIVE PAIN SENSATION IN PATIENTS

J. Büntzel[1], K. Kisters[1], J. Büntzel[2]

[1]Medizinische Klinik, St. Anna Hospital Herne;
[2]HNO-Klinik, Südharz Klinikum Nordhausen, Germany

Objective: Partial pharyngectomy or tonsillectomy is known as the standard procedures with the most severe problems in post-operative pain management in the field of ENT cancer surgery. Might be there a correlation between the individual pain sensation and the serum magnesium level of the affected patients?

Materials and Methods: Between July and December 2013, 82 consecutive pharyngectomy/ tonsillectomy patients (37 male, 44 female) of the ENT-clinic at Nordhausen were asked to specify their postoperative pain on a visual analogue scale (VAS, 0 to 10) twice a day over a maximal span of a week. The magnesium serum level was measured pre-operatively. All patients received a standard analgesic treatment with metamizole or paracetamole.

Results: Regarding the serum level our population showed a normal distribution- 12 patients <0.80 mmol/l (group A), 17 patients 0.80-0.85 mmol/l (group B), 28 patients 0.85-0.90 mmol/l (group C) 16 patients 0.90-0.95 mmol/l (group D) and 8 patients >0.95 mmol/l (group E). The mean VAS of group A was 2±1.871 points, of group B 0.956±1.016 points, of group C 1.688±1.595, of group D 0.906±1.186 points and of group E 1.344±2.066 points. The increasing of VAS in group A becomes significant between day 4 and 6 after surgical resection (p=0.01), when the first wound debris dissolves.

Conclusion: Our data points to a particular pain sensibility in the neuropathic range of patients, whose serum magnesium level is decreased. The value of magnesium as co-analgesic drug should be tested for this group.

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HYPERCALCEMIA AT THE END OF LIFE

J. Büntzel, O. Micke, H. Büntzel, K. Kisters

Klinik für HNO-Erkrankungen, Kopf-Hals-Chirurgie, Interdisziplinäre Palliativstation, Südharz Klinikum Nordhausen GmbH, Nordhausen, Germany

Objective: The loss of electrolyte's homeostasis is a generally accepted bad prognostic sign in patients with advanced tumor diseases. When it is necessary to treat hypercalcemia in end-of-life care?

Materials and Methods: Between January 1st and July 31st, 2014 we have registered 9/238 patients, who were admitted at our palliative care unit because of excessive hypercalcemia, mental confusion, paired with hallucinations and aggressive behaviour. Our analysis is descriptive, retrospective and in case report form.

Results: All patients had no brain metastases (MRI). The serum calcium concentration was >2.7 mmol in all cases. The clinical main symptoms were hallucinations and aggressive behaviour without any psychiatric diseases in the anamnesis of the patient. The onset time of symptoms was <48 hours. All patients received bisphophonates and midazolam IV. The hypercalcemia was corrected in 3/9 patients after 5 days, the sedation could be finished and the patients deserved the PCU without any psychiatric signs. Six patients died because of multi-organ dysfunction and basic cancer disease.

Conclusion: Psychotic reactions without brain metastasis could be results of hypercalcemia at the end of life. In such cases it is necessary to start with bisphosphonates and palliative sedation.

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GPX2 UNDEREXPRESSION INDICATES POOR PROGNOSIS IN PATIENTS WITH UROTHELIAL CARCINOMAS OF THE UPPER URINARY TRACT AND URINARY BLADDER

I. Chang[1,2], C. Hun[1], C. Li[3]

[1]Institute of Biotechnology and Chemical Engineering, I-Shou University, Kaohsiung, Taiwan;
[2]Department of Pathology, E-DA Hospital, I-Shou University, Kaohsiung, Taiwan; (3)Department of Pathology, Chi-Mei Foundation Medical Center, Tainan, Taiwan

Specific *Objective:* Urothelial carcinoma (UC) is the most common tumor type of urinary tract. Despite the less frequent prevalence of upper tract urothelial carcinoma (UTUC) compared with urinary bladder urothelial carcinoma (UBUC), tumorigenesis of UC arising from both areas may share a similar molecular pathway. Oxidative stress is believed to be one of the important etiologies in carcinogenesis that has not been systemically investigated in UC. Through data mining from a published transcriptomic database of UBUCs (GSE31684), glutathione peroxidase 2 (GPX2) was identified as the most significant gene showing

stepwise downregulation from early tumor development to progression and metastasis among those responsive to oxidative stress (GO:0006979), as one of the key antioxidant enzymes, GPX2 is one of the mammalian glutathione peroxidase family. We therefore analyze GPX2 transcript and protein expressions and their associations with clinicopathological factors and survivals in our well-characterized cohort of UC.

Methods: Real-time RT-PCR assay was used to detect GPX2 mRNA level in 20 fresh UBUC specimens. Immunohistochemistry evaluates by using H-score was used to determine GPX2 protein expression in 340 UTUCs and 295 UBUCs, respectively. The mRNA and protein expression statuses were further correlated with clinicopathological features. The prognostic significance of GPX2 protein expression was further evaluated for disease-specific survival (DSS) and metastasis-free survival (MeFS).

Summary of Results: Decrease of GPX2 transcript level was associated with both higher pT and positive nodal statuses in 20 UBUCs (all $p<0.05$). GPX2 protein underexpression was also significantly associated with advanced pT status (both $p<0.001$), lymph node metastasis (UTUC, $p<0.001$; UBUC, $p=0.004$), high histological grade (UTUC, $p=0.041$; UBUC, $p=0.035$), vascular invasion (UTUC, $p<0.001$; UBUC, $p=0.02$), frequent mitoses (UTUC, $p=0.007$; UBUC, $p=0.042$) in both groups of UCs. GPX2 underexpression not only predicted dismal DDS and MeFS at univariate analysis, but also implicated worse DDS (UTUC, $p=0.002$; UBUC, $p=0.029$) and MeFS (UTUC, $p=0.001$; UBUC, $p=0.032$) in multivariate analysis. *Conclusion:* GPX2 underexpression is associated with advanced tumor status and implicated unfavorable clinical outcome for both patients of UTUCs and UBUCs. Our study discloses that GPX2 plays an important role in tumor progression in UCs and may serve as a potential prognostic biomarker of UCs.

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EARLY DETECTION OF LUNG CANCER

H. Dienemann

Thoraxklinik, Heidelberg University, Heidelberg, Germany

In Germany lung cancer is the most common cause for cancer-related mortality. In 2014, there will be an estimated 48.000 new cases of lung cancer diagnosed in this country and over 44.000 individuals are expected to die from this disease. Most patients will be diagnosed with advanced disease. The National Lung Screening Trial (NLST) demonstrated that low-dose screening has the potential to reduce lung cancer related mortality in high-risk individuals, defined by smoking history and age. With respect to validation of biomarkers a biospecimen repository including blood, sputum and urine samples was established; the specimens were collected from more than 10.000 participants. Since the prevalence of lung cancer in asymptomatic volunteers is low when they are selected based on the NLST criteria the use of biomarkers may further support the interpretation of low-dose CT. However, none have been validated for clinical use so far, whereas some evidence suggests that molecular biomarkers can provide additional information to clinical prediction models, *e.g.* in identifying high-risk individuals who should undergo further screening. In addition, molecular biomarkers have been investigated to define their role for differentiating between benign and malignant pulmonary lesions. Preliminary data has confirmed the potential utility of markers obtained from surrogate tissues like airway, blood and sputum to discriminate lung cancer patients from high risk controls. In a recent systematic review, 45 published trials of molecular biomarkers for the early detection of lung cancer have been identified, however, most of them have been phase I or II and none has been able to demonstrate the utility of any marker in the early detection of lung cancer. Some evidence from the NLST biomarker repository suggests that molecular markers may provide some complementary information to clinical and radiographic data in patients with pulmonary nodules identified on CT scans. In contrast, clinical modelling (using variables like race or ethnic group, education, body mass index, COPD, history of cancer and others) has been shown to predict the risk of lung cancer development with high accuracy and was more efficient at identifying persons for lung cancer screening, as compared with the NLST criteria.

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PREDICTIVE MOLECULAR PATHOLOGY: A PREREQUISITE OF PERSONALIZED MEDICINE

M. Dietel

Institute of Pathology, Charité, Humboldt University Berlin, Germany

Diagnostic molecular pathology is a major part of tissue based diagnostics and clinical management of infectious diseases and tumors as well as in the pharmaceutical development of new anti-cancer drugs. To read a patient's tissue as "deeply" as possible and to obtain com-bined information on morphological, genetic, proteomic as well as epigenetic grounds is both challenge and chance of modern anatomic pathology. Applications of new techniques play an increasing role in the routine process of tissue-based

diagnostics and in translational cancer research. The major up-coming challenges are

• to directly detect a broad spectrum of microorganisms in surgical specimens,

• to precisely and reproducibly diagnose malignant tumors, even rare lesions, and to establish internationally accepted diagnostic algorithms,

• to define the individual prognosis of the actual patient,

• to asses the probability of metastases, *e.g.* in case of clinical state M0 at time of tumor diagnosis and

• to predict response/resistance of individual tumors.

Due to continuous technical developments in immunohistochemistry and *in situ* hybrization assisted by different molecular and computational techniques the power of diagnostic histopathology has increased dramatically during the last decade. The new techniques are performed under standard operating procedures and continuous quality control. Combined application of the different approaches will further improve the importance of histological diagnoses and their predictive accuracy. In oncology, the application of new targeted compounds, *e.g.* therapeutic antibodies or kinase inhibitors, has achieved promising results in the clinics. However, the targeted drugs are efficacious only in a limited number of tumors that express the target molecules. A promising novel diagnostic technology is based on multi-gene analyses, which for, example, can predict the outcome/response to chemotherapy. In summary, all efforts should be directed to improve the tissue-based diagnosis and predictive relevance and to provide the clinicians with all those information needed for optimal treatment.

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MAGNETIC TISSUE ENGINEERING FOR VOICE REHABILITATION – FIRST STEPS IN A PROMISING FIELD

S. Dürr[1,2], C. Bohr[1], M. Pöttler[2], S. Lyer[2], R.P. Friedrich[2], R. Tietze[2], U. Eysholdt[1], M. Döllinger[1], C. Alexiou[2], C. Janko[2]

[1]Department of Phoniatrics and Pediatric Audiology, University Hospital Erlangen, Germany; [2]Department of Otorhinolaryngology, Head and Neck Surgery, Section for Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftungsprofessorship, University Hospital Erlangen, Germany

Introduction: The voice is one of the most important instruments for communication between humans. Voice is the result of intact and well working vocal folds. A defect of these structures causes dysphonia, which is associated with a clear reduction of quality of life. Therefore, tissue engineering of the vocal folds more and more comes into view. There are multiple different approaches trying to solve that challenging problem. We will present our first results applying magnetic nanoparticles for Magnetic Tissue Engineering. *Materials and Methods:* Vocal fold fibroblasts (vff), which play a central role in the structure of the vocal folds, were isolated from fresh rabbit larynges and cultured. For magnetization, cells were incubated with various amounts of superparamagnetic iron oxide nanoparticles (SPION). The loading of the cells with SPION was determined by Prussian Blue Staining. Biocompatibility was analyzed by the classical toxicological plate photometric assays WST-1 and LDH reflecting cellular metabolic activity and damage of the plasma membrane, respectively. The mitochondrial membrane potential of the cells was verified by DiIC1(5)-staining in flow cytometry. *Results:* Isolated cells from rabbit vocal folds show a viable fibroblastic morphology and cells are able to proliferate in culture. Cells can be successfully loaded with SPION, whereas optimal iron loading of the cells and the avoiding of cytotoxicity represents a balance act in Magnetic Tissue Engineering. Comprehensive experiments with various concentrations of SPION give an idea of an optimal SPION concentration where magnetization of the cells was achieved nearly in the absence of cytotoxicity. *Conclusion and Discussion:* As a first step of implementation of nanotechnology in the field of tissue engineering of the vocal folds, we tested SPION regarding their compatibility with isolated vff of rabbits. Certainly, to put this new and innovative technique onto a firm base, further investigations including genotoxicity and nanoparticle degradation, have to be performed. Magnetic Tissue Engineering meaning the loading of cells with magnetic nanoparticles forming three dimensional structures in a magnetic field will be a promising approach in the future.

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PERSONALISED TREATMENT FOR CANCER: HOW BIOMARKERS CAN HELP

M. Duffy

Clinical Research Centre, St Vincent's University Hospital and UCD School of Medicine and Medical Science, University College Dublin, Dublin 4, Ireland

The current approach to administering systemic therapy to cancer patients is largely empirical. Consequently, it is likely

that many patients with aggressive disease are undertreated while many with indolent disease are overtreated. In addition, for those patients who receive treatment, only a proportion derives clinical benefit while many suffer from adverse side effects. Most serious of all, a small number suffer from severe toxic effects, which in very rare cases, may be fatal. With recent developments in targeted therapies and molecular diagnostics, we are beginning to move from the traditional "trial and error" approach to a position involving a personalized approach, *i.e.*, giving the right drug at the right dose to the right patient. In order to achieve this situation, we need:

• Strong and independent prognostic biomarkers that can separate patients with indolent disease from those with aggressive forms,

• Biomarkers to prospectively predict response or resistance to specific therapies in order that the right patients receives the right drug(s),

• Biomarkers to identify patients likely to develop severe toxic side effects from therapy under consideration for administration.

Amongst the best validated prognostic biomarkers are AFP, HCG and LDH in patients with non-seminomatous germ cell tumors, PSA in prostate cancer, CEA in colorectal cancer, uPA/PAI-1, Ki67, Oncotype DX and MammaPrint in breast cancer. Although several other serum and tissue biomarkers have been shown to provide prognostic information, they are not widely used in the clinic. Similarly, although levels of circulating tumor cells have been shown to be prognostic in several different cancers, their measured is not currently performed for clinical use. In addition to the established therapy predictive biomarkers such as estrogen receptors (ER) for identifying patients with breast cancers likely to benefit from hormone therapy and HER2 for the selection of breast cancer patients likely to respond to anti-HER2 therapies, several new predictive biomarkers have become available in recent years. These include KRAS mutational ststus for the identification of patients with advanced colorectal cancer unlikely to benefit from anti-EGFR antibodies (cetuximab or panitumumab), EGFR mutational ststus for selecting patients with advanced non-small cell lung cancer (NSCLC) for treatment with tyrosine kinase inhibitors (gefitinib or erlotinib), BRAF mutations for selecting patients with advanced melanoma for treatment with vemurafenib and ALK translocation for identifying patients with NSCLC likely to benefit from crizotinib. The practice of personalised treatment for cancer will have major implications for pharmaceutical companies, diagnostic companies, and patients. For pharmaceutical companies, the availability of prognostic and predictive biomarkers will result in having enriched populations for participation in clinical trials. This in turn should reduce the cost and shorten the time of trials. In addition, the availability of toxicity markers should considerably reduce the number of adverse drug reactions and thus save pharmaceutical companies from possible litigation and the risk of having to withdraw drugs from the market. For diagnostic companies, personalised treatment will require a major change in emphasis. Traditionally, these companies have focused on serum-based screening and monitoring biomarkers. In order to become involved in the personalized treatment of cancer, an expanded test menu that includes prognostic, predictive and toxicity biomarkers will be necessary. This will require tissue-based as well as blood-based tests. Collaboration with academic researchers and pharmaceutical companies may be necessary to achieve these ends. The biggest beneficiary of personalized medicine should be the patient. The availability of prognostic and predictive biomarkers should help avoid under-treatment of aggressive tumors, overtreatment of indolent tumors, enhance drug efficacy and decrease toxicity, thus leading to a more individualized approach to cancer treatment.

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PRELIMINARY RESULTS OF A MULTICENTRE-STUDY FOR URINARY BLADDER CANCER ANTIGEN (UBC) RAPID AS TUMOUR MARKER FOR URINARY BLADDER CANCER

T.H. Ecke, C. Arndt, O. Lux, T. Otto, S. Hallmann, J. Ruttloff, H. Gerullis

Department of Urology, HELIOS Klinikum Bad Saarow, Germany

Introduction and *Objective:* UBC rapid is a test detecting fragments of cytokeratins 8 and 18 in urine. They belong to the frequently overexpressed cytokeratins in tumor cells. Up to date there are no clear data for bladder cancer patients and healthy controls without including factors for elevated cytokeratins in urine. We present first results of a phase II multi-center study measuring UBC rapid in bladder cancer patients and healthy controls.

Methods: Clinical urine samples were used from 56 patients with tumors of the urinary bladder (31 low grade

and 25 high grade tumors) and from 21 healthy controls. Urine samples were analyzed by the UBC rapid point-of-care (POC) system and evaluated both visually and quantitatively using the concile Omega 100 POC reader. For visual evaluation, different thresholds of band intensity for considering a test positive were applied. Sensitivities and specificities were calculated by contingency analyses.

Results: We could show that pathological concentrations of UBC rapid are detectable in urine of bladder cancer patients. The calculated diagnostic sensitivity for UBC rapid in urine was 72% for high grade, but only 29% for low grade bladder cancer. The specificity was 85.7%. Pathological levels of UBC rapid in urine were higher in patients with bladder cancer in comparison to the control group.

Conclusion: UBC rapid can differ between bladder cancer patients and control group. Further studies with a higher amount of samples will show how valuable these results are.

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HE4: RISK OF MALIGNANCY AND PREDICTIVE FACTOR FOR OPERABILITY?

H. EI Braicu[1], L. Mecke[2], B. Hellmeyer[4], G. Mueller[5], E. Nohe[6], C. Rodekamp[1], J. Sonnenschein[6], R. Bonness-Zaloum[4], C. Chekerov[1], C. Beteta[1], U. Selck[3], J. Torsten[3], J. Sehouli[1]

[1]Department of Gynecology, Charité Medical University Berlin, Germany;
[2]Department of Gynecology, VivantesAuguste Victoria Clinic, Berlin;
[3]Department of Gynecology,Vivantes Clinic Neukölln, Berlin;
[4]Department of Gynecology, Vivantes Clinic Friedrichshain, Berlin;
[5]Department of Gynecology, Vivantes Clinic Hellersdorf, Berlin;
[6]Department of Gynecology, Vivantes Clinic am Urban, Berlin, Germany

Ovarian cancer is the first cause of death due to gynecological malignancies. This poor prognosis is mostly due to lack of early diagnosis and screening modalities. Transvaginal ultrasound and biomarkers are the method of choice in the clinical routine. HE4 is the most reliable biomarker together with CA125 for the diagnosis and monitoring of ovarian cancer patients. Unlike CA125, HE4 is not increased in endometriosis and several benign gynecological diseases, this making it more suitable in premenopausal patients. Within the CharitéVivantes Network, a prospective study on pelvic mass patients is ongoing. We intend to include 920 pelvic mass patients in whom a surgical intervention is planned. Patients' enrollment started in August 2013 and until now 760 patients have been included prospectively, 250 of them representing the discovery cohort. The preliminary results showed that HE4 is more suitable for diagnosis in premenopausal patients, whereas within the postmenopausal subgroup, CA125 has a better sensitivity and specificity than HE4 or IOTA logarithms in detecting ovarian malignancy.

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THE NEW TK 201 ELISA PROVIDES PROGNOSTIC INFORMATION IN PATIENTS WITH BREAST CANCER

K.J. Kumar[1,2], A.C. Aronsson[2], G. Pilko[3], M. Zupan[4], K. Kumer[5], T. Fabjan[5], J. Osredkar[5], S. Eriksson[1,2]

[1]Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, VHC, PoBox 7011, SE 75007, Uppsala, Sweden;
[2]AroCell AB, VirdingsAllé 32B, SE-754 50 Uppsala, Sweden;
[3]University Medical Centre Ljubljana, Institute of Oncology, Ljubljana, Slovenia;
[4]University Medical Centre Ljubljana, Blood transfusion Centre, Ljubljana, Slovenia;
[5]University Medical Centre Ljubljana,Instituteof Clinical Chemistry and Clinical Biochemistry, Ljubljana, Slovenia

Background: Thymidine kinase (TK1) is an enzyme involved in DNA precursor synthesis and its expression is correlated with cell proliferation. TK leaks out into the blood from rapidly proliferating cells, particularly in case of cancer cells. Serum TK activity levels have since many years been used for prognosis and monitoring of leukemias and lymphomas. More recently measurement of serum TK activity and TK1 protein levels, using new methods, have been done with promising results also in case of different solid tumor diseases.

Study Aims: Here we describe the first clinical results with the new sandwich TK 210 ELISA, analysing serum samples from 92 breast cancer patients with known TNM clinical staging and 110 age matched healthy blood donor controls.

Materials and Methods/Patients: Serum TK1 protein levels were measured with a sandwich ELISA based on two anti TK1 monoclonal antibodies produced against peptides from the C-terminal region of human TK1. Recombinat TK1 was used as calibrator, spiked in a normal human serum matrix. Both the calibrators and serum samples were preincubated in the AroCell serum dillution buffer for 60 min prior to transfer to the monoclonal antibody coated ELISA plates. The TK 210 ELISA (Research use only kits from

AroCell AB)were then performed in a routine fashion with a biotinylated second monoclonal antibody. Patient sera were collected at the University Medical Center Ljubljana where the diagnosis and clinical staging were performed. Sera from 92 breast cancer patients were analysed as well as sera from 110 blood donors, 52 females and 58 males with an age distribution between 16 to 86 years. CA 15-3 values were also determined in a majority of the breast tumor samples.

Results: The functional sensitivty of the TK 210 ELISA was 0.35 ng TK1 per ml serum and 2 of the 110 sera from healthy blood donors were above this value (*i.e.* 0.55 and 2.62 mg TK1/ml, respectively). There were 41 T1 breast cancer samples with a mean of 0.40±0.42 ng TK1/ml, 47 T2 samples (mean 1.36±2.8 ng TK1/ml) and 9 T3 samples (mean 0.53±0.58 ng TK1/ml). The CA 15-3 values in these three patient groups were 23.5±13, 104±164 and 49±67, respectively. These TK1 as well as CA 15-3 values were significantly different between the T1,T2 and T3 stage sub groups. When the T2 stage breast cancer patients were sub-divided into different metastatic sub-groups, there were 27 M0 samples (mean 0.46±0.49 ng TK/ml), 5 M1 samples (mean 0.67±0.54 ng TK1/ml) and 13 M2 samples with a mean of 2.80±3.78 ng TK1/ml. The CA 15-3 values in the three groups were 22±16, 54±12 and 241±148, respectively. The M1 and M3 subgroups showed highly significant differences both with TK1 and CA 15-3. There was a subgroup of 5 samples among the T2 patients with high TK1 values (9.81, 9.51, 9.83, 5.72 and 4.73 ng TK1/ml, respectively).

Conclusion: The result of this first clinical evaluation of TK 210 ELISA strongly indicates that this assay can provide valuable prognostic information concerning patients with breast cancer and that it shows similarities with CA 15-3 but also differences, particularly in early stages of disease. Further studies will define the predictive and monitoring capacity of the new TK 210 ELISA.

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SCREENING AND EARLY DETECTION OF GASTROINTESTINAL TUMORS

S. Faiss

Gastroenterologie & Interventionelle Endoskopie, Asklepios Klinik Barmbek, Hamburg, Germany

Colorectal cancer is a leading cause of cancer death in Western countries, and since a long time screening programs have been shown to reduce the mortality of this disease. Current strategies for reducing the toll from colorectal cancer focus on early detection and removal of potential precancerous lesions. Therefore, colonoscopy is currently regarded as the gold standard for the detection of polyps and cancers in the colon as well as in the rectum and is the preferred method of screening for colorectal cancer in Europe and in the United States. In the last years some studies focused on the problem of the so called missed cancers or interval cancers in the colorectum confirming some older data with a relatively high rate of missed colorectal lesions in back-to back colonoscopies and follow-up studies. Furthermore, recent studies highlighted new problems (*e.g.* incomplete polyp resection, inadequate withdrawal time, inadequate adenoma detection rate, the behaviour of sessile serrated adenomas) that might be responsible for missed colorectal lesions and interval carcinomas. In contrast to Japan and other Asian countries, the incidence of gastric cancers in Europe is less. In addition, with the reduced prevalence of *H. pylori* in Western countries the incidence of gastric adenocarcinomas will decrease more and more. On the other hand, adenocarcinomas (Barrett's carcinoma) of the distal oesophagus are detected more frequently in the last years. Therefore, surveillance of patients with histologically confirmed Barrett's oesophagus including virtual chromoendoscopy and magnification/high-resolution endoscopy is necessary to detect candidates with high-grade dysplasia or early Barrett's adenocarcinomas, which could be treated endoscopically by endoscopic submucosal dissection (ESD) and/or radio-frequence ablation techniques. Pancreatic adenocarcinoma is one of the most life-threating carcinomas in humans. So far, no reasonable screening and/or early detection programme is available. However, in the last years more and more studies focus on the detection, surveillance and/or early operative therapy of precancerous cystic lesions of the pancreas like IPMN (branch and main duct type) and mucinous cystic neoplasia (MZN).

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FAMILIAL PANCREATIC CANCER

V. Fendrich, D.K. Bartsch

University Hospital Marburg, Department of Surgery, Marburg, Germany

Familial pancreatic cancer (FPC) is defined by families with at least two first-degree relatives with confirmed pancreatic ductal adenocarcinoma (PDAC) that do not fulfill the criteria of other inherited tumor syndromes with an increased risk for the development of PDAC, such as hereditary pancreatitis or hereditary breast and ovarian cancer. FPC is autosomal dominant inherited and presents with a heterogeneous phenotype. Although the major gene defect has yet not been identified, some important germline mutations in the

BRCA2-, PALB2- and ATM-genes are defined in a part of FPC families. It is suggested by experts to include high risk individuals in a screening programme with a multidisciplinary approach under research protocol conditions. However, neither biomarkers nor reliable imaging modalities for the detection of high-grade precursor lesions are yet available. Most screening programs are currently based on endoscopic ultrasound and magnetic resonance imaging and first data demonstrated that precursor lesions (PanIN, IPMN) of PDAC can be identified. Timing and extent of surgery are still a matter of debate. The present review focuses on the clinical phenotype of FPC, its histopathological characteristics, known underlying genetic changes, genetic counseling and screening.

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PROGNOSTIC SIGNIFICANCE OF SERUM TUMOR MARKERS IN PATIENTS WITH ADVANCED-STAGE NSCLC TREATED WITH PEMETREXED-BASED CHEMOTHERAPY

O. Fiala[1], M. Pesek[2], J. Finek[1], V.M. Matejka[1], L. Holubec[1], Y. Bortlicek[3], O. Topolcan[4]

Departments of [1]Oncology and Radiotherapy, [2]Pneumology and [4]Nuclear Medicine, Medical School and Teaching Hospital in Pilsen, Charles University in Prague, Czech Republic; [3]Institute of Biostatistics and Analyses, Masaryk University, Brno, Czech Republic

Background: Tumor biomarkers represent an effective tool for diagnostics and follow-up monitoring of patients with non-small cell lung cancer (NSCLC). We focused on the predictive and prognostic role of the seven following tumor biomarkers: carcinoembryonic antigen (CEA), cytokeratin-19 fragments (CYFRA 21-1), MonoTotal, neuron-specific enolase (NSE), chromogranin A, thymidine kinase (TK) and squamous cell carcinoma antigen (SCCA) in patients with advanced-stage NSCLC treated with pemetrexed-based chemotherapy.

Methods: Totally 114 patients with advanced-stage (IIIB or IV) non-squamous NSCLC treated with pemetrexed-based chemotherapy (monotherapy or combination with platinum derivative) were included. Comparison of patients'survival (PFS and OS) according to the level of assessed tumor markers was performed using the log-rank test.

Results: We observed a significantly shorter overall survival (OS) for patients with high pretreatment levels of CYFRA 21-1 (10.3 *vs*. 23.4 months; $p<0.001$), NSE (1.6 *vs*. 13.5 months; $p=0.003$) and TK (11.3 *vs*. 23.4 months; $p=0.003$).

Conclusion: CYFRA 21-1, NSE and TK are feasible biomarkers for the estimation of patients'overall prognosis, however none of the measured serum tumor markers could specifically predict the efficacy of pemetrexed-based chemotherapy.

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OCTREOTIDE IN THE TREATMENT OF MALIGNANT THYMOMA – CASE REPORT

O. Fiala[1], M. Pesek [2], J. Finek[1], V.M. Matejka[1], L. Holubec[1], D. Slipkova[3], O. Topolcan[3]

Departments of [1]Oncology and Radiotherapy, [2]Pneumology and [3]Nuclear Medicine, Medical School and Teaching Hospital in Pilsen, Charles University in Prague, Czech Republic

Background: Thymomas are the most common mediastinal tumors. Systemic therapy for patients with unresectable or recurrent thymomas is a challenging field in current oncology research. There is some evidence that somatostatin analogues combined with corticoids may have a role in the treatment of advanced malignant thymoma; however the role of these agents has not been fully evaluated.

Case Report: A 39-year-old man with metastatic thymoma was administered a long-acting depot injection form of octreotide. An octreotide scan before the treatment initiation revealed low uptake. CT control after three months of treatment revealed a marked regression of pleural metastases, while primary tumor mass remained stable. The treatment response lasted for 9 months.

Conclusion: We describe the interesting case of marked clinical and radiological response of advanced malignant thymoma to treatment with octreotide in a heavily pretreated patient, even though an octreotide scan before the treatment revealed low uptake.

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LOCOREGIONAL TREATMENT IN LIVER METASTASES

R. Fischbach

Radiologie und Neuroradiologie, Asklepios Klinik Altona, Hamburg, Germany

Surgical resection or local thermoablation are the only potentially curative treatments in isolated liver metastasis. However, only few patients are resectable or candidates for local tumor destruction by radiofrequency or microwave

ablation at the time of diagnosis. Despite improvements in systemic chemotherapy with new substances and biologic agents, overall survival and the number of resectable cases after chemotherapy remain disappointing. Locoregional transarterial therapies like transarterial chemoembolization (TACE) and selective internal radioembolization therapy (SIRT) exploit the predominant arterial supply of liver malignancies compared to the portalvenous supply of the normal liver parenchyma. Using the transarterial route drug eluting particles or microparticles containing beta-emitting radiation (Yttrium-90) can be directed into the tumor vasculature. Regional drug application with embolization allows for much higher hepatic concentration of cytotoxic agents resulting in significantly improved response rates, even in chemorefractory disease. 90 Y radioembolization shows a profound local efficacy of high doselocal radiotherapy reducing the burden of liver metastases, eventually leading to prolonged progression free survival and overall survival. Radioembolization is mainly used to slow down hepatic progression in salvage situations and after multiple lines of systemic chemotherapy-including biologic agents have failed. Indication, technique and results of radioembolization will be presented in different disease entities. The results of prospective clinical trials in patients with non-resectable liver metastases from colorectal carcinoma show improved response to systemic chemotherapy in combination with radioembolization. Especially if radioembolization is used at an early line of therapy, prolonged disease control can be achieved. It can be expected that radioembolization will be used with increasing frequency for augmentation of local response of systemic chemotherapy, hopefully leading to higher likelihood for potentially curative resection or ablation.

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VALUE OF CA125 IN OVARIAN CANCER FOLLOW UP: AN ILLUSION OR REALITY?

C. Fotopoulou

Gynaecological Cancer Centre and Ovarian Cancer Action Research Centre, Imperial College Healthcare Trust London, Hammersmith Campus, United Kingdom

The value of conventional tumor markers such as CA125 has been heavily challenged over the last years by failing to show any beneficial impact on survival in the follow-up of patients with invasive epithelial ovarian cancer in a large randomized trial of early *versus* delayed treatment of women with ovarian cancer in complete remission after first-line platinum-based chemotherapy. Also numerous analyses attempting to identify the role of CA125 in predicting malignancy or even resectability of ovarian tumors have generated rather contradictory and inhomogeneous results. Nevertheless, CA125 continues to represent a major backbone of the post treatment follow up of ovarian cancer patients and to represent a major pole of anxiety for these patients. Even though current evidence clearly shows that initiation of early systemic treatment is of no survival benefit, data are only very scarce regarding surgery at relapsed disease at a time point early enough to be able to obtain a complete cytoreduction. Since only 6% of the patients received surgery at relapsed in the major randomized trial which revealed CA125 to be not beneficial, the question of CA125 based monitoring at follow up to identify patients as ideal surgical candidates remains unanswered. The results of the currently ongoing DESKTOP trial will open novel pathways in the follow up of patients with platinum sensitive disease if surgery at relapse will identified as beneficial for patients survival and quality of life.

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IMMUNOHISTOCHEMICAL EVALUATION OF THE ROLE OF TP53 MUTATION IN CERVICAL CANCER

C. Freier[1], A. Stiasny[1], C. Kuhn[1], D. Mayr[2], C. Alexiou[3], C. Janko[3], I. Wiest[3], U. Jeschke[1], B. Kost[1]

[1]LMU Munich, Department of Obstetrics and Gynaecology, Munich, Germany;
[2]LMU Munich, Department of Pathology, Munich, Germany;
[3]ENT-Clinic, Section for Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftung, University Hospital Erlangen, Germany

Background: Human papillomavirus (HPV) induced cervical cancer is driven by virus specific oncoproteins E6 and E7. The E6 protein of HPV types 16 and 18 interacts with the E3 ubiquitin-protein ligase, resulting in the proteolysis of p53. In addition, E6 binds CBP/p300 and decreases the ability to activate p53-responsive promoter elements. Variable levels of E6 mRNA have been found in both cervical intraepithelial neoplasia and cervical cancer. The aim of this study was to analyse the mutation of the TP53 gene in cervical cancer patients and to correlate the mutation to clinical parameters and prognosis.

Materials and Methods: Paraffin sections of 250 cases of cervical cancer derived from the Department of Obstetrics and Gynaecology, LMU Munich, were analysed. As primary antibody a specific antibody against mutated p53 protein (ab32049, Abcam®) was used. The polymer staining method and diaminobenzidine were applied for further development. Distribution and intensity of the staining were evaluated with

a semi-quantitative immunohistochemical score (IRS) both in the nucleus and cytoplasm. Correlation of the staining with grading, staging and survival analysis was evaluated with the statistical program SPSS.

Results: A total of 66% of studied cervical cancers were expressing the mutated p53 protein (*i.e.*, more than 10% of the cancer cells stained). Significant differences were found in squamous epithelial tissue with a median expression of p53 with an IRS 4 compared to IRS 0 in adenocarcinomas. A significantly higher expression of mutated p53 was found in G2 patients compared to G3 patients. According to this finding the overall survival was better in patients expressing the mutated p53 protein in the nucleus.

Discussion: Despite the central role of p53 in the hallmarks of cancer, *TP53* mutation and expression status is not yet used for the prognosis of cervical cancer. Interestingly, we found a very high mutation rate of the *TP53* gene in a cancer type where p53 is initially inactivated *via* the oncoprotein E6 during the development of cervical cancer. An unexpected finding is the correlation of p53 mutation alone with a better survival. This could possibly be due to a lack of cell cycle arrest in tumors expressing mutated p53 resulting in a better clinical response, but could need further correlation to the E6 protein expression.

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CCL22 EXPRESSION AND FOXP3 INFILTRATION IN HUMAN BREAST CANCER

C. Freier[1], C. Kuhn[2], S. Endres[1], D. Mayr[3], K. Friese[2*], D. Anz[1*], U. Jeschke[2]

[1]Abteilung für Klinische Pharmakologie, Medizinische Klinik und Poliklinik IV - Klinikum der Universität München, Germany;
[2]Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe - Klinikum der Universität München, Germany;
[3]Pathologisches Institut - Klinikum der Universität München, Germany
*Both authors contributed equally

Regulatory T cells (Treg) are a subpopulation of T cells with the capacity of down-regulating the immune response promoting tumor-induced immune suppression. Infiltration of tumors with Treg is associated with bad prognosis in many patients. Several chemokines have been described to be responsible for Treg accumulation in tumors and therefore, represent potential targets for cancer therapy. CCL22 has been described to specifically recruits human Tregs *in vitro* and to recruit Treg in human cancers. We aimed to investigate whether CCL22 is expressed in human breast tumors in order to evaluate whether this chemokine may be involved in the recruitment of Treg to the cancer tissue.

Methods: More than 100 paraffin samples of human breast cancer were stained for CCL22 and FoxP3. The expression level was evaluated in a semi-quantitative manner.

Results: We found that FoxP3+ cells infiltrated 50% of the breast tumor samples. Moreover, we observed difference in the FoxP3+ cells infiltration of ductal, lobular and tipple negative tumors. CCL22 expression was heterogeneous from no expression at all, to a diffuse expression. In most of the cases, immune cells expressed CCL22. However, tumor cells expressed CCL22 in the tumor tissue.

Conclusion: Our results demonstrate that CCL22, a chemokine that specifically attracts Treg, is expressed in human cancers and that the Foxp3+ cells infiltration was tumor type dependent. It will be interesting to evaluate this chemokine as a target for tumor therapy.

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TUMOUR MARKERS IN ORAL AND OROPHARYNGEAL CANCER

R.E. Friedrich

Oral and Maxillofacial Surgery, Eppendorf University Hospital, University of Hamburg, Hamburg, Germany

Oral and oropharyngeal cancer comprise a variety of entities. The majority of these cancers are of squamous cell origin that give rise to carcinoma. This predominance of squamus cell carcinoma (SCC) in the malignant neoplasms arising in the upper aerodigestive tract differs from the more frequent diagnosis of adenocarcinoma in other body regions, in particular the lower aerodigestive tract. The biological characteristics of squamous cells are the coverage of body surfaces by regeneration and desquamation. Epithelial cells with glandular differentiation can often be characterized by specific products excreted into the vicinity. This biological difference must be observed in the development of tumor markers dedicated to distinguish carcinoma of the oral and oropharyngeal region. However, in both types of epithelia, both inflammation and neoplasm can cause an increase of cellular breakdown products that are measurable in tissue, blood or lymphatics. Furthermore, tumor volumes in this region are usually relatively small. Relationships between volume and alteration of a marker's serum level are less pronounced than in tumors of the trunk. Therefore, the calculations of cut-off levels for certain markers represent a great challenge to all fields of medicine involved in the management of cancer partients. Management of oral/oropharyngeal SCC is predominantly based on ablative

surgery. Survival rates of these SCC have not markedly improved during the last decades, despite advances in reconstructive surgery and in the field of oncology. Inspection and imaging modalities are the first line diagnostic measures in detection and surveillance of neoplasm in the upper aerodigestive tract. Serological tumor markers are currently not an established tool to monitor SCC. Early trials to establish the measurements of cellular breakdown products (*e.g.* tissue polypeptide antigens) failed a significant correlation to stage grouping or prognosis in these carcinomas. However, imaging of tumors in the head and neck region after therapy can pose important problems. Alterations of anatomy after surgery and inflammation response to multiple therapies can give rise to major diagnostic difficulties in terms of identifying local or regional recurrence. Clinical experience to date also shows that there are important limits to the application of imaging modalities like magnetic resonance imaging and positron emission tomography for timely diagnosis. Recently, circulating tumor cells (CTC) were investigated in SCC of the upper aerodigestive tract and showed a prognostic impact. This report gives an overview on the development of tumor markers in the oral and oropharyngeal region with special reference to the requirement profile for markers preferentially identified in serum.

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RECURRENT ODONTOGENIC MYXOMA IDENTIFIED ON COMPUTED CONE BEAM TOMOGRAMS

R.E. Friedrich

Oral and Craniomaxillofacial Surgery,
Eppendorf University Hospital, University
of Hamburg, Hamburg, Germany

Odontogenic myxoma (OM) is a rare mesenchymal tumor arising predominantly in the alveolar process of the jaws. Therapy is tumor resection with safety margins. Ablative surgery for OM is often associated with loss of teeth and impaired masticatory function. In the frontal region of the alveolar arch, resection can cause extensive bone loss and consequently a debilitating facial appearance. OM show a tendency for local recurrence. This report details the follow-up control of an OM of the anterior maxilla. Local resection intended teeth preserving excochleation due to the decision of the patient to refuse en-block resection. The patient was regularly investigated clinically and radiologically. Cone beam computed tomography (CBCT) enabled us to identify local recurrence rapidly and lead to prompt surgical intervention. We present the radiographic features of OM on CBCT and discuss alternative surgical strategies to gain local tumor control in OM.

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EXTENSIVE EXTRACRANIAL MENINGIOMAS IN NEUROFIBROMATOSIS TYPE 2

R.E. Friedrich[1], C. Hagel[2]

[1]Department of Oral and Craniomaxillofacial Surgery,
[2]Institute of Neuropathology, University of Hamburg,
Hamburg, Germany

Purpose: The purpose of this report is to detail rare cases of neurofibromatosis type 2 (NF2) with symptomatic extracranial meningiomas.

Materials and Methods: This is a clinical report about 2 patients with NF2 detailing clinical and radiological findings. Results of ocular findings, applied imaging techniques, and pathological findings of the space-occupying lesions are presented.

Results: The first patient, a 31-year-old male with established NF2 diagnosis and multiple intracranial tumors and tight-sided blindness, developed a slowly growing swelling of the right neck in close proximity to the mandibular angle. Imaging of the neck revealed solid masses encompassing the carotid arteries. Initially, the patient refused surgery but recurred for tumor debulking procedure 2 years later. A modified neck dissection was used to reduce the bulky tumor mass extending down to the right clavicle. During the next three weeks the patient experienced several excessive bleedings from carotid artery rupture. Finally, the artery was obliterated, without neurological complications. Later, further tumor was reduced *via* a navigation-assisted transoral route. The patient survived for several years and deceased with evidence of intracranial tumor progression. The second patient, a 53-year-old male, developed several subcutaneous tumor masses of the left frontal and parietal regions. He reported a painful pressure inside the left orbit, headache, and impaired motility of the left eye. He showed a slight proptosis. A lateral orbitomy was used to access the tumor. The solid masses were removed, firmly adhering to the bone and orbital soft tissues. After surgery, the patient reported improved eye mobility and reduced headache. Both tumors proved to be meningiomas.

Conclusion: In these patients, the extracranial meningiomas, rather than the intracranial primaries, were symptomatic and were surgically treated. Head and neck surgeons should be aware of the rare possibility that solid tumors of this region could be extracranial meningiomas.

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CHERUBISM: A CASE REPORT WITH SURGICAL INTERVENTION

R.E. Friedrich[1], J. Zustin[2], T. Grob[1,3]

[1]Department of Oral and Maxillofacial Surgery, [3]Institute of Pathology, Eppendorf University Hospital, University of Hamburg, Hamburg, Germany; [2]Institute of Pathology, University of Münster, Münster, Germany

Cherubism is a rare benign, autosomal-dominantly hereditary fibro-osseous condition affecting predominantly the jaws. Symmetrical cyst-like expansions of the jaws cause the characteristic facial swellings. The disease is often associated with severe malpositioning of teeth. The gene for cherubism is SH3BP2 located on chromosome 4p16.3. The repeated experience of self-limiting disorder has resulted in a wait-and-see strategy about therapeutic options. Indeed, cessation and regression of even large bone enlargements can be expected in early adulthood. Nevertheless, severe facial disfiguring and functional impairment can make necessary a surgical intervention. This report details the surgical procedures in a patient with progressive and disfiguring jaw expansions at the end of adolescents and the limited effect of surgically assisted orthodontic tooth movement in a patient with disease-associated tooth retention and hypodontia.

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MAXILLARY RESECTION AND CONSECUTIVE OSSEOUS RECONSTRUC-TION INCLUDING TOOTH TRANSPLANTATION

R.E. Friedrich[1], H.A. Scheuer[2], W. Höltje[1]

[1]Department of Oral and Craniomaxillofacial Surgery, [2]Orthodontics, Eppendorf University Hospital, University of Hamburg, Hamburg, Germany

Odontogenicmyxoma (OM) is a rare tumor arising in the jaws. The tumor is supposed to be odontogenic in origin due to the frequent localization of the tumor inside the jaws in close relation to teeth. The aim of this report is to detail the course of a patient who developed OM of the maxilla. She underwent adequate ablative surgery and reconstruction, including tooth transplantation to the original tumor site. Six years after first diagnosis of OM she developed a local recurrence in close proximity to the teeth transplanted to the reconstructed maxilla. Once again, a partial maxillary resection was performed, with no reconstruction. The patient has been free from tumor recurrence for over twenty years. We discuss the current hypothesis on OM pathogenesis and the possible impact of division-active cells on tumor regrowth.

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EXPRESSION OF DOG1 (ANO1) IN POORLY DIFFERENTIATED CARCINOMAS OF THE HEAD AND NECK

R.E. Friedrich[1], T. Wunder[2], S. Bartel-Friedrich[3], J. Zustin[4]

[1]Department of Oral and Craniomaxillofacial Surgery, [2]Institute of Anatomy, Eppendorf University Hospital, University of Hamburg, Hamburg, Germany; [3]Department of Otorhinolaryngology, University of Halle-Wittenberg, Halle a. d. S., Germany; [4]Institute of Pathology, University of Münster, Münster, Germany

DOG1, also known as discovered on GIST1, anoctamin-1 (ANO1), and transmembrane member 16a (TMEM16a), is a calcium-activated chloride channel expressed in a variety of normal and neoplastic tissues. DOG1 is a specific marker for gastrointestinal stromal tumor. In the head and neck region, DOG1 is a sensitive discriminator for acinus cell carcinoma. Presently, only a few publications present data concerning the expression of DOG1 in head and neck cancer of squamous cell origin. According to one study, the expression of DOG1 in squamous cell carcinoma of the head and neck appears to be associated with a poor prognosis. Up to now, reports on DOG1 expression in head and neck carcinoma arerestricted to few cases. The aim of this study was to analyze the expression pattern of an anti-DOG1 antibody in poorly differentiated carcinoma of the upper aerodigestive tract.

Materials and Methods: A total of 91 specimen of35 patients with carcinomas of the upper aerodigestive tract were investigated for DOG1 expression. Inclusion criteria for this study was poorly or undifferentiated malignancy of the head and neck.Samples of the same resection site that showed moderate or well differentiated squamous cell carcinoma were also enrolled. Immunoreactivity in carcinomas was estimated using a visual score (0: negative; 1: basal positive, 2:parabasal positive, 3: completely positive, 4: basal and parabasal positive).

Results: Fifteen out of 91 specimen were immunoreactive for DOG1 antibody (15.2%). DOG1 positive tissues were diagnosed in 10 patients (28.8%). Within the group of DOG1 positive carcinoma, 15 of 36 samples were positively stained (43.3%). DOG1 immoreactivity in individuals varied considerably, *e.g.* only 2 out of 8 specimen in a case of

lymphangiosiscarcinomatosa were DOG1 positive. Interestingly, DOG1 was identified in 3 of 6 undifferentiated nasopharyngeal carcinoma.In DOG1-positive cases, basal and parabasal staining pattern were predominant staining patterns. Completely stained samples were rare (n=3).

Discussion: This study revealed for the first time the expression of DOG1 in poorly differentiated carcinoma the upper aerodigestive tract. DOG1 is a valuable tumor marker in several malignancies of mesenchymal origin. In selected entities of the head and neck this antigen is also expressed and can be used in the differential diagnosis, *e.g.* acinus cell carcinoma of salivary glands. Differential diagnosis of poorly differentiated carcinoma of the head and neck can still be regarded as a problem and immunoreactivity of poorly differentiated carcinoma can provide indispensable assistance for diagnosis. The percentage of DOG1 positive carcinoma is high concerning the number of patients, compared to results of previous study on head and neck cancer tissues. However, DOG1 is not constantly expressed within a sample. DOG1 expression was found only in a subset of entities and the expression pattern appeared to be not related to grading. Even within samples of a single resection tissue, the expression varied substantially. Possibly, DOG1 expression can be used as a prognostic factor in the management of poorly differentiated carcinoma of the head and neck.

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INSULIN-LIKE GROWTH- FACTOR RECEPTOR IN ODONTOGENIC MYXOMA

R.E. Friedrich[1], C. Hagel[2], J. Zustin[3]

[1]Department of Oral and Maxillofacial Surgery, [2]Institute of Neuropathology, Eppendorf University Hospital, University of Hamburg, Hamburg, Germany; [3]Institute of Pathology, University of Münster, Münster, Germany

Odontogenicmyxoma is a rare neoplasm in the maxillofacial region. OM is predominantly a lesion of the jaws. The tooth-bearing regions are frequently affected. The association of OM with the dentition is a major fundamental reason to hypothesize that the neoplasm arises from odontogenic precursor cells. However, the pathogenesis of OM is obscure. The aim of this study was to further characterize OM by means of immunohistochemical identification of antigens.

Materials and Methods: Formalin fixed, paraffin embedded specimen of five cases with OM were immunohistochemically stained for detection of podoplanin, hyaluronan, nestin, CD 133, and IGF1R.

Results: None of the specimens were immunoreactive to antibodies identifying nestin, CD133, podoplanin and hyaluronan. All specimen showed some nuclear and cytoplasm staining for IGF1R.

Discussion: The OM is known to be poorly defined by immunohistochemical detection of proteins. We were not able to confirm a recent report on nestin staining in OM. However, IGF1R immunoreactivity possibly refers to IGF1R-positive cells identified in phases of odontogenesis.

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OPTIC PATHWAY GLIOMA IN PATIENTS WITH NEUROFIBROMATOSIS TYPE 1

R.E. Friedrich[1], M. Nuding[1], M. Feucht[2], V.F. Mautner[3]

Departments of [1]Oral and Craniomaxillofacial Surgery, [2]Ophthalmology, [3]Neurology, Eppendorf University Hospital, University of Hamburg, Hamburg, Germany

Introduction: Neurofibromatosis Type 1 (NF1) is an autosomal dominant inherited disease affecting multiple organs. The phenotype varies considerably. The optic pathway glioma (OPG) is a neuro-opththalmological disease that can occur sporadically and in NF1. OPG occurs in about 15% of NF1 patients and is a defining criterion to establish NF1 diagnosis. The clinical course of OPG in NF1 may vary substantially. The aim of this study was to analyse the prevalence of OPG in a large center for NF1 patients, to characterize the clinical course of OPG, to address the association of unknown bright objects (UBO) and OPG, and to determine the impact of therapy on clinical outcome, in particular visual acuity (VA).

Materials and Methods: The study is based on the files of 1.827 patients with evidence for NF1. First, the radiological findings of these patients were evaluated. In 925 patients magnetic resonance images (MRI) of the head were available for analysis that were performed to investigate the optic pathway (total number of MRI is 1948). Further 50 patients had only cranial computed tomograms (CCT), (total number of patients is 975). MRI criteria for OPG were applied according to Listernick *et al*. The radiologic localization of OPG was classified: unilateral/bilateral/chiasmatic/pre-/postchiasmatic/chiasmatic/chiasmatic and postchiasmatic/optic pathway completely affected. MRI with OPG were investigated for UBO. Clinical findings focused on the course of VA in symptomatic or asymptomatic patients and compared these to MRI findings. Therapy was classified as chemo- or radiotherapy or surgery, and various combinations of these modalities. Visual diagnostic prior to therapy was used as the reference finding. Chi-Square test was used for analysis.

Results: The total number of OPG was 134 (13.75%; MRI=26/925, CCT=8/50). Seventy-seven had a

favourable(asymptomatic) and 57 (ca. 43%) patients showed unfavourable(symptomatic) course of disease (female: 29 (ca. 41%)). Symptomatic and asymptomatic OPG occurred in 28 (44%) and 36 (56%) of 64 males (w/m=1.1:1). Mean age of patients with favourable course was 11.6 years (ys), (median 7.4; SD 11.2; n=76). Sixteen patients of this group were >18 ys of age at the time of diagnosis. Restricting age of diagnosis to patients <18 ys, arithmetic mean age was 7.3 ys at first diagnosis (SD 4.5). Symptomatic OPG (n=57, mv=9) were 7.6 ys old (median: 6.1 ys, SD 6.92) at the first diagnosis. In this group 2 patients developed symptomatic OPG at unusually higher ages (25 and 44 ys, resp.). Mean age of patients <18 ys (n=46) was 6.4 ys (SD 3.5) in this group. The most frequent initial finding was strabism (n=12/25%) in symptomatic OPG (n=48, mv=9) and the main cause for amblyopia. Amblyopia did not differed in patients with and without therapy ($p>0.5$). *Protrusiobulbi* occurred in 9 of 28 treated patients ($p=0.072$ (Pearson [*]), 0.092 (Fisher's exact test [**])) and constituted 6 patients of the surgically treated group. Funduscopic findings in patients with and without therapy did not differ significantly ($p=0.517$[*] bzw. 0.636[**]). None of the following findings differed significantly between treated and untreated patients: *protrusiobulbi*, endocrine abnormalities, *amblyopia*, *strabism, ptosis*, nystagmus, *macrocephalus, hydrocephalus*, headache, and funduscopic findings: papillary pallor/ opticusatrophy. Median value of VA remained constant in the worse seeing eye and in the best seeingeye increased constantly in the patient group without therapy during the observation period (0.75_initial up to 1.0_end).In treated patients, therapies differed largely and allowed no statistically significant evaluation of the therapy on outcome. In general, impaired vision ofthe affected eye(s) was noticed in the vast majority of patients during the observation period. There is no correlation between symptomatic and asymptomatic OPG and UBOs ($p=0.942$; Phi-coefficient=–0.007). Radiological localization was sufficiently precise to allow detailed analysis in 128 cases (mv=6). In 14 (27%) of symptomatic patients the complete visual pathway was affected. In only 2 (2.6%) patients this finding was present in asymptomatic patients. The glioma was restricted to one side in 27 (35.5%) asymptomatic and in 9 (17.3%) symptomatic OPG patients. Bilateral pre-chiasmatic OPG were found in 9.6% of symptomatic and 22.4% of asymptomatic patients. About 45% of all patients (both groups) showed pre-chiasmatic involvement. OPG restricted to the chiasma occurred in both groups essentially in the same frequency: 7 patients each.

Discussion: Most OPG in NF1 affected children are asymptomatic and show a favourable course allowing to refrain from therapy. A favourable course was documented in about 60% of patients. The majority of symptomatic OPG (n=31/48; mv=9) were diagnosed prior or equal to their seventh year of life. However, it has to be mentioned that OPG can take an unfavourable course after this period of life (n=7/48; mv=9). This variability of clinical course severely impairs the health care professionals in giving advice to patients and relatives. Furthermore, this wide spectrum of tumor biology is a handicap to develop therapeutic strategies in OPG. Tumour localization had a minor value as a predictor of tumor biology. Unilateral or bilateral OPG showed a trend for better clinical course. Large glioma along the visual pathway correlated in many cases with an unfavourable course of VA. Therapy for these patients was difficult and frequently these patients had to face severe impairment of vison. According to the literature the most important clinical criterion to diagnose OPG is the investigation of VA. This is the reason why a consensus of VA criteria/methods is requested by several authors in order to allow the comparison of therapies. However, in this study VA was not the only parameter to determine the severity of OPG concerning VA. The impact of amblyopia on VA was apparent in this study but could not further be specified. Magnetic resonance imaging with application of contrast media is currently the gold standard to diagnose OPG. Funduscopic findings may have predictive value for the course of VA in NF1-associated OPG. Furthermore, a *protrusiobulbi* should alert the clinician to look for tumor progression. Spontaneous regression of OPG is a rare observation that can be associated with improved VA. A further rarity in this study was new symptomatic OPG in adults. These findings underline the unpredictability of tumor biology in OPG. Therefore, even asymptomatic adult OPG patients with NF1 should be investigated regularly. UBO are not associated with OPG and there is no predisposition to develop astrocytoma. Chemotherapy is said tobe effective in treatment of OPG in NF1. According to our small collective available for analysis this statement could not be substantiated. Prospective long-term studies at specialized centers applying standardized diagnostic criteria and therapies could allow more precise information about the effectiveness of therapeutic options in NF1 associated OPG.

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MRI AND CT AS NEW DIAGNOSTIC BIOMARKERS IN ONCOLOGY

T. Friedrich, T. Hackert, H. Lubenau, L. Grenacher

DIR Universität Heidlberg; VAXIMM, Mannheim, Germany

Background: Personalized medicine takes an increasing importance in the field of modern oncology. Due to response rates between 20 up to 70 percent for standardized therapies

depending on the etiology of the cancer and only 30 percent in metastatic diseases individual oncologic concepts are rapidly increasing. So diagnostic biomarkers as stratifying markers become more and more important. Beside the established biomarkers as serum parameters, clinical measurements etc. diagnostic imaging modalities keep entering in the oncologic follow-up pathways. To overcome todays limitations of "pure" morphologic measurements functional imaging methods are now part of the routine protocols.

Purpose: In this presentation we present two new functional imaging methods as diagnostic biomarkers and their relevance in therapeutic concepts for adenocarcinoma of the pancreas. The first method is dual-energy CT perfusion of resectable pancreatic cancer. The second method is dynamic-contrast-enhanced MRI (DCE-MRI) as a new biomarker for a new concept of oral DNA vaccination targeting VEGF-Receptor-2for locally advanced, irresectable pancreatic cancer (Phase I trial).

Materials and Methods: For the first study we evaluated 25 patients with pancreatic carcinoma on a 64-slice dual-energy CT scanner (Siemens, Erlangen) and evaluated the perfusion parameters assessing perfusion, permeability and blood volume for normal and malignant parenchyma by overlaying color coded perfusion maps. In the second study we performed a randomized, placebo-controlled, double blind dose–escalation study including 45 patients with local irresectable pancreatic cancer. DCE –MRI was performed on a 1.5 Tesla MRI (Siemens, Erlangen) on day 0, 38 and 3 months after treatment followed by pixel-by-pixel analysis based onTofts model and Ktrans calculations. Clinical parameters included established biomarkers CA 19-9, VEGF-A and collagen IV were compared with the diagnostic markers.

Results: In study 1 all carcinomas could be in the color-coded perfusion maps of dual-energy CT. Perfusion, permeability, and blood volume values were significant lower in pancreatic carcinomas compared to healthy pancreatic tissue. In two patients morphologic invisible small tumors could be identified only with the additional color coded perfusion map. For the DCE-MRI at day 38 mean changes in Ktranswere -18% *versus* +2% in the placebo group. MRI after three months showed mean changes of-18% *versus* +3% in the placebo group. These patients showed significant decreasing ktrans values at day 38 ($\alpha<0.05$, Wilcoxon test). MRI data corresponded with increasing VEGF-A and collagen VI while no markable effects could be observed with response to RECIST-criteria.

Conclusion: Dual Energy CT as well as MRI with DCE-measurement are promising radiologic tools as diagnostic biomarkers for the evaluation of therapies in malignant pancreatic tumors. In addition to that, data suggests that VXM 01 can induce VEGFR 2 specific T-cell response and impacts tumor perfusion and dual-energy CT could be used as a relevant tool to differentiate early local recurrence to increase the survival of patients with pancreatic carcinomas.

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CIRCULATING TUMOR MARKERS IN THE FOLLOW-UP OF GASTROINTESTINAL TRACT MALIGNANCIES: GUIDELINES *VS*. PRIMARY STUDIES IN THE CLINICAL PRACTICE

M. Gion[1], C. Trevisiol[2], G. Rainato[2], A. Fabricio[1]

[1]Centro Regionale Specializzato Biomarcatori Diagnostici Prognostici e Predittivi, Dipartimento di Patologia Clinica – Azienda ULSS 12 Veneziana, Venezia;
[2]Istituto Oncologico Veneto IOV - IRCCS, Padova, Italy

Tumor markers may have a different clinical role in different clinical scenarios due to their intrinsic limitations, represented by a poor sensitivity for early cancer and a poor specificity, being increased also in conditions different from cancer such as benign diseases. These drawbacks may limit their effectiveness for the diagnosis of a primary tumor, as a result of the confounding effect of the occurrence of benign diseases. Conversely, tumor markers may be effective during the follow-up after the curative treatment of the primary tumor when the probability of relapse is high. Indeed, tumor markers are effective because they are frequently the first sign of a relapse, anticipating clinical signs or positive imaging. However, in spite of their effectiveness, the role of tumor marker determination during the follow-up is under debate in several malignancies since the early detection of the relapse is not necessarily beneficial to the patient when effective therapies for the metastatic disease are not available. For this reason clinical practice guidelines tend to provide restrictive recommendations. Nevertheless tumor markers are extensively used in the clinical practice leading to a high rate of inappropriateness. This is at least in part due to the different approach of clinical studies and guidelines. In general, clinicians relay on published evidence to take clinical decisions. However, it may occur that guidelines and clinical studies reach different conclusions concerning tumor markers. Primary studies look at tumor marker effectiveness assessing their diagnostic performances. A tumor marker can show excellent values of sensitivity and specificity, or even very good positive and/or negative predictive values in a clinical study. Looking at that study, the marker could be recommended since its efficacy is proven. Therefore, according to clinical studies, tumor markers should be frequently used, since their effectiveness is proven. On the other side, clinical practice guidelines explore the usefulness of a biomarker on relevant outcomes, such as survival.

Therefore, an effective tumor marker that has not been proven to be also useful, cannot be recommended. In order to elucidate the position of the scientific community on tumor marker application in clinical practice, we have performed a comprehensive search of clinical practice guidelines. A systematic MEDLINE (PubMed) literature search was carried out to identify documents potentially containing recommendation statements intended to optimize patient care. The following key words (synonyms and mesh terms) were used: cancer, guideline, consensus conference. Moreover, a hand search was performed in web sites of guidelines repositories (National Guideline Clearinghouse, GIN library), guidelines producers (NICE, SIGN, CCO, NHMRC, SNLG) and scientific societies (AIOM, ESMO, ASCO, NCCN, NACB, EGTM).

The content of guidelines concerning gastrointestinal tract malignancies has been extracted from the documents, synoptically summarized and discussed. Only few tumor markers are recommended for the clinical use. They are well known markers discovered 20-30 years ago directly associated to tumor bulk. Many of them are helpful in patient management, but they cannot be used in isolation to take therapeutic decisions. In general, they triggers further imaging. In the follow-up of colorectal cancer a proper use of CEA is reported to improve survival anticipating the detection of liver metastases and thus increasing the probability of their radical resection. Recent review articles have been also identified and examined. The results are compared with those of guidelines, highlighting the differences between information provided by guidelines and those found in primary studies.

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EPITOP DETECTION IN MONOCYTES AS LIQUID BIOPSIES FOR DIAGNOSIS OF TUMOR DISEASES

M. Grimm, S. Schmitt, P. Teriete, T. Biegner, A. Stenzl, J. Hennenlotter, H.J. Muhs, A. Munz, T. Nadtotschi, K. König, J. Sänger, O. Feyen, H. Hofmann, S. Reinert, J.F. Coy

Klinik für Mund-, Kiefer- und Gesichtschirurgie Universitätsklinikum Tübingen, Tübingen, Germany

Background: Biomarkers allowing the characterization of malignancy and therapy response of oral squamous cell carcinomas (OSCC) or other types of carcinomas are still outstanding. The biochemical suicide molecule endonuclease DNaseX (DNaseI-like 1) has been used to identify the Apo10 protein epitope that marks tumor cells with abnormal apoptosis and proliferation. The transketolase-like protein 1 (TKTL1) represents the enzymatic basis for an anaerobic glucose metabolism even in the presence of oxygen (aerobic glycolysis/Warburg effect), which is concomitant with a more malignant phenotype due to invasive growth/metastasis and resistance to radical and apoptosis inducing therapies.

Methods: Expression of Apo10 and TKTL1 was analysed retrospectively in OSCC specimens (n=61) by immunohistochemistry. Both markers represent independent markers for poor survival. Furthermore, Apo10 and TKTL1 have been used prospectively for epitope detection in monocytes (EDIM)-blood test in patients with OSCC (n=50), breast cancer (n=48), prostate cancer (n=15), and blood donors/controls (n=74).

Results: Positive Apo10 and TKTL1 expression were associated with recurrence of the tumor. Multivariate analysis demonstrated Apo10 and TKTL1 expression as an independent prognostic factor for reduced tumor-specific survival. Apo10+/TKTL1+ subgroups showed the worst disease-free survival rate in OSCC. EDIM-Apo10 and EDIM-TKTL1 blood tests allowed a sensitive and specific detection of patients with OSCC, breast cancer and prostate cancer before surgery and in after care. A combined score of Apo10+/TKTL1+ led to a sensitivity of 95.8% and a specificity of 97.3% for the detection of carcinomas independent of the tumor entity.

Conclusion: The combined detection of two independent fundamental biophysical processes by the two biomarkers Apo10 and TKTL1 allow a sensitive and specific detection of neoplasia in a noninvasive and cost-effective way. Further prospective trials are warranted to validate this new concept for the diagnosis of neoplasia and tumor recurrence.

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PATIENT-CENTRED CARE AND SHARED DECISION-MAKING IN ONCOLOGY

M. Härter

Department of Medical Psychology, Center for Psychosocial Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

In the last years, patient participation has become increasingly important in cancer care. Many patients want to play an active role in decisions about their care. An approach which takes these needs of patients into account is the model of shared decision-making (SDM) in which the patient and the clinician work together actively and equally and share information in order to come to a shared medical decision (1). SDM is a recommended strategy in preference-sensitive decisions, which occur frequently during cancer treatment. Decisions are considered preference-sensitive when more than one effective treatment option is available or possible side effects are likely to burden the patient's everyday life. Several interventions

strategies to increase the adoption of SDM exist (*e.g.*, trainings for clinicians and patients, decision support interventions, decision aids). A body of evidence indicates that patient decision aids support SDM in cancer care. These tools enhance decisional comfort, knowledge of treatment options and satisfaction with the decision in patients. They reduce the proportion of patients who act passively in the decision making process and the number of patients experiencing indecision after consultations (2, 3). However, few health providers feel adequately trained to implement SDM in practice, and physicians appear to overestimate the number of patients who prefer a passive role in decision making (4). In contrast to studies evaluating PDA, the evidence is less clear regarding SDM-training programs to enhance the SDM competencies of physicians. Few of the existing training programs were evaluated (5). Furthermore, SDM has been promoted on political and health policy level. Nevertheless, SDM has not been implemented into routine practice. Studies revealed barriers and facilitators for the implementation of SDM.6The talk will focus on effective SDM interventions SDM in oncology and how to implement them in medical school as well as in routine care.

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THE ADNATEST DETECTION AND MOLECULAR CHARACTERISATION OF CIRCULATING TUMOR CELLS: A PROGNOSTIC AND PREDICTIVE BIOMARKER IN CANCER?

S. Hauch

AdnaGen GmbH, Langenhagen, Germany

CTCs have a central role in the metastatic cascade, tumor dissemination and progression. New experimental and clinical data suggest that the epithelial-to-mesenchymal transition plays an important role in the generation of CTCs and the acquisition of resistance to therapy. CTCs seem to be a heterogeneous population of cells with different phenotypes and biological value. The detection and more importantly, the molecular characterisation of CTCs using the commercially available AdnaTests (AdnaGen GmbH, Langenhagen, Germany) for various tumor entities enable clinicians to monitor changes in the expression profile of CTCs during therapy as well as the effectiveness of a targeted therapy. This information provides independent prognostic and predictive factors as well as the opportunity for an improved personalized anticancer treatment. In addition, real-time monitoring could lead to the early identification of development of resistance to the therapeutic. In summary, the molecular characterisation of CTCs provides important information for identification of potential therapeutic targets and for understanding resistance to therapies. It could also offer new insights into the biology of tumor dormancy and tumor cell dissemination. This may open new opportunities for the early detection of metastatic spread and its successful treatment.

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CYTOTRACK CTC ENUMERATION AND HER2 CHARACTERIZATION

A.S. Haugaard[1], H. Stender[1], K. Holmstrøm[2], T. Møller[2], A. Frandsen[1], S. Nejlund[1], T. Hillig[1], G. Sölétormos[1]

[1]Department of Clinical Biochemistry, CTC Center of Excellence, Nordsjællands Hospital, Hillerød, Denmark; [2]Bioneer A/S, Hørsholm, Denmark

Background: Over-expression of human epidermal growth factor receptor-2 (HER2) in breast cancer patients is indicative of a poor prognosis and influences therapy selection. Therefore, molecular profiling of primary tumors is routinely performed in current clinical practice. HER2 profiling of circulating tumor cells (CTCs) may also be performed and may prove useful not only in therapy selection, but also in therapy monitoring of breast cancer patients. CytoTrack technology combines immunofluorescence scanning and enumeration of CTCs with the possibility of downstream characterization of individual CTCs. HistoFlex is an automated analysis platform that allows for rapid sequential analysis of individual biomarkers in tissues using immunohistochemistry (IHC) and on cells using immunocytochemistry (ICC) methods and may thus, be used for CTC profiling.

Methods: In this test the compatibility of the CytoTrack CTC enumeration method with manual ICC and HistoFlex was evaluated. Cancer cell lines MCF-7 and SK-BR-3 were

incubated with antibodies against pan-cytokeratin for fluorescent detection and enumeration with CytoTrack. Subsequently immunofluorescent staining with antibodies against HER2 was performed by either manual ICC (1 day) or by HistoFlex (1 hour).

Results: A positive HER2 signal was observed on the cell membrane of SK-BR-3 cancer cells, compared to weak/none signal on the MCF-7 cells. The manual ICC and the automated HistoFlex procedures provided similar results.

Conclusion: Both manual ICC and HistoFlex procedures were compatible with the CytoTrack CTC enumeration method and can be used for subsequent HER2 characterization of CTCs. However, HistoFlex provides faster results with reduced hands-on-time.

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CLINICAL VALUE OF SQUAMOUS CELL CARCINOMA ANTIGEN (SCCAG) IN ANAL CANCER

C. Henkenberens[1], H. Toklu[1,2], C. Tamme[3], F. Bruns[1]

[1]Department of Radiation Oncology, MHH, Hannover, Germany;
[2]Department of Internal Medicine, Robert-Koch Hospital, Gehrden, Germany;
[3]End- und Dickdarmzentrum (edh), Hannover, Germany

Background: Squamous cell carcinoma antigen (SCCAg) is a tumor marker that is expressed by anal cancer but its clinical utility in diagnosis and follow-up remains controversial. Because it may offer additional information to the clinician, we investigated retrospectively the value of pre- and posttreatment serum squamous cell carcinoma antigen levels in 24 consecutive patients with squamous cell carcinoma of the anus who received concurrent chemoradiation in the past five years in our institution.

Patients and Methods: From 2010 till 2014 pretreatment serum SCCAg measurement was performed in 24 consecutive patients with suspected squamous cell carcinoma of the anal canal and/or margin before local excision was performed to confirm the diagnosis histologically. Then, all patients received a combined modality radiotherapy and chemotherapy using 5-fluorouracil and mitomycin C as first-line treatment. Sequential serum SCCAg measurement after chemoradiation was performed in 21 patients, additionally to the regular follow-up examinations (imaging of the pelvis and rectoscopy) at first at three-month interval. SCCAg pretreatment values were compared with posttreatment values at the last follow-up examination regarding outcome and disease recurrence.

Results: The median pretreatment levels of SCCAg according to UICC tumor stages were 1.6 μg/L in stage I tumors (T1N0M0; n=3), 1.1 μg/L in stage II tumors (T2-3N0M0; n=11), 1.1 μg/L in stage III tumors (N+ status; n=10); no patient had metastatic disease at diagnosis (stage IV). The median posttreatment values at the time of the last follow-up examination decreased in stage I-II (stage I=1,0; stage II=0,80) while a slight SCCAg increase in stage III was observed due to one false positive value and one simultaneously recurrent/metastatic disease. Of the 16 patients who had normal SCCAg levels, 11 (68.8%) achieved a complete remission after initial treatment. One patient developed local recurrent disease. He received a salvage abdominoperineal resection and is still disease-free. Two patients presented with distinct metastases of the liver, respectively of the lung. Two patients with initial lymph node metastases developed recurrent lymph node metastases and distinct metastases of the skin, respectively of the lung. 7of 8 (87.5%) patients who had initially elevated SCCAg levels achieved a complete remission. One patient developed inoperable ulcerative recurrent lymph node metastasis and died to lethal arterial bleeding. Two patients had increased post-chemoradiation SCCAg levels: One increased value was false positive, when further examination excluded recurrent or distant disease; and in one patient the increased tumor marker led to the diagnosis of metastatic and recurrent disease.

Conclusion: Pretreatment SCCAg levels in our collective with squamous cell carcinoma of the anal canal treated with chemoradiation were not appropriate to predict the clinical outcome. This emphasizes the importance of regular clinical follow-up examinations (including imaging) and confirms clinical practice. Yet, increased SCCAg levels after chemoradiation are suspicious for recurrent and/or metastatic disease and provide additional information to the clinician.

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ASSOCIATION OF CA 27-29 AND CIRCULATING TUMOR CELLS BEFORE AND AT THE END AS WELL AS TWO AND FIVE YEARS AFTER ADJUVANT CHEMOTHERAPY IN PATIENTS WITH EARLY STAGE BREAST CANCER – THE SUCCESS TRIAL

P. Hepp[1], U. Andergassen[2], B. Jäger[3], E. Trapp[2], M. Alunni-Fabbroni[2], T. Friedl[3], N. Hecker[4], R. Lorenz[4], P. Fasching[5], A. Schneeweiss[6], T. Fehm[1], W. Janni[3], B. Rack[2]

[1]Department of Gynecology and Obstetrics, University Hospital Düsseldorf;
[2]Department of Gynecology and Obstetrics, University HospitalUlm;
[3]Department of Gynecology and Obstetrics, University HospitalMunich LMU;

[4]Frauenärztliche Gemeinschaftspraxis Braunschweig;
[5]Department of Gynecology and
Obstetrics, University Hospital Erlangen;
[6]National Center for Tumor Diseases, University Hospital
Heidelberg, SUCCESS Studiengruppe, Germany

Background: Evidence for the prognostic value of circulating tumor cells (CTCs) in early stage breast cancer is swiftly increasing. An alternative approach to identify patients (pts) at risk for recurrence is based on the detection of the MUC1 based tumor marker CA27-29. Here we report the association of these two prognostic markers before and immediately after chemotherapy(CHT) as well as at the two and five years follow-up.

*Methods:*The SUCCESS trial compared fluorouracil, epirubicin and cyclophosphamide followed by Docetaxel (FEC-Doc) *vs*. FEC followed byDoc, Gemcitabine (FEC-DocG) and 2 *vs*. 5 years treatment with zoledronic acid in 3754 pts with N+ or high risk early stage breast cancer. CA27-29 was measured with the ST AIA-PACK CA27-29 reagent (Tosoh Bioscience, Belgium). The cutoff for CA27-29 positivity was >31 U/ml. CTCs were assessed with the CellSearchSystem (Veridex, USA). The cutoff for CTC positivity was ≥1 CTC/15ml full blood. The relationship between CTC positivity and CA27-29 positivity was assessed based on Cramer's V, which measures the association between two nominal variables that varies from 0 (no association between the variables) to 1 (complete association).

Results: Both CA27-29 and CTC data were available for 1981 pts before CHT, 1602 pts immediately after CHT, 1159 pts two years after CHT and 707 pts five years after CHT. Before CHT, 7.9% of pts were CA27-29 positive and 21.3% were CTC positive. 2.4% of pts were simultaneously CA27-29 and CTC positive. Comparing the frequency of positive results for either test a weak, nevertheless significant association was found (p=0.0015; Cramer's V=0.063). Immediately after CHT, 21.0% of pts were CA27-29 positive and 22.8% were CTC positive. 4.2% of pts were simultaneously CA27-29 and CTC positive. At this time no significant association between CA27-29 and CTC was found (p=0.162; Cramer's V=0.035). Two years after CHT, 2.8% of pts were CA27-29 positive and 18.6% were CTC positive. 0.7% of pts were simultaneously CA27-29 and CTC positive. There was again no significant association between CA27-29 and CTC at this time (p=0.349; Cramer's V=0.028). Five years after CHT,7.5% of pts were CA27-29 positive and 8.5% were CTC positive. 1.8% of pts were simultaneously CA27-29 and CTC positive. A weak, but significant association between positive CA27-29 and CTC results was in this case found (p<0.001; Cramer's V=0.164).

Conclusion: In this study we showed that positivity for CTCs and CA27-29 were significantly associated before CHT and 5 years after CHT; however, no correlation was found immediately or two years after CHT during the course of early stage breast cancer. It therefore seems reasonable to further evaluate the prognostic value of CTCs and CA27-29 as a combined prognostic test.

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WHAT'S THE RELATIONSHIP BETWEEN SYMPTOM CONTROL, PATIENT REPORTED OUTCOME MEASURES (PROM) AND TUMOR MARKERS IN RECURRENT OVARIAN CANCER?

F. Hilpert

Klinik für Gynäkologie und Geburtshilfe,
Universitätsklinikum Schleswig-Holstein
Campus Kiel, Deutschland

The clinical course of recurrent ovarian cancer is initially very heterogeneous but ends ultimately in a common disease with heavy symptoms, high tumor burden, resistance and death.Therapy follows primary the palliative aspects of symptom control, objective response or disease stabilization, and after that the improvement of survival. The tumor marker CA 125 is an established laboratory surrogate parameter for the tumor burden in diagnosis and treatment monitoring. New results show that this objective marker can be set into context with the subjective estimation by PROM. It appears, depending on the specific setting in recurrent disease, that the clinical evaluation of CA 125 can have negative implications on the quality of life of patients on the one hand, but also can be a useful clinical tool. The missing link between tumor marker and PROM in the all day clinical use seems to be the individual symptomatology. The differentiated consideration of this "ménage à trois" is helpful to improve quality of life of our patients.

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LIQUID BIOPSY: MUTATIONAL STATUS OF CIRCULATING NUCLEIC ACIDS AS A NOVEL TOOL FOR THERAPY PREDICTION AND PROGNOSIS IN CANCER PATIENTS

S. Holdenrieder

Institute of Clinical Chemistry and Clinical Pharmacology,
University Hospital Bonn, Germany

The concept of "companion diagnostics" in the treatment of cancer patients with "targeted therapies" implies the pretherapeutic therapy stratification on basis of tissue

mutation status such as EGFR mutations for TKI therapies in lung cancer and absence of K-Ras mutations for cetuximab therapy in colorectal cancer. However, therapy response rates and duration are still limited due to primary or secondary resistances. Recently genetic heterogeneity including spatial and temporal variability within a tumor or between primary tumor, lymph node and distant metastases was uncovered explaining the difficulties of one-time molecular examinations in tissue biopsy material. Therefore the need for continuous monitoring of the overall mutation status in the patient body as stratification tool for therapy modification became evident.

Nowadays, sensitive blood-based diagnostics identifying mutations in circulating tumor cells (CTC) and in circulating cell-free plasma DNA (cfDNA) are available. They could overcome the genetic heterogeneity, as circulating cfDNA reflects the cancerous DNA changes in the whole body. As this concept of "liquid biopsy" is only minimally invasive it can be used to complement tissue biopsy for patient stratification and for the serial monitoring of successfully treated and newly occurring resistant cell clones at an individual level. BEAMing (beads, emulsion, amplification and magnetics) technology allows the sensitive quantification of specific mutations and has recently shown great potential for therapy monitoring, early recurrence detection and resistance monitoring in colorectal and lung cancer.

Here we show the highly sensitive detection and quantification of K-Ras mutations in plasma DNA of 32 patients with pancreatic cancer receiving chemotherapy. Most remarkably, K-Ras mutation status in plasma was highly predictive for response to chemotherapy and for prognosis of progression-free and overall survival while K-Ras tissue status was not. During the course of therapy, the amount of mutated plasma DNA correlated with therapy response and tumor recurrence. Our findings underline the high clinical relevance of this "liquid biopsy" approach for the individualized guidance of cancer patients.

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ONCOLOGICAL BIOMARKERS IN THERAPY MONITORING OF LUNG CANCER

S. Holdenrieder[1], J. von Pawel[2], D. Nagel,[3] P. Stieber[3]

[1]University Hospital Bonn, Institute of Clinical Chemistry and Clinical Pharmacology, [2]Asklepios Lung Hospital Gauting; Department of Thoracic Oncology, [3]University Hospital Munich, Institute of Clinical Chemistry, Germany

Background: Although cancer biomarkers are widely used in therapy monitoring of lung cancer patients, there are only rare systematic evaluations of their clinical significance.

Aim: We analyzed lung biomarker kinetics in patients with non-small cell (NSCLC) and small cell lung cancer (SCLC) on their power to specifically identify patients with poor and good response to systemic chemotherapy.

Methods: Lung biomarkers CYFRA 21-1, CEA, NSE and ProGRP were evaluated in prospectively collected serum samples of 311 patients with advanced NSCLC with 1st-line and 161 with 2nd-line chemotherapy, and 128 patients with SCLC (1st-line chemotherapy). Absolute marker levels and relative changes at time of staging after two treatment cycles were correlated with concurrent radiological response to therapy.

Results: Absolute levels and kinetics of CYFRA 21-1 and CEA at staging discriminated highly significantly between non-progressive and progressive patients in NSCLC (1st-/2nd-line therapies). Best ROC curves for identification of progression were obtained for CYFRA 21-1 levels (AUC 81.3% and 79.6%). Because several patients with tumor response had increasing values, sensitivity for detection of progression was only 31% and 22% (CYFRA 21-1; 1st/2nd-line), and 17% and 12% (CEA; 1st-/2nd-line) at 90%-specificity for non-progression. For detection of remission, sensitivity was 29% and 55% (CYFRA 21-1; 1st/2nd-line), and 31% and 21% (CEA; 1st-/2nd-line) at 90%-specificity for non-remission. In SCLC, ProGRP, NSE and CYFRA 21-1 levels at staging discriminated well between response groups while kinetics were less meaningful. Best ROC curves for non-response were obtained for the combinations of NSE and CYFRA 21-1 (AUC 91.3%) and ProGRP and CYFRA 21-1 (88.3%). Concerning kinetics, sensitivity for detection of non-remission at 90%-specificity was 20%, 40%, 10%, and 30% for ProGRP, NSE, CYFRA 21-1 and CEA kinetics, respectively; for detection of remission, sensitivity was 49%, 13%, 19% and 39% for ProGRP, NSE, CYFRA 21-1 and CEA kinetics, respectively.

Conclusion: Lung cancer biomarker levels are well suited to estimate the response to systemic therapy in NSCLC and SCLC patients. Kinetic interpretation is particularly useful for response detection, while increases in some well-responding patients limit the specific detection of progression. For interpretation of oncological biomarker monitoring studies it has to be generally considered that they are hampered by i) the questionable accuracy and clinical relevance of radiological staging (type of imaging, inter-observer variance, RECIST criteria, tumor size in CT *vs.* activity in PET, classification of "no change"), the type of progression (primary *vs.* metastases, slow *vs.* fast, single *vs.* multiple new lesions, different response of multiple lesions) and the lead time of biomarkers that may lack a correlation with imaging at a given time point but anticipate later progression.

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THE FIRST CLINICAL EXPERIENCE WITH CABAZITAXEL APPLICATION IN THE IIND LINE OF CASTRATION-RESISTANT PROSTATE CANCER TREATMENT

L. Holubec[1,2], J. Katolicka[3], O. Fiala[2], J. Finek[2]

[1]Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague, Plzen;
[2]Department of Oncology, Faculty Hospital and Medical Faculty Pilsen;
[3]Department of Oncology and Surgery, Faculty Hospital at Saint Ann, Brno, Czech Republic

Introduction: Docetaxel represents standard treatment in the Ist line of castration-resistant prostate cancer (CRPC), and results of studies of the IIIrd phase recommend application of several drugs for the IInd line of CRPC. The exact profile of a patient for whom the drugs are meant, as well as the sequence which would bring the best possible results, have not been defined so far.

Patients and Methods: The group includes 16 patients of 56-84 years of age with diagnosis of prostate adenocarcinoma, castration-resistant disease with castration level of testosterone, who were treated with docetaxel in the first line of CRPC therapy. Docetaxel was applied for the period of 3-26 months within the first line, and 75% of the patients were treated with docetaxel for longer than 6 months (3-24 months). Doses of 25mg/m^2 of cabazitaxel were applied intravenously every three weeks.

Results: The only undesirable effects of the grade III-IV recorded were hematological toxicity, leucopenia of GIII and neutropenia of GIII without any infection complications, and non-hematological toxicity was minimum. Gleason Score (GS) of 8-9 was assessed histologically with 9 patients, and all these patients' response to docetaxel applied in the Ist line for period longer than 6 months (6-11 months) was good. The follow-up therapy with cabazitaxel has taken place in this subgroup for 3-15 months so far, and PSA has dropped by more that 50%, which means partial remission in 5 patients. Only 3 patients out of the total number of 16 patients died within the course of assessment, and in of both of them visceral organs and lymphatic ganglia were affected metastatically.

Conclusion: Through our first clinical experience, cabazitaxel demonstrated good effect in the IInd line of the treatment in case of patients with GS>7 as well as patients with a good long-term response to the docetaxel treatment in the Ist line of CRPC.

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COMBINED ORAL METRONOMIC AND BIODIFFERENTIATING ANTI-ANGIOGENIC THERAPY WITH GASTROENTERO-PANCREATIC PATIENTS WITH NEUROENDOCRINE TUMORS (GEP-NETS)

L. Holubec, J. Finek, O. Fiala, V.M. Matejka, P. Mrazkova, V. Bajciova, E. Sedlackova

Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague, Plzen, Czech Republic, Dept. of Oncology, Faculty Hospital and Medical Faculty Pilsen, Dept.of Medical Imaging, Faculty Hospital and Medical Faculty Pilsen, Dept.of Oncology, Charles University Prague, Pediatric Oncology Department, Univ. Hosp. Brno, Czech Republic

Background: The outcome of patients with metastatic, progressive or recurrent neuroendocrine tumors remains poor despite the several novel therapeutic approaches. The study aims to assess contribution of experimental bio-differentiating and antiangiogenic therapy with gastro-enteropancreatic neuroendocrine tumor patients (GEP-NETs), along with metronomic application of low doses of cytostatics, after failure of any form of oncologic therapy available. The target group were patients with multiple distant metastases of GEP-NETs.

Patients and Methods: Therapy according to the COMBAT schema (combined oral metronomic and bio-differentiating antiangiogenic treatment) was started with 12 patients (8 men and 4 women). Such a therapeutic schema is based on combination of peroral antiangiogenic doses of celecoxib and fenofibrate along with metronomic doses of chemotherapeutics (cyclophosphamide and temozolomide) together with pro-differentiating character of vitamin D /25 OH D(3), 1-25 OH D(3). One bio-chemotherapy cycle lasted 77 days, the patients were treated continually and they were given analogs of somatostatin receptors throughout the therapy. The total therapy responses were assessed after 3-5 cycles of bio-chemotherapy. Further on, toxicity of the therapy was monitored and tumor markers of chromogranin A (CgA), NSE, TPS, and TK were assessed.

Results: Partial remission occurred in 4 (33%) out of the total number of 12 patients, 5 (42%) patients resulted in stable disease, and progression of the disease was detected in 3 (25%) patients only. The median of the therapy response period was 15 months. The average value of CgA before the therapy was 651.9 ng/mL, after 3-5 cycles of bio-chemotherapy it decreased to 451.3 ng/mL (p<0.0313). The therapy tolerance was very good.

Conclusion: The therapeutic results achieved appear to be promising for application of bio-differentiating and

antiangiogenic therapy along with metronomic application of low doses of cytostatics with GEP-NET patients. Pursuant to the pilot results, a multicentric clinical study of phase II has been initiating currently.

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PANITUMUMAB IN THERAPY OF PATIENTS WITH ADVANCED METASTASIZING COLORECTAL CARCINOMA - PERSONAL CLINICAL EXPERIENCE

L. Holubec, O. Fiala, V.M. Matejka, V. Liska, O. Topolcan, J. Finek

Dept. of Oncology, Univ. Hospital and Medical Faculty Pilsen, Czech Republic

Background: Targeted biological therapy as a part of personalized medicine becomes standard therapy for oncology patients. In case of patients with wild-type of K-RAS gene, Panitumumab appears to be an effective possibility of the targeted therapy.

Study Aims: The authors present results of a study which analyzes a group of 41 patients with advanced metastasizing colorectal carcinoma (mCRC) who underwent palliative bio-chemotherapy and were given Panitumumab in the second and further lines of the therapy.

Patients and Methods: A data file of 41 advanced mCRC patients (30 men, 11 women; median age of 61 years) was evaluated. The primary aim of this study was to assess overall response rate (ORR) and time to progression (TTP), the secondary objective was to assess bio-chemical response rate through tumor markers (CEA, CA 19-9, TPS, and TK) assessment, to evaluate life quality of the patients and to assess toxicity of the therapy. ORR was assessed with all the patients in compliance with RECIST criteria after 6-9 cycles of the bio-chemotherapy.

Results: The therapy response rate was assessed with 36 patients. The overall response rate was achieved in 66% of the patients /CR-1 pts (3%); PR-12 pts (23%); SD-11pts (30%)/. Median TTP was 22.4 weeks. In case of bio-chemical response rate assessment, the CEA, CA 19-9, and TPS tumor markers showed statistically significant decline of values comparing the values before the therapy and at the time of ORR achievement (CEA $p<0.0266$; CA 19-9 $p<0.0075$; TPS $p<0.0002$). Regarding the TK tumor marker, no significant decline was recorded ($p<0.1671$). The main undesirable effect appeared to be dermal toxicity (grade II-III with 56% of the patients). No serious infusion response occurred within our group of patients.

Conclusion: In patients with wild type of K-RAS gene, Panitumumab can be used as a highly effective alternative therapy of mCRC with very good tolerance and minimum of undesirable effects. Assessment of the tumor markers: CEA, CA 19-9, and TPS is a suitable way to check effectiveness of the targeted biological therapy.

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THE IMPORTANCE OF EARLY TUMOR SHRINKAGE (ETS) AND DEEPNESS OF RESPONSE (DPR) IN ASSESSING THE EFFICACY OF SYSTEMIC ANTICANCER TREATMENT

L. Holubec, O. Fiala, V. Liska, P. Mrazkova, J. Bauer, J. Finek

Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague, Plzen; Department of Oncology, Department of Medical Imaging, Faculty Hospital and Medical Faculty Pilsen; Czech Republic

Background: There are four biological target products routinely approved for metastatic colorectal cancer (mCRC) treatment on the basis of clinical studies results: monoclonal EGFR antibodies (cetuximab, panitumumab) and an antibody used against VEGF (bevacizumab, aflibercept). Positive therapy effect is mostly connected with concurrent use of chemotherapy. The efficacy of target anticancer therapy is regularly evaluated using the following indicators: objective response rate (ORR), progression free survival (PFS) and overall survival (OS). The change in the tumor burden extent is assessed by the cumulative change in the size of the target tumor lesions using imaging methods. Here are the most frequently used WHO and RECIST criteria. The main problem of these criteria is that they use different definitions of response rate evaluation. Generally, the existing results of these evaluations do not confirm a direct correlation between the objective response rate (ORR) and survival (PFS or OS). Another problem of these methods is that the results of the assessment do not correlate with the biological activity of tumor growth, since it is a static evaluation of clinical status.

Purpose: This oral presentation provides an overwiev of results related to new possibilities for evaluating the efficacy of anticancer therapy using the concept of "deepness of response" (DpR) and the concept of "early tumor shrinkage" (ETS) in patients with metastatic colorectal cancer.

Conclusion: The results of numerous post-hoc and exploratory analyses of clinical studies consistently suggest

that ETS and DpR are important variables in assessing the efficacy of systemic anticancer treatment.
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TREATMENT OF CETUXIMAB-RELATED SKIN TOXICITY IN A PATIENT WITH METASTATIC COLORECTAL CARCINOMA – A CLINICAL CASE REPORT

L. Holubec[1], J. Říčař[2], K. Pizinger[2], O. Fiala[1], H. Korunková[1], P. Mrázková[3], J. Fínek[1]

[1]Department of Oncology and Radiotherapy, [2]Department of Dermatology and Venereology, [3]Department of Medical Imaging, University Hospital and Faculty of Medicine in Pilsen, Charles University in Prague, Czech Republic

Background: Cetuximab, a monoclonal anti-EGFR antibody, represents a targeted biological treatment that is becoming a standard of personalized medicine in patients with metastatic colorectal carcinoma. Extent of skin toxicity is the most significant predictive factor of cetuximab efficacy. A case report demonstrating a treatment possibility for the cetuximab-related dermal toxicity is presented.

Case Report: Authors report on a 57 year-old female patient suffering from metastatic colorectal carcinoma who underwent palliative bio-chemotherapy in the schedule of cetuximab+ FOLFIRI. Six weeks after therapy initiation, a grade 3 papulopustular rash was detected. Early local treatment using vitamin K1 0.1% cream in combination with oral doxycycline and antihistamines enabled resuming of the chemotherapy.

Conclusion: Appropriate therapeutic management of skin toxicity associated with cetuximab treatment enabled to continue the antineoplastic therapy with no interruptions and improved the patient's prognosis.
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TOXICITY OF MITOXANTRONE LOADED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPION) IN A HT-29 TUMOUR SPHEROID MODEL

A. Hornung, M. Pöttler, R.P. Friedrich, S. Dürr, J. Zaloga, I. Cicha, C. Janko

Department of Otorhinolaryngology, Head and Neck Surgery, Section for Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftung Professorship, University Hospital Erlangen, Germany

Introduction: Magnetic Drug targeting (MDT) using superparamagnetic iron oxide nanoparticles (SPION) loaded with chemotherapeutic drugs represents a new promising therapeutic strategy for cancer treatment. Although nanoparticle mediated effects on cell monolayers have been extensively investigated previously, more relevant *in vitro* models are needed to transfer the experimental data to the *in vivo* situation. Here, the absorption, penetration and effect of different concentrations of mitoxantrone (MTO), SPIONMTO and unloaded SPION in 3D tumor spheroids of HT-29 colon carcinoma cells were investigated.

Materials and Methods: Tumour spheroids were generated by seeding dispersed HT-29 cells on agarose coated cell culture wells and grown for 72 hours. Then, fluid MTO, SPIONMTO and unloaded SPION were added to the spheroids. Proliferation of the spheroids was monitored by transmission microscopy. Every 24 hours pictures were taken and spheroid morphology and growth were analysed by calculating the areas of the spheroids *via* ImageJ software.

Results and Discussion: The data revealed that unloaded SPION have no influence on the proliferation of the tumor spheroids compared to the untreated controls, while both fluid MTO and SPIONMTO inhibited proliferation of the spheroids in a dose and time dependent manner. In comparison to fluid MTO, the effect of SPIONMTO on spheroid growth was slightly delayed.

Conclusion: Both fluid MTO and SPIONMTO effectively inhibit spheroid growth, indicating that the chemotherapeutic agent is capable of penetrating into three dimensional cellular structures. However, in further investigations it has to be analysed if MTO infiltrates in its bound form (SPIONMTO) or if it has to be released from the nanoparticles before infiltrating.
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HORIZON 2020

J. Kinkorová

Faculty Hospital Plzen, Czech Republik

Horizon 2020 is the biggest EU programme for research and innovation ever with a budget more than €80 billion of funding for the next 7 years (2014 to 2020). Horizon 2020

follows achievements in the previous programme, 7th Framework programme for research and technological development (2007 to 2013). Horizon 2020 differes in the structure from FP7, by coupling research and innovation, aiming to achieve this with its emphasis on excellent science, industrial leadership and tackling societal challenges. The goal is to ensure Europe produces world-class science, removes barriers to innovation and makes it easier for the public and private sectors to work together in delivering innovation. Horizon 2020 consists of three priorities:(1) Excellent Science. This will raise the level of excellence in Europe's science base and ensure a steady stream of world-class research to secure Europe's long-term competitiveness. It will support the best ideas, develop talent within Europe, provide researchers with access to priority research infrastructure, and make Europe an attractive location for the world's best researchers. (2) Industrial Leadership. This will aim at making Europe a more attractive location to invest in research and innovation (including eco-innovation), by promoting activities where businesses set the agenda. It will provide major investment in key industrial technologies, maximise the growth potential of European companies by providing them with adequate levels of finance and help innovative SMEs to grow into world-leading companies. (3) Societal Challenges. This reflects the policy priorities of the Europe 2020 strategy and addresses major concerns shared by citizens in Europe and elsewhere. A challenge-based approach will bring together resources and knowledge across different fields, technologies and disciplines, including social sciences and the humanities. Health, demographic change and wellbeing is financially significantly supported societal challenge. Europe will be for this period facing many "heatlh" challenges; chronic diseases such as cardiovascular disease (CVD), cancer, diabetes, neurological and mental health disorders, overweight and obesity and various functional limitations are major health and societal problems for the future. In Europe CVD annually accounts for more than 2 million deaths and costs the economy more than EUR 192 billion while cancer accounts for a quarter of all deaths and is the number one cause of death in people aged 45-64. Over 27 million people in the EU suffer from diabetes and the total cost of brain disorders (including, but not limited to those affecting mental health) has been estimated at EUR 800 billion.

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EFFICACY ORIENTATED SEQUENTIAL POLYCHEMOTHERAPY (EOSPC) AND OVERALL SURVIVAL (OS) OF PATIENTS SUFFERING FROM EXOCRINE PANCREATIC CANCER (PACA) – AN EVALUATION OF 438 PATIENTS TREATED BETWEEN 1997 – 2014

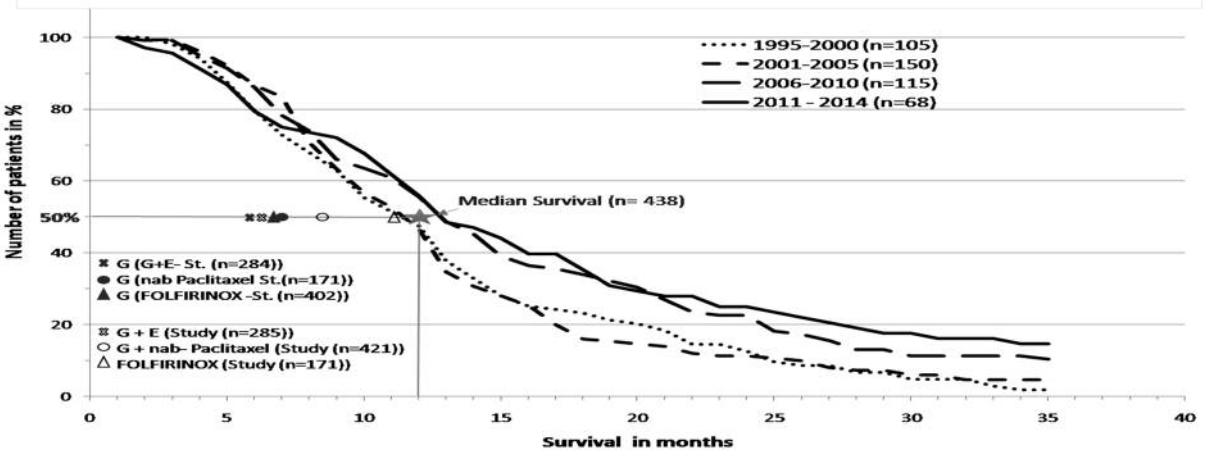
R. Klapdor, M. Bahlo, M. Brenzinger, S. Klapdor

Medical Unit, ZeTDT GmbH, Hamburg, Germany

Background: In 2013 two new prospective randomized clinical therapy studies have been published, presented as a breakthrough in palliative chemotherapy of advanced exocrine paca patients (median OS for Gemcitabine(Gem)/ FOLFIRINOX 6.8 *vs*. 11.1 months, for Gem *vs*. Gem+nab-Paclitaxel 6.7 *vs*. 8.5 months). Now,since 1997 our group favoured the concept of an EOSPC of paca patients. The concept based on the one hand on our experience, that serial determinations of the serum tumor markers CA 19-9 and CEA in addition to the imaging methods (IM) CT/MRT allow a more rapid detection of a tumor answer to therapy than IM alone, that means a more rapid detection of initial tumor response to therapy (response *vs*. progression) as well as a more rapid detection of a new progress after initial tumor regression under palliative chemotherapy. As a consequence, a serial determination of valid tumor markers in addition to the IM should allow to shorten ineffective treatments in order to try not only a 1st - line therapy in a patient, but also a 2nd - or 3rd - line treatment in order to improve the OS, to reduce the costs of treatment by reducing the time of ineffective treatment and last not least to decrease the rate of potential side effects of ineffective chemotherapy for the patients. On the other hand, our concept based on the introduction of new drugs, like oxaliplatin and irinotecan in addition to 5FU, into colorectal oncology, which might offer also beneficial effects in paca patients as 2nd- or 3rd - line therapies after 1st-line therapy with a gemcitabine based regimen, even if not – till today - officially approved for paca at this time. We now analysed the results of our treatment concept in our patients since 1997 with respect ot overall survival.

Methods: We analysed the treatment results of alltogether 438 patients with proven locally advanced, recurrent of metastasized exocrine pancreatic cancer since 1997, basing on a follow-up with serial determinations of the tumor markers CA 19-9 and /or CEA every 2-4 weeks in combination with bimonthly performed imaging methods (CT or MRT). Already in 1997 we started our EOSPC concept, as previously described (*e.g*. R. Klapdor *et al*.: Anticancer Res *23*: 841-44, 2003). Drugs or drug combinations used as 1st-, 2nd- or 3rd - or 4th- line therapie were: Gemcitabine, 5-FU, Mitomycin-C, combinations with oxaliplatin or irinotecan *e.g*. as previously described, in single patients taxanes as well as Gem+Erlotinib since 2007 and FOLFIRINOX and Gem+nab-paclitaxel in 2013/2014.

Results: Our results confirm a significantly higher rate of 2nd- and 3rd-line therapies in our patients. The OS for all 438 patients treated since 1997 amounted to 12 months, nearly unchanged for the periods 1997-2000, 2001-2005, 2006-2010 and 2011-2014 (OS 11, 11, 12 and 12 months). Analyses for



Figure, Abstract No. 57

1-year and 2-year survival also showed comparable results for the whole group compared to the different time periods.

Conclusion: The actual evaluation of 438 of our paca patients treated on the basis of our concept of EOSPC demonstrates, that the OS data for the "new" drug regimens Gem+nab-paclitaxel as well as FOLFIRINOX, published in 2013, are not higher than the OS of our 438 patients, treated with the availabe drugs between 1997 and 2014 following our EOSPC concept. On the other hand, our results suggest, that the involvement of paca patients in the generally accepted Gemcitabine control arms as done in the FOLFIRINOX- and nab-paclitaxel- or Gem+Erlotinib study might be probably seen as a prognostic negative factor with respect to survival of these paca patients, as already suggested in our previous publication (R. Klapdor: Anticancer Res *27*: 1789-1794, 2007) (Figure).

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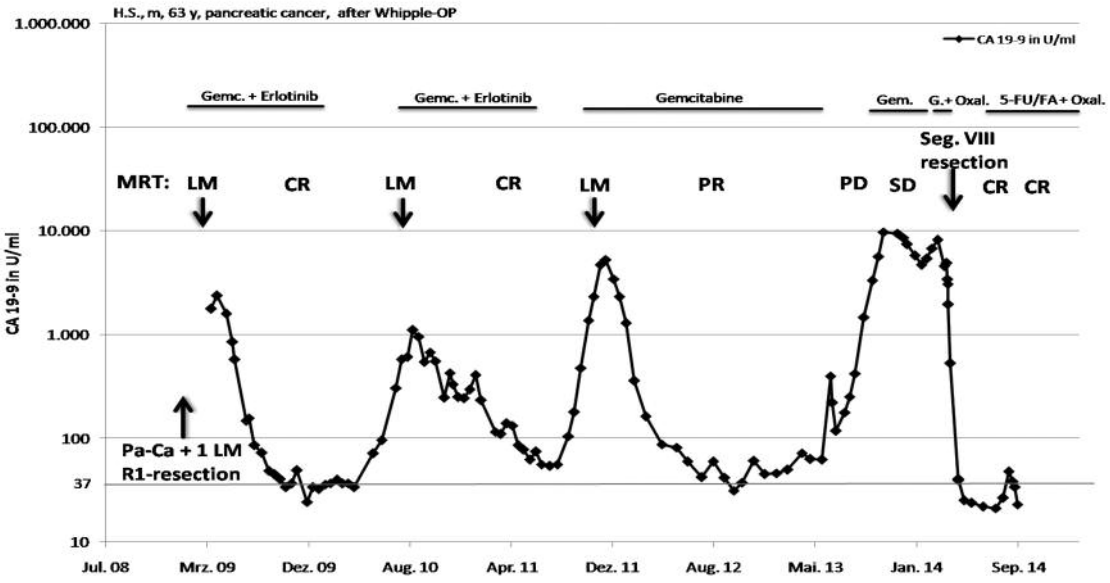
CURE OF METASTATIC PANCREATIC CANCER (PACA) DISEASE BY RESECTIVE SURGERY FOLLOWED BY FIVE YEARS PALLIATIVE CHEMO-THERAPY AND ACTUAL SEGMENT VIII LIVER RESECTION ? – A CASE REPORT

R. Klapdor[1], S. Klapdor[1], M. Fard-Aghaie[2]

[1]Internal Unit, ZeTDT GmbH,
[2]Dep. of Surgery, Asklepios Barmbek, Hamburg, Germany

First resective operation: In a 63 years old male patient pylorus preserving pancreatic head resection and segment 7 liver resection was done in April 2009 because of a pT3, pN0, M1 (unilocal, segment VII), G2, L0,V1,R1 (UICC IV) PACA disease, after painless jaundice and cholangitis as first clinical symptoms in his illness history. *5-years palliative chemotherapy:* Because of even postoperatively elevated CA 19-9 serum levels (1732 U/ml) and detection of a small tumor lesion in segment VIII by postoperative MRT palliative chemotherapy (pall. chemo) was started with Gemcitabin+Erlotinib (Gem+E) resulting in Rash II and in a complete remission (CR) of tumor disease with decrease of CA19-9 below 37 U/ml and vanishing of the liver metastasis in the MRT. Pall. chemotherapy was stopped in March 2010. In July 2010, serum CA 19-9 increased again (up to 1104 U/ml), the MRT showed again a tumor lesion in liver segment VIII. A second treatment period with Gem+E (July 2010 – end of June 2011) showed again a good tumor response (Rash II, vanishing of tumor lesion, decrease of CA 19-9 to 56 U/ml). Treatment was interrupted because of a therapy resistant ulcer of the 5th toe, later requiring amputation. In December 2011, serum CA 19-9 again increased (up to 9501 U/ml), the MRT showed two small tumor lesions within liver segment VIII. We started a Gem monotherapy (1.12.2011- 20.6.2013), again resulting in a good tumor response, with decrease of serum CA 19-9 to 37 U/ml and at least partial remission (PR) (nearly CR) in the imaging methods. Treatment had to be interrupted because of necessary eye operations. Initially after starting this Gem treatment we also noticed a skin reaction very similar to the Rash symptoms seen in the course of the first two therapy sequences, probably due to steroid applications simultaneously given with the gem infusions as done also during the first two treatment cycles. At the end of 2013, serum CA 19-9 again increased (up to 8227 U/ml), the MRT resulted again in 2 increasing tumor lesions in segemt VIII. A new Gem monotherapy resulted again in a decrease of serum CA 19-9



Figure, Abstract No. 58

(down to 4686 U/ml), however only a transient decrease, followed by a new increase (up to 8227 U/ml). The start of a combined chemotherapy (Gem+Oxaliplatin, 2 cycles) induced again a decrease of serum CA 19-9 (to 4919 U/ml CA 19-9). However, we now proposed a segmental liver resection, as a new whole body investigation did not show any other tumor lesion than within segment VIII. A PET-CT also confirmed the liver lesions in segment VIII, but no other lesions. Segment VIII resection (March, 27, 2014) allowed complete tumor resection. The pathologists confirmed liver metastasis of a PACA. Serum CA 19-9 decreased within 5 weeks below 37 U/ml (initial half life time 30 hours). An adjuvant chemotherapy (5FU/FA+ Oxaliplatin) is done since more than 4 months. Till today there is no sign of tumor recurrence in the MRT, no new increase of CA 19-9. The future follow-up has to demonstrate whether we really have a cure of a metastatic PACA disease five years after primary resection. Furthermore this case report should stimulate discussion on interventional surgery of liver metastasis in these patients and on factors that might be responsible for such a kind of metastasis restricted for 5 years to only one liver segment (Figure).

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COMPARISON OF 3 DIFFERENT IMMUNOASSAYS FOR DETERMINATION OF CA 19-9 IN PANCREATIC CANCER PATIENTS - ANOTHER ASPECT OF POTENTIALLY CLINICAL RELEVANCE

R. Klapdor, M. Bahlo

Internal Unit, ZeTDT GmbH, Hamburg, Germany

Introduction: CA 19-9 represents the most important serum tumor marker for serological follow up of exocrine pancreatic cancer since about 30 years. Furthermore, several publications stress the prognostic relevance of preoperative elevation of CA 19-9, of the degree of decrease after resective surgery, of the initial decrease kinetics of serum CA 19 after beginning of palliative therapy, or they stress *e.g.* the prognostic relevance of complete responses of serum CA 19-9 (decrease below the generally accepted cut-off level of 35-37 U/ml). For determination of CA 19-9 analytical assays of different manufacturers are offered. It is generally accepted that the absolute CA 19-9 serum levels measured by the various assays may not be transferred from one assay to another. We now would like to focus the interest to another aspect of potentially clinical relevance.

Methods: During the past 6 months we measured the CA 19-9 concentrations in 110 sera of pancreatic cancer patients, range between 1 and 10.000 U/ml, using the folowing analytical systems: KRYPTOR (BRAHMS), ADVIA Centaur (Bayer Diagnostics) and mini-Vidas-System (Biomerieux) and analysed the measured data for various correlations.

Results: The results demonstrate that the ADVIA Centaur and the mini-Vidas-System resulted in higher serum levels compared to the KRYPTOR in the case of CA 19-9 concentrations higher than 80-100 U/ml. Below 80-100 U/ml

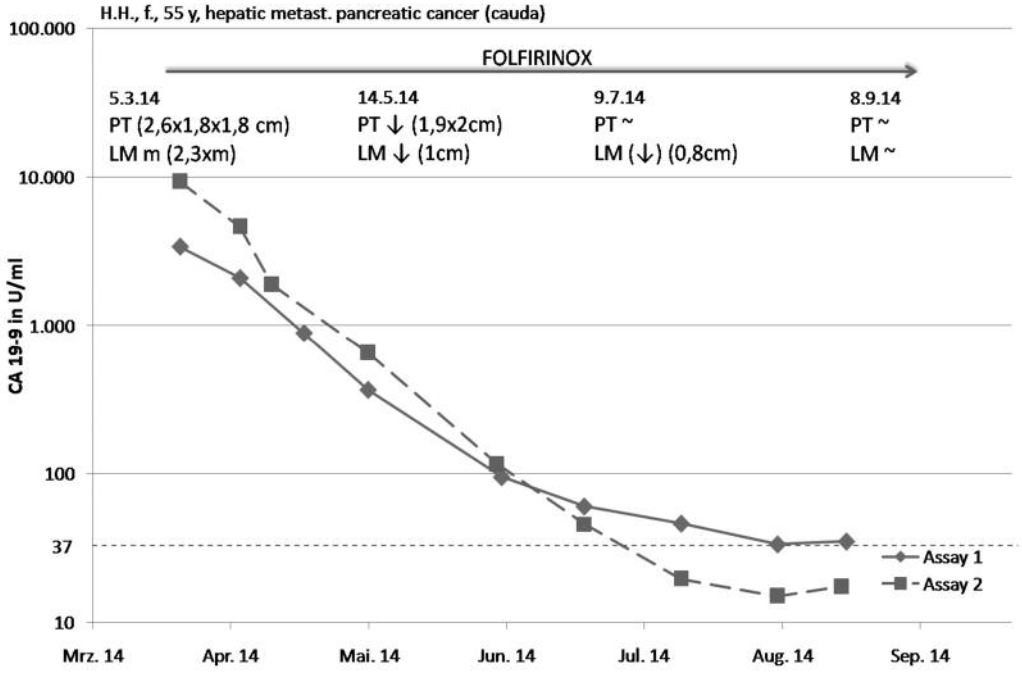

Figure 1, Abstract No. 59

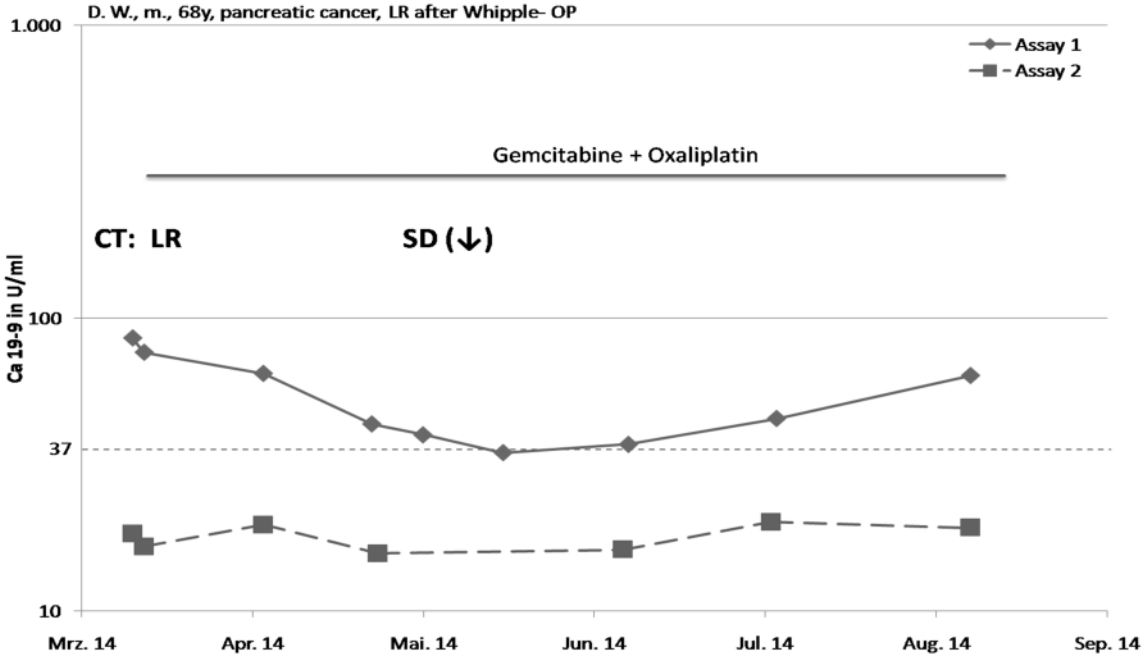

Figure 2, Abstract No. 59

there is a trend to lower levels compared to the KRYPTOR, with in most cases lower levels below the cut-off of 35-40 U/ml. In contrast, CA 19-9 determinations at two different times with the KRYPTOR *e.g.* revealed a very strict correlation over the whole range of CA 19-9 levels (1-10.000 U/ml).

Conclusion: Our results suggest that even nowadays different methods for determination of serum CA 19-9 offered on the market not only cannot be used interchangeably with respect to the absolute serum levels measured, but probalby also kinetic data or response analyses as potentially prognostic parameters may not be transferred from one assay to another. From these data further follows, that "Ring"studies should include values in the the range next to the cutt-offs in addition to lower and higher serum concentrations, and that in single patients it might be of value to try *e.g.* a second CA 19-9 assay in order to find an elevated CA 19-9 serum concentration in a cancer patient. As long as no generally accepted reference material is available further studies should also stress this topic in order to increase the acceptance of CA 19-9 as a relevant tumor marker by the clinicians (Figures 1 and 2).

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CA 19-9 AND CEA AS SEROLOGICAL TUMOR MARKERS (TM) FOR THE DIAGNOSIS AND FOLLOW-UP OF EXOCRINE PANCREATIC CANCER (PAPA)

R. Klapdor, M. Bahlo

Internal Unit, ZeTDT GmbH, Hamburg

Serum *CA 19-9* was introduced into clinical routine in 1983 in order to optimize diagnosis and follow-up of exocrine PACA disease (sensitivity 80-90% at time of primary diagnosis at that time, specificity 95%). However, clinical praxis soon demonstrated that even CA 19-9 does not represent an ideal TM or biomarker: *no organ specificity, no tumor specificity, no early diagnosis*. 5-10% of the patients do not produce CA 19-9 at all, the serum concentration increases with tumor load, till today not allowing detection of real early stages; on the other hand CA 19-9 secretion into the serum/weight unit of a tumor may vary enormously from one tumor disease to the other, preventing a narrow correlation between serum concentration of CA 19-9 and stage of the disease in the imaging methods (IM) in PACA disease. Therefore even nowadays colleagues and patients might follow the recent recommendations in the *German guide lines for exocrine PACA (2013)* which do not recommend CA 19- determinations for screening, early or earlier diagnosis and do not mention at all CA 19-9 with respect to follow-up after resective or during palliative therapy of PACA. Singular high dose hook effects or Hama phenomena, unknown assay-changes within a lab, insufficient own clinical experience, serum determinations in too long time intervals, budget problems or other aspects might underline the 2013 guide line proposals, as well as insufficient knowledge of observed differences in the extend of a tumor answer measured by TM and IM resp. Serum CA 19-9 concentrations *e.g.* may indicate progressive disease (PD) even if the IM still indicate a stable disease in the case of slowly growing tumors; on the other hand a stable disease (SD) or minor response (MR), in singular cases also a partial regression (PR) of serum CA 19-9 does not exclude a SD or retarded PD measured by imaging methods. In these cases the CA 19-9 response indicates a therapy induced antitumoral effect and an inhibitory influence on tumor growth, however only in the sense of more or less retardation of tumor growth, not strong enough to induce tumor regression or to inhibit further tumor growth. Surely, also to our experience serum CA 19-9 does not fullfill the criteria of an ideal TM. We also know that a TM cannot replace the IM. However, as a functional parameter an every 2-4 weeks determined serum CA 19-9 often reacts more sensitive and more rapid during *follow-up* of a tumor disease than the IM performed every 2 months. *E.g.* a new increase of serum CA 19-9 (2-3 determinations without relevant changes in γGT and/or pancreatic enzymes) nearly in all of these patients indicate new tumor progression during adjuvant as well as palliative treatment, often weeks or some months earlier than the IM, thus *e.g.* allowing an earlier interruption of an ineffective chemotherapy and a minimization of potential therapy induced side effects, a reduction of costs by avoidance of ineffective treatment and the the chance for a 2nd-, 3rd- or forth line therapy, even in the case of a rapidly growing tumor as known from exocrine PACA. Basing on these experiences we are treating our patients since more than fifteen years on the basis of an efficacy orientated sequential poly-chemotherapy (EOSPC), resulting in a significantly higher rate of 2nd-, 3rd- and 4th-line therapies than reported in other studies, followed by an overall survival of 12-14 months, an OS significantly longer than *e.g.* the median OS of 6-7 months in the Gemcitabine arms of the previous as well as the modern prospective randomized clinical treatment studies. Furthermore, CA 19-9 determinations might improve an *earlier diagnosis* at least in those patients, in which abdominal or back pains are not investigated by US or CT over weeks or months. Even nowadays a lot of patients show CA 19-9 levels of 1000 U/ml and more (up to 100000 U/ml) at the time of primary diagnosis. Similar considerations are known for CEA as a second TM in PACA disease. Serum CEA may be a relevant TM in the case of CA 19-9 negativity. However, in general CEA is not as sensitive as CA 19-9 in PACA. Our

retrospective analysis of up to now 110 PACA patients *e.g.* resulted at time of admission and end of the disease resp. in the following data: CA 19-9 neg. in 22% and 6% resp., CEA in 54% and 26% resp., CA 19-9 + CEA neg. in 13% and 4% resp. In 4% (4/110) of these patients we found however a striking different course of CA 19-9 and CEA (decreasing CA 19-9 and increasing CEA or *vice versa* under palliative chemotherapy), probably indicating different antitumoral activities of the cytostatic treatment on tumor cell clones producing CEA or CA 19-9. The question whether other TM like CA 125 or cytokeratins might have clinical relevance in PACA patients with normal CA 19-9 + CEA levels at time of admission or in advanced stages of the disease, we actually study in a retrospective evaluation using our serum bank.

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HOME PARENTERAL NUTRITION – THE GUIDELINES 2012/2013 SEEM TO SUPPORT OUR USE OF NUTRTION THERAPY IN CRITICAL ILL PANCREATIC CANCER PATIENTS

S. Klapdor[1,2], R. Klapdor[1]

[1]Internal Unit, ZeTDT GmbH;
[2]PiM Patient im Mittelpunkt GmbH, Hamburg, Germany

Pancreatic cancer patients often suffer from malnutrition and cachexia due to exocrine pancreatic insufficiency, tumor compression of the stomach/duodenum, pains, vomiting and anorexia induced by the tumor itself and/or by antitumoral therapies or various combinations. These problems will become more and more of clinical relevance, in relation to improvements of survival during recent years as a consequence of an earlier diagnosis, of more effective drugs or drug combinations available, of a more subtile follow-up and of the increasing development of efficacy orientated sequential multimodal therapy concepts. Nutrition advice, adequate intake of pancreatic enzymes to the meals and addition of oral enteral supplements are generally accepted as therapeutical options. Home Parenteral Nutrition (HPN), however, needs further propagation, probably due to more time consuming handling, potential problems/side effects that might occur, to budget problems due to some contradictory results in the literature. In our experience over the past 15 years in our pancreatic cancer patients we see an indication for HPN in addition to dietary advice, adjusted enzyme therapy and oral enteral supplements especially a) in order to stabilize or improve the nutrition status and life quality in patients with nutritonal problems after resective surgery on the one hand with the aim to stabilize or improve the nutrition status and life quality, on the other hand in order to start adjuvant therapy in our patients with such kind of postoperative problems still in time, b) in order to ameliorate and improve severe side effects of adjuvant or palliative chemotherapy in order to avoid undesired interruptions of palliative therapies, especially in patients with hints for an effective chemotherapy and c) in order to rapidly switch to a 2nd- and / or 3rd- line treatment regimen in the case of increasing nutrition problems due to progressive tumor disease in the case of ineffective first-line therapy. To our experience one should start HPN if indicated relatively early in the development of malnutrition or cachexia, especially in pancreatic cancer patients known to show a rather rapid progressive disease, with a relatively short median overall survival of 6-8 months in most of the official treatment studies. We already could demonstrate that continuous and adequate nutritional advice and support are able to significantly improve the nutrition status in these pancreatic cancer patients (see *e.g.* E. Richter *et al.*, Anticancer Res *32*: 2111-2118, 2012). Furthermore, on the basis of our long-time experience we belive that early active nutrtional advice and support, including HPN might have contributed to the results of our concept of an efficacy orientated sequential polychemotherapy of pancreatic cancer patients, practiced by us since about 15 years, with an median overall survival of about 12 months over the whole time period The results of some typical follow-up courses will underline our experience.

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PANCREATIC DISEASES AND VITAMIN D SUPPLEMENTATION

S. Klapdor[1], P. Roth[2], R. Klapdor [1]

1Internal Unit, ZeTDT GmbH, Hamburg, 2MWZ Labor Limbach, Heidelberg, Germany

For some decades it was recommended to supplement vitamin D in patients with exocrine pancreatic insufficiency (chronic pancreatitis, pancreatic cancer, resective surgery) by i.m. aplication of fatty adic soluble preparations (ADEK Falk® or others) basing on the assumption that an insufficient uptake of orally given vitamin D will not allow a normalization of serum Vitamin D in these patients. In 2010 we could however demonstrate that i.m. application of ADEK preparations with 10.000 IE per injection do not allow normalisation in most of the patients. On the contrary oral application of vitamin D allows to normalize the serum concentrations in the case of adequate oral substitution on the basis of serum vitamin D controls (up to 20.000 IE per day, in very single patients also up to 40.000 IE/day). We now report on long term results over up to 4 years.

Patients and Methods: In 4 controls (C) and 22 pancreatic patients (n=5 chronic pancreatitis (CP), n=8 pancreatic cancer (PC) and n=9 after curative resective surgery (PR)) we measured the serum concentrations of 25(OH)D3 (ng/ml), 25 (OH)D2 (ng/ml), 3-c-epi25(OH)D3 (ng/ml), 24,25(OH)2D3 (ng/ml), 1.25 DHCC (ng/ml), as well as serum calcium, PO4, alkaline phosphatase (AP), creatinin, plasma PTH and urine crosslinks. The median vit. D supplementation was 40.000 IU/ week (7.000 – 140.000 IU/week) in pancreatic patients.

Results: Serum 25(OH)D3 increased significantly in all pancreatic patients and controls resp. into the normal range during oral vit. D supplementation. Normalization was measured even in all patients with total pancreatectomy in their history. The vit. D metabolites showed an expected behaviour like a significant increase of 1.25 DHCC within the normal range. Serum calcium showed a slight increase within the normal range, 24 h calcium in the urine remained in the normal range, serum creatinin and serum phosphor remained unchanged, plasma PTH and urinary crosslinks showed a decrease of the medians, in some cases also normalization of initially significantly elevated levels. Median serum AP only showed a trend to slightly decreased levels (n.s.) although, in some patiens, a significant decrease was observed, partly combined with significant decreases of plasma PTH.

Conclusion: Our oral vitamin D supplementation was well tolerated without side effects. Only one patient mentioned slight gastrointestinal symptoms at the beginning of the supplementation period. After dose adaptation the supplementation dose remained stable over years. These results emphasize to look in these patients for parameters of vitamin D metabolism in order to avoid skeletal problems by adequate vitamin D supplementation. These data, however, might also be of interest with respect to recent discussions focussin to potential relevance of vitamin D metabolsim for cancer prevention, immunologic diseases, cardial function or treatment of diabetes mellitus.

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DETERMINATION OF INTERLEUKIN 4, 5, 8 AND 13 IN SERA OF PATIENTS WITH BREAST CANCER BEFORE TREATMENT AND ITS INFLUENCE ON CIRCULATING TUMOUR CELLS

A. König[1], B. Rack[1], T. Vilsmaier[1], K. Friese[1], W. Janni[2], U. Jeschke[1], T. Weissenbacher[1], SUCCESS Study Group

[1]Department of Gynaecology and Obstetrics, Klinikum Ludwig-Maximilians-Universität, Munich, Germany; [2]Department of Gynaecology and Obstetrics, University Hospital Ulm, Ulm, Germany

Background: Circulating tumor cells (CTCs) in women with breast cancer are an indication for the prognosis for reduced disease free survival (DFS), distant DFS, breast cancer-specific survival and overall survival (OS) before starting of systemic treatment. Using the Cell Search System (Veridex, Raritan, NJ) to detect CTCs is a useful but also an expensive and time consuming method. So the intention of this study was the evaluation of cytokine profiles as marker for CTC-involvement.

Methods: All analysed sera were obtained from women with breast cancer who participated in the phase I SUCCESS study. The analysis of CTCs, the time of blood sampling and the methodology were prospectively designed. The definition of the prognostic value of the CTCs was a scientific objective of the study protocol. There were two groups of patients: 100 women with a positive result for circulating tumor cells and 100 women being negative for circulating tumor cells. These groups were matched into pairs of two. The criteria to match the patients were histo-pathological grading, lymph node status, hormone receptor type, and TNM classification and survived breast cancer patients *versus* deceased tumor associated patients. A recently developed multi cytokine/chemokine array (Meso Scale Discovery®, Rockville, USA) was used to screen the serafor the TH2 cytokines: IL-4, IL-5, IL-8 and IL-13. The analysis of the correlation results from using the Spearman correlation coefficient and the Mann-Whitney-U-Test.

Results: By comparison of the patients being CTC positive and negative, there was no result in the group of the patients with detected CTCs. In patients being CTC negative, secretion of Interleukin 8 and Interleukin 13 was increased (IL-8: p=0.017; IL-13: p=0.045) but only if the progesterone receptor was negative. For further analysis, patients with tumor associated death were compared to patients still alive. A correlation between hormone receptor negativity and increasing of Interleukin-4 was found (IL-4 in case of progesterone receptor negative: p=0.015; IL-4 in case of estrogen receptor negative: p=0.045). IL-5 levels indicated a significant correlation regarding patients with lymph node involvement and patients without positive lymph nodes. IL-5 was increased in patients with positive lymph nodes and positive Her-2-receptor status (IL-5, positive lymph nodes and Her2-positive: p=0.042). Moreover Interleukin-4 was increased in patients with positive progesterone receptor status and negative estrogen receptor status (IL-4: p=0.024).

Conclusion: TH2-cytokines are significantly modified in patients being CTC negative and progesterone receptor expression. Interleukin4 is increased in patients with negative hormone receptor status. We suppose that increasing of Interleukin4 depends on hormone receptor status. In literature a correlation between IL-4 and apoptosis resistance is described. So we can suspect that increasing of IL-4 is responsible for the aggressively in these cases.

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THYROID HORMONES AND VITAMIN D IN BREAST CANCER PATIENTS WITH MUTATIONS IN THE BRCA1 OR BRCA2 GENE

T. Kolben, T. Hary, L. Holdt, T.M. Schwarz, C. Goess, R. Wuerstlein, U. Jeschke, N. Harbeck, N. Ditsch

Klinik und Poliklinik für Gynäkologie und Geburtshilfe, Klinikum der Ludwig-Maximilians-Universistät, Großhadern, Munich, Germany

Purpose: The thyroid hormones free triiodothyronine (fT3) and free thyroxine (fT4) and thyroid stimulating hormone (TSH) which has a regulatory effect on the thyroid gland play an important role in the process of differentiation and proliferation of breast tissue. Current investigations showed an increased occurrence of hypothyroidism in BC patients. Increased expression of the Vitamin D receptor in BC tissue seems to be associated with increased overall survival in breast cancer (BC) patients. Instead, there are indications that point to a decreased level favoring the proliferation of tumor tissue. Up to now, this association of thyroid and Vitamin D blood levels has hardly been focused on BRCA1 or BRCA2 mutation carriers with BC. In former studies we could demonstrate correlations between the IHC expression of thyroid and Vitamin D receptors and BC prognosis. The purpose of this study was to evaluate the association of thyroid gland function and Vitamin D blood levels with the occurence of BC in patients with mutations in the BRCA1 and BRCA2 genes.

Patients and Data Collection: At the department of hereditary breast and ovarian cancer of the Ludwig-Maximilians-University Hospital of Munich 40 BC patients (10 patients with mutations in the BRCA1 gene, 10 with mutations in the BRCA2 gene, and 20 with sporadic BC as a comparison control) were selected for the analysis of the following lab values: fT3, fT4, TSH and Vitamin D. The primary diagnosis was made between 21 and 62 years of age. The patients of the control group were matched by age. Additionally, anamnestic data were evaluated in regard to disorders of the thyroid gland and primary BC diagnosis.

Results: Concerning the thyroid function no association could be demonstrated in our collective of BRCA mutation carriers neither in terms of hypo- nor hyperthyroidism. There was a tendency of increased values of Vitamin D in BRCA2 mutation carriers in comparison to sporadic BC patients (p=0.066). The grading of the tumors in the BRCA2 group (G1: 12.5%; G2: 75%; G3: 12.5%) had a lower grading than the tumors in the control group (G1: 12.5%; G2: 37.5%; G3: 50%). In the group of patients with BRCA1 mutations the incidence of the primary diagnosis of BC during pregnancy was elevated (30% *vs*. 0%) in comparison to the control group. The higher incidence of triple-negative breast cancer subtypes (60%) as well as high grade tumors (G3) (80%) in patients with BRCA1 mutations as it is known from larger collectives could be confirmed in our collective.

Conclusion: No association between the thyroid hormones and BC in BRCA mutation carriers could be found. There is no difference in comparison to patients with sporadic BC. A tendency of elevated Vitamin D in BRCA2 mutation carriers could be observed. Another finding was that the tumor grading in the BRCA2 group tended to be of lower differentiation grade than in the control group. This observation could be consistent with the ability of Vitamin D to inhibit growth and induce differentiation. In contrast, a deficiency of Vitamin D is correlated with an increased risk of BC.

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MOLECULAR SIGNATURES IN OVARIAN CANCER: THE NEXT BIG THING

S. Kommoss

Department für Frauengesundheit, Universitätsklinikum Tübingen, Germany

Ovarian carcinoma is composed of several histological types. 75% of patients are presenting with a high grade serous carcinoma, the other four major histological subtypes are low-grade serous, endometrioid, clear cell, and mucinous carcinomas. These five major histologic types of ovarian carcinoma differ with respect to genetic risk factors, precursor lesions, molecular alterations, stage at presentation, response to chemotherapy and clinical behavior and are best considered to be distinct disease entities. Regardless of these obvious differences between histologic types of ovarian carcinoma, the disease is still treated according to a “one size fits all” approach that has delayed the development of novel targeted therapies in ovarian cancer for so long. The Integration of novel type-specific therapies is still hampered by a poor understanding of the molecular heterogeneity of the disease and of the genes and/or pathways that are altered in these molecular subtypes. New and novel therapeutic approaches to human ovarian cancer will very likely be dependent on our understanding of the heterogeneity of ovarian cancer. With the insight into the molecular heterogeneity and genes or pathways altered in ovarian neoplasms growing, molecular testing becomes more and more relevant in these days. Molecular signatures in ovarian cancer may not only have the potential to act as a diagnostic aid but may furthermore be able to identify special subgroups of patients, which might benefit from new targeted treatments.

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MOLECULES OF THE EPIDERMAL GROWTH FACTOR (EGF) RECEPTOR FAMILY - DEAD END OR PROMISING BIOMARKER FOR OVARIAN CANCER THERAPY

C. Kurzeder

Department of Gynecology and Gyn. Oncology, University Essen, Essen, Germany

Ovarian cancer is the most lethal gynaecologic malignancy. Standard treatment consists of surgical debulking followed by platinum based chemotherapy. Various members of the EGFR family and their corresponding downstream signaling molecules have been found to be overexpressed or amplified in ovarian cancer patients. Since these pathways are crucial drivers of cancer proliferation the prognostic and predictive value of these molecules is in focus of current research and targeted interventions are currently being explored in clinical trials. Whereas preclinical models could demonstrate antitumor activity of monoclonal antibodies and tyrosine kinase inhibition targeting EGFR and HER2 signaling clinical trials in unselected cohorts of patients yielded disappointing results. Pertuzumab is a monoclonal antibody directed against Her2 and inhibits ligand activated heterodimerization with other receptors of the EGFR family. In a retrospectively defined subgroup of platinum resistant ovarian cancer patients according to HER3 mRNA expression level treatment with Pertuzumab improved PFS when added to gemcitabine chemotherapy [Makhija, 2010]. The 2-part PENELOPE trial (NCT01684878) is prospectively investigating pertuzumab therapy added to single-agent CT in this population.

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TUMOR THERAPY CONTROL BY CONVENTIONAL TUMOR MARKERS I

R. Lamerz

Institut/Klinik: 2nd Department of Internal Medicine, LMU-Klinikum-Campus Grosshadern, University of Munich, Germany

Specific Objective: The advent of targeted therapies supported by increasing molecular genetic and epigenetic investigations and increasing regular guidelines from international and national societies have reduced recommendations for conventional tumor markers. This was promoted by their limited evidence of prognostic and predictive relevance in contrast with their easy and relatively inexpensive request but needed reasonable interpretation. The objective was to look for recent publications concerning application of conventional tumor markers for three tumor entities of hepatocellular carcinoma (HCC), testicular germ cell tumors (GCT) and multiple myeloma (MM) as supportive tools in comparison with own experiences in the follow-up of treated patients.

Methods: Besides the observation of actual guidelines for the tumor entities some relevant international or national publications concerning relevant tumor marker applications were concerned and some few selected own tumor marker follow-up cases presented using own AFP/hCG radio-immuno-assays applied for up to 25 years under former German quality control protocols besides commercial AFP-L3 and DCP assays and besides FLC- and standard laboratory determinations of the central clinical laboratory. Summary of

Results: In HCC there are different opinions concerning TM inclusion in screening *versus* AASLD and EASL GL, newer preliminary assay combination data including prognosis, prediction and treatment control according to own clinically helpful follow-up experiences. In GCT, the value of AFP/hCG and LDH determinations is since long accepted evidence for inclusion in staging, prognosis and monitoring of therapy control and recurrence (IGCCCG, IGCCCG-2, TM un/favourable decline), whereas in MM initial and follow-up serum electrophoresis/immunofixation, (un)involved serum-Img and FLC determination and the international staging system (ß2m, albumin) are relevant controls besides pre-therapeutic bone marrow investigation, genetic classification (FISH) and imaging.

Conclusion: The use of selected tumor markers in follow-up of patients during and after therapy including palliation may be of value in combination with patients' regular necessary laboratory parameters, history and physical examination and hint at therapy response (ideally in continuous graphical follow-up presentation), but any critical treatment change decision should always depend on guideline-related imaging confirmation.

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TUMOR THERAPY CONTROL BY CONVENTIONAL TUMOR MARKERS II

R. Lamerz

2nd Department of Internal Medicine, LMU-Klinikum-Campus Grosshadern, University of Munich, Germany

Specific Objective: The advent of targeted therapies supported by increasing molecular genetic and epigenetic investigations and increasing regular guidelines from

international and national societies have reduced recommendations for conventional tumor markers. This was promoted by their limited evidence of prognostic and predictive relevance in contrast with their easy and relatively inexpensive request but needed reasonable interpretation. The objective was to look for recent publications concerning application of conventional tumor markers for two tumor entities of colorectal carcinoma (CRC) and pancreatic ductal adenocarcinoma (PDAC) as supportive tools in comparison with own experience in the follow-up of treated patients.

Methods: Besides the observation of actual guidelines for the tumor entities some international or national publications concerning relevant tumor marker applications were concerned and some few selected own tumor marker follow-up cases presented using commercial CEA or CA 19-9 immuno-assays of the central clinical laboratory. Summary of

Results: In CRC, CEA is useless for screening, but of value in prognosis and prediction, postoperative survival in stage II and III and monitoring in active disease (NACB GL 2008, EGTM GL2014) and in after-care stage II/III (S3 GL 2013) with predictive probability of recurrence in stage II< III and correlation with radiologic imaging (CEA, CA 19-9). In PDAC, CA 19-9 is not recommended for screening, not alone for operability/resectability evaluation and monitoring response to therapy. It is of value at start of therapy in locally advanced metastatic disease and 1-3 monthly during active treatment, but not alone for definition of disease recurrence, furthermore of prognosis for risk stratification (ASCO 2006, EGTM 2010); according to others, it is of benefit for postoperative prognosis and benefit prediction of adjuvant therapy.

Conclusion: The use of CEA in CRC and of CA 19-9 in PDAC in follow-up of patients during and after therapy including palliation may be of value in combination with patients' regular necessary laboratory parameters, history and physical examination and hint at therapy response (ideally in continuous graphical follow-up presentation), but any critical treatment change decision should always depend on guideline-related imaging confirmation.

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VIROTHERAPY OF PANCREATIC CANCER - CURRENT STATE AND FUTURE TRENDS

U.M. Lauer

University Hospital Tübingen, Department Internal Medicine I (Gastroenterology, Hepatology, Infectious Diseases), Tübingen, Germany

Pancreatic cancer constitutes a highly significant clinical problem. Accordingly, novel therapeutic approaches are desperately needed. Oncolytic virotherapy employs live viruses with selective tropism for cancer cells (so-called oncolytic viruses - OVs) as a novel anti-cancer therapeutic approach for many types of cancers, including pancreatic cancer. In addition, many of these cancer-selective OVs have the potential to be combined with currently available therapies resulting in increased therapeutic benefits compared to single agent therapies. No cross-resistances are observed so far. Recent preclinical data point out that at least some OVs employed in combinatorial chemovirotherapic approaches do not undergo a reduction of their respective replicative viral capacities, do not deteriorate chemotherapy-induced biological effects such as therapy-induced senescence (TIS) nor require an intact apoptosis pathway for the execution of oncolytic cell death. These are important prerequisites in overcoming chemoresistance in pancreatic cancer cells *via* induction of a massive oncolytic tumor cell death causing the concurrent release of both tumor and viral antigens which then induces a profound host immune response against the tumor cells. Preclinical results point out that induction of ER stress could also be an important component of oncolytic cell death and that addition of stimuli of this stress response significantly could augment OV activities. Furthermore, the recombinant nature of OVs enables the construction and validation of infectivity-enhanced, armed (*e.g.*, with suicide genes), targeted and thereby oncolysis-enhanced and even more efficient tumor-specific virus constructs. Especially OVs being targeted to disrupt tumors undergoing epithelial to mesenchymal transition (EMT) may help to reduce the population of pancreatic cancer stem cells that are contributing to treatment resistance and tumor metastasis. However, the microenvironment of pancreatic cancer makes it difficult for any systemic therapies, including oncolytic virotherapy, to access cancer cells. Breaching the stromal barrier is a prerequisite to improve the delivery and efficacy of OVs. Significant therapeutic benefit might be obtained by using strategies that aim to deplete the desmoplastic stroma. These approaches have to be combined with OV delivery to the pancreatic tumor sites, either by direct intra-tumoral injection or intra-peritoneal, intra-arterial and intra-venous administration. Early clinical trials (phase I/II) with OVs have shown that oncolytic virotherapy is both a safe and feasible option for pancreatic cancer treatment in humans. However, there is still a great deal of work for optimizing OVs in the treatment of pancreatic cancer.

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HYPERTHERMIA IN TARGETED TUMOUR THERAPY USING MAGNETIC NANOPARTICLES - FIRST RESULTS

S. Lyer[1], C. Janko[1], R. Tietze[1], J. Zaloga[1], W. Schmidt[2,3], C. Alexiou[1], S. Dürr[1]

[1]Department of Otorhinolaryngology, Head and Neck Surgery, Section for Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftungsprofessorship, University Hospital Erlangen, Germany;
[2]Department of Phoniatrics and Pediatric Audiology, University Hospital Erlangen, Germany;
[3]Siemens CT, Germany

Introduction: Personalized tumor therapy is offering various possibilities for an improved cancer treatment. Common to all different approaches of patient specific tumor treatment is the ambitious aim of improving the efficiency and reducing the side effects of cancer therapy. Nanoparticles are also offering various possibilities for an improved cancer treatment and can serve as a platform for targeted therapy including ferrofluid based hyperthermia. Recently, it could be shown, that Abraxane® a nanoparticulate formulation approved for the treatment of pancreatic cancer in combination with Gemcitabine functionalized with two peptides can be, significantly targeted to tumors. It could be also shown, that magnetic nanoparticles could be accumulated in tumors with high efficiency with magnetic fields by Magnetic Drug Targeting (MDT) or when functionalized with the luteinising hormone-releasing hormone. Since it is known for some chemotherapeutics, that their toxic effect can be enforced by hyperthermia, it was the aim of this study to lay a basis for the combination of targeted chemotherapy and hyperthermia.

Materials and Methods: Cell culture: Two different cell lines were used: VX-2, a rabbit squamous cell carcinoma and SCC-15, a human squamous cell carcinoma of the tongue. Using Real-time cell analysis (RTCA) and conventional WST-1 assay, we examined the effect of magnetic iron oxide nanoparticles (MIONs) and MIONs loaded with the chemotherapeutic agent mitoxantrone. Additionally, a magnetic field was applied (0.4 Tesla, 5 minutes) to simulate Magnetic Drug Targeting in cell culture. Finally, we conducted the experiments under hyperthermic conditions (45˚C) in an incubator. Animal experiments: VX-2 rabbit tumors were implanted subcutaneously at the left hind limb of three female New Zealand rabbits. For inducing hyperthermia in the tumors, ca. 1ml MIONs (SEONLA) was injected intratumorally. After waiting for 30 min the hind limbs of the animals were placed into a newly designed alternating magnetic field system and temperature was measured intratumorally for 90 to 100 minutes. *Results:* The concentration of SPION and therefore of the chemotherapeutic agent in the cells was increased by the magnetic field, which accordingly caused higher toxicity. The application of hyperthermia additively raised the toxic effect. The animal experiments showed, that with the utilized combination of iron oxide particles and alternating magnetic field system the intratumoral temperature could be increased by 4.7˚C to 6.7˚C in three tumor bearing rabbits after intratumoral application of magnetic iron oxide nanoparticles.

Conclusion: The cell culture experiments show, that the effect of Magnetic Drug Targeting can be enhanced by local hyperthermia, as it is already known from *e.g.* radiotherapy. The animal experiments suggest that with the new developed device, which can be use in mice, rats and rabbits, it is possible to heat up tumors after intratumoral application. For reaching higher temperatures or the same temperatures with lower iron concentrations in the tumor, the magnetic field between the two pancake coils should be chosen higher, than that used for the present work. In further experiments we aim at investigating, if it is possible to achieve similar intratumoral temperatures after using MDT for accumulating nanoparticles in the tumors.

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SYSTEMATIC FOLLOW-UP AND THERAPY MONITORING IN GYNECOLOGICAL MALIGNANCIES - IS IT REALLY USEFUL?

S. Mahner

University Medical Center Hamburg-Eppendorf, Department of Gynecology, Hamburg, Germany

At first sight it seems self-evident to closely follow-up on patients with malignant disease and high risk of recurrence. Early detection of recurrence can lead to secondary surgical resection, initiation of second line systemic treatment and a follow-up examination without evidence of disease is reassuring both, the patient and the physician. Nevertheless, routine follow-up can have major impact on patients' quality of life, *e.g.* in a situation of an asymptomatic patient and rising tumor marker with no evidence of disease. Does this justify initiation of second line treatment? Is serologic follow-up always better than radiologic? And what is the ideal follow-up interval. Most of these question remain unsolved, despite significant advances in both, tumor markers and radiologic techniques. Therefore, current guidelines for diagnosis and treatment of gynecologic malignancies do not recommend regular systematic tumor marker follow-up. At the same time, in clinical routine, this aspect is probably the single most violated guideline recommendation.

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DO WE REALLY NEED SCREENING IN GYNECOLOGY?

S. Mahner

University Medical Center Hamburg-Eppendorf, Department of Gynecology, Hamburg, Germany

Screening for gynecologic malignancies is one of the biggest success stories of the past century. Pap-smears to screen for cervical dysplasia as well as mammography screening are well established screening methods in many countries. In contrast – screening for endometrial or ovarian cancer could not be established yet. Also, with the introduction of HPV testing, debates are ongoing whether this could replace traditional PAP test. Since screening inevitably also leads to false positive finding and improvement of mortality through screening is often difficult to detect, even with modern radiologic and molecular screening methods, the question to screen or not to screen for gynecologic malignancies remains open.Based on current evidence, national and international guidelines cannot recommend universal screening for all gynecologic malignancies. Future screening studies therefore need to focus on differential risk assessments and cost-efficiency analyses.

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PROTON RADIATION TREATMENT FOR PANCREATIC CANCER: WHAT CAN WE EXPECT?

O. Micke[1], A. Haidenberger[2]

[1]Department of Radiotherapy and Radiation Oncology, Franziskus Hospital, Bielefeld, Germany;
[2]Department of Radiotherapy, Agatharied Hospital, Hausham, Germany

Purpose: Radiotherapy, primarily in combination with chemotherapy, has been established as a standard treatment in locally advanced exocrine pancreatic carcinoma. Still, the majority of patients continue to succumb to the disease process. In general, the appliced radiotherapy dose is limited by the surrounding organs of risk, *i.e.* small bowel, kidneys and spinal cord. Proton beam therapy (PBT) offers the possibility by its unique physical properties to deliver high doses to the target volume with a maximal sparing of normal disease. This may offer a new opportunity to improve local control and overall survival in locally advanced pancreatic cancer. Admittedly, clinical experience on PBT in pancreatic cancer is still very limited.

Methods: In a pilot study at the Rinecker Proton Therapy Center in Munich patients with locally advanced pancreatic cancer treated with spot scanning proton therapy were evaluated. As comparison, data from 152 patients with locally advanced pancreatic adenocarcinoma treated with conventional 3D-conformal radiotherapy at the Münster University Hospital and the Franziskus Hospital Bielefeld were used. This patient collective received a combined radiochemotherapy, consisting of hyperfractionated accelerated conformal radiotherapy with a total dose of 44.8 Gy that was applied in ten fractions per week of 1.6 Gy and of a concomitant chemotherapy with 5-fluorouracil or gemcitabine.

Results: From July 2010 through January 2013, 49 patients with inoperable pancreatic cancer were treated with hypofractionated spot scanning proton beam therapy (PBT). Treatment consisted of 54 Gy (RBE) in 18 fractions. 12 patients (25%) had metastatic disease in the liver or lung and a total dose of 40 Gy (RBE) in 10 fractions was applied to the pancreas and an individual stereotactic schedule was applied to the metastases in apnea. 29 patients (59%) received gemcitabine 300 mg/m^2 weekly concomitant during PBT. Overall, PBT was feasible and safe. However, the mean follow-up was limited for only 7.5 months (range 3-28). During follow-up, there were no grade 3 or 4 toxicities. RTOG grade 2 and 1 toxicity was observed in 14 and 5 patients, respectively. Local control rates achieved were excellent with 100% after 6 months and 88% after 12 months. In all patients a tumor response in terms of tumor volume reduction was verified through MRI and PET/CT scans. The crude survival rate after 12 months was 50%. The local control rates were considerably superior compared to the Münster/Bielefeld collective. Still, the overall survival rates after 12 months are similar. Notably, the comparison of both collectives is difficult due to the different sample size and length of follow-up period. Hence, besides these promising results of PBT it must be awaited whether the excellent local control rates are related to better overall survival rates or secondary resections.

Conclusion: The use of PBT for locally advanced pancreatic cancer is feasible and safe. The results are promising concerning excellent local tumor control. The number of treated patients is still limited and further investigation is required. In particular, a comparison with more advanced photon therapy techniques like intensity modulated arc therapies is needed.

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WHY DID I GET CANCER? CASUAL ATTRIBUTION AMONG CANCER PATIENTS

O. Micke[1], R. Mücke[2], J. Büntzel[3]

[1]Department of Radiotherapy and Radiation Oncology, Franziskus Hospital, Bielefeld, Germany;

[2]Department of Radiotherapy, Lippe Hospital, Lemgo, Germany;
[3]Department of Otolaryngology, Südharz Hospital, Nordhausen, Germany

Purpose: Patients with cancer often have their personal theories about what may have caused their disease. Coping response to disease and treatment is directed by such cognitive representations of cancer. These common-sense beliefs of patients have indeed proven useful in predicting both behavioural and psychological responses to their disease. They have predicted decisions to seek health care and compliance with medical interventions or the adherence to complementary and alternative medicine (CAM). Therefore, understanding more about which cancer patients identify causal attributions for their disease could be also relevant to the success of health promotion interventions. However, the existing studies on this important topic are inconsistent and have been limited by small samples sizes. Therefore, the current study was carried out, to achieve more data in this field.

Methods: A total of 200 patients from two different centres with different tumor diagnoses in curative or palliative setting were evaluated by standardized semi-structured interviews. Patients were asked their personal causal attributions and the possible resulting changes of lifestyle or values. In addition, tumor and disease parameters were documented. There were predominantly patients with breast cancer, with head and neck cancer and with prostate cancer. 60 % of patients were treated.

Results: Overall, interviewed patients had a mean number of 1.87 of causal attributions (range; 1-4). The most cited causal attributions (more than one answer possible) were tobacco (n=89), alcohol (n=61), stress (n=58), lack of immune system (n=43), diet (n=35) genetics (n =27), cancer history (n=26), environment (n=23), sun (n=7), medication/hormonal substances (n=6), lack of preventive care (n=4), viral or other infections (n=3), radiation (n=0), a punishment from God or other higher being (n=0) and unknown (n=33). Most patients (n=31; 65.5%) want to change their habits (more than one answer possible): Primarily to quit smoking (n=69) and drinking (n=35), reduce stress (n=58), enhance immune system (n=57), taking vitamins/micronutrients (n=53), more sportive activities (n=46), allocation to complementary and alternative medicine (CAM) (n=42), allocation to spirituality/religiosity (n=34), and not specified (n=55). Categorizing causal attributions of patients in "blame" (n=88) or "no blame" (n=98) showed that blame was associated with higher fears of recurrence in patients. There was a correlation between self-blame and perceived control over future health and a higher medical caregiver burden.

Conclusion: The majority of cancer survivors reported specific causal attributions and many had contemplated about the "why me". Understanding and assessing causal attributions could aid in the understanding of survivor's adjustment and psychosocial well-being. The personal beliefs of cancer patients are associated with compliance with recommended health practices, reprioritization of goals, changed lifestyle and values, and spiritual/religious development as well as with the allocation to complementary and alternative medicine (CAM).

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A PANEL OF SIX SERUM BIOMARKERS IS USEFUL AS HELP FOR THE DIAGNOSIS OF LUNG CANCER

R. Molina, R.M. Marrades, J.M. Augé, J.M. Escudero, X. Filella

Hospital Clinic, IDIBAPS, Medical School, University of Barcelona, Spain

Background: Lung cancer (LC) is the most frequent and fatal human cancer. Its prognosis is directly related to early diagnosis. In this study, we hypothesized that a combined panel of six serum tumor markers (TM) is useful in the diagnosis of patients with clinical suspicion of LC.

Methods: We investigated the diagnostic utility of a combined panel of six serum TM (CEA, CA125, SCC, CYFRA 21-1, NSE and ProGRP) in 3,144 consecutive individuals referred to our institution because of the clinical suspicion of LC.

Results: Using standard clinical procedures, LC was confirmed in 1,828 patients (58.2%; 1,563 with NSCLC (85.5%) and 265 with SCLC (14.5%)) and excluded in 1,316 individuals (41.8%)). The sensitivity of the TM panel investigated here for the diagnosis of LC was 89%, its specificity 82%, its NPV 84% and its PPV 87%. In patients with early stages of LC these figures were, respectively, 83%, 82%, 94% and 57% for NSLC stage I/II and 94%, 82%, 99% and 29%for intra-thoracic SCLC. Finally, two TM (NSE and ProGRP) significantly differentiate NSCLC from SCLC (AUC 0.894 and 0.861, respectively).

Conclusion: This study is the first to show that a panel of six serum TM can be useful in the clinical management of LC, either as a first step in the screening of high risk populations (its high sensitivity and NPV can help to exclude LC) and/or the diagnostic assessment of patients with a clinical suspicion of LC (high specificity, high PPV), even in early stages of the disease, plus a significant capacity to differentiate NSCLC and SCLS, which may require different therapeutic strategies.

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HE-4 IN OVARIAN CANCER THERAPY MONITORING: COMPARISON WITH CA 125

R. Molina, J.M. Escudero, J.M. Auge, X. Filella, P. Fuste

Laboratory of Biochemistry, Gynecology department, Hospital Clinic, Barcelona, Spain

Background: The main clinical utility of tumor markers are follow-up and therapy monitoring. CA 125 is the tumor marker of choice in ovarian cancer, but another new tumor marker, HE-4 has been reported recently. The advantages to use several tumor markers in the follow-up have been only scarcely studied.

Objective: to compare the clinical utility of CA 125 and HE-4 in therapy monitoring of patients with ovarian cancer.

Methods: Serial CA125 and HE4 serum levels were determined in 119 patients treated for ovarian cancer during a period of two years. All patients had a minimum of 4 determinations during a period of at least 6 months. Therapy responses were calculated using the criteria of the EORTC. Treatment was chemotherapy, using different chemotherapy regiments. CA 125 and HE4 cut-offs were 35 U/mL and 120 pmol/L, respectively. We considered as response when pretherapy tumor marker serum levels decrease to at least 50% in two serial determinations during at least 3 weeks. Similar criteria were used to indicate progression: increase of at least 50% in two serial determinations.

Results: CA 125 was correlated with tumor response in 83%, HE-4 in 85% and any of them in 92% of our patients. Similar results were found when mucinous tumors were excluded: any of the tumor markers was useful in 96% of our patients. It is interesting to indicate that CA 125 was better than HE-4 (higher concentrations, previous changes, *etc*.) in 33% of our patients, HE-4 better than CA 125 in 29% of patients and similar in the remaining 30% of patients with ovarian cancer. However, it is interesting to notice that changes in the predominant tumor marker appeared in 25% of patients during therapy.

Conclusion: Both tumor markers may be used in the follow-up of patients with ovarian cancer during therapy. The reason of changes in the tumor marker pattern could be further evaluated.

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CIRCULATING TUMOR CELLS AND CIRCULATING NUCLEIC ACIDS AS PROGNOSTIC AND PREDICTIVE FACTOR IN BREAST CANCER PATIENTS

V. Müller

Department of Gynaecology, University Medical Center Hamburg Eppendorf, Hamburg, Germany

Tumor cell dissemination by hematogenus spread is a key process in the metastatic cascade of breast cancer. Evidence has emerged that the detection of circulating tumor cells (CTC) in blood can provide important prognostic information and might help to monitor efficacy of therapy. The prognostic value of CTC detection in early and metastatic breast cancer has been demonstrated in several large patient cohorts. Moreover, several prospective studies were launched to examine if CTC enumeration and/or characterization can help to improve the management of breast cancer patients: STIC CTC Metabreast (France) and Endocrine Therapy Index (USA) assess the CTC-guided endocrine therapy *vs*. chemotherapy decision in metastatic breast cancer patients; SWOG0500 (USA) and CirCe01 (France) assess the CTC count changes during treatment in metastatic patients; DETECT III (M1 patients, Germany) and Treat CTC (cM0(i+)(European Organization for Research and Treatment of Cancer/Breast International Group) assess the use of anti HER2 treatments in HER2-negative breast cancer patients selected on the basis of CTC detection/ characterization. The DETECT studies are prospective, multicenter, open-label clinical trials designed for patients with HER2-negative MBC and evidence of CTC in the peripheral blood. Overall, about 2000 patients with HER2-negative MBC will be screened for CTC to be able to recruit 120 patients for DETECT III (start: February 2012), 400 patients for DETECT IV- everolimus cohort (start: December 2013) and 120 patients for DETECT IV-eribulin cohort (start: second half of 2014). DETECT III is a two-arm study for patients with HER2-positive CTCs, randomized to treatment of physician's choice therapy (chemotherapy or endocrine treatment) with or without additional HER2-targeted treatment therapy with lapatinib. DETECT IV combines tow single-arm studies aimed at patients with HER2-negative CTCs. Postmenopausal patients with hormone-receptor-positive MBC will be treated with the mTOR-inhibitor everolimus in combination with an endocrine therapy of physician's choice (everolimus cohort), whereas patients with triple-negative or hormone-receptor-positive MBC and indication to chemotherapy will receive eribulin (eribulin cohort). In addition to CTC, DNA, mRNA and microRNA are released and circulate in the blood of cancer patients. More recently, several studies were published indicating the potential use of circulating nucleic acids for cancer screening, prognosis and monitoring of the efficacy of anticancer therapies.

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IMMUNOCYTOCHEMICAL CHARACTERIZATION OF DISSEMINATED TUMOR CELLS FROM BONE MARROW OF BREAST CANCER PATIENTS

J.N. Mumm, A. Vogl, A.C. Kölbl, K. Friese, U. Jeschke, U. Andergassen

Department of Obstetrics and Gynecology, Ludwig-Maximilians University of Munich Munich, Germany

Background: The occurrence of circulating tumor cells (CTCs) in the blood and disseminated tumor cells (DTCs) in the bone marrow of patients with epithelial breast cancer is mostly linked with a worse prognosis for overall survival (OAS). But detection and characterization of those cells is still a technical challenge, as the number of tumor cells is rather small in respect to the surrounding blood or bone marrow cells. Here we present a method for DTC detection from bone marrow samples based on immunocytochemistry, using breast cancer associated glycosylation molecules as marker for detection and characterization. This approach emanates from the fact, that altered glycosylation is a main feature of tumor cells and that such modifications of carbohydrate chains on the cell surface could help to distinguish tumor cells from normal cells.

Methods: The combinations of marker epitopes was first applied to blood samples of healthy donors, to which tumor cells of breast cancer cell lines were added. A pan-cytokeratin (CK) marker antibody was used to distinguish tumor cells from normal blood cells, as CK is a widely published marker for epithelial cells. For further characterization of tumor cells either Tn or O-Acetyl-GD3 were used in an immunofluorescence double staining. These marker combinations were then applied to 27 bone marrow samples of breast cancer patients, which was withdrawn during tumor surgery.

Results: The sample in which most cells stained positive for CK/Tn and CK/O-AC-GD3, was from a patient who certainly had remote metastases. The two samples of triple negative breast cancer patients surprisingly only yielded a few positively stained cells, an astonishing fact regarding the aggressive tumor biology of this breast cancer subtype.

Conclusion: These really preliminary result shows, that a certain characterization of tumor cells can be achieved by a double staining of bone marrow samples with CK and a glycosylation marker. These results should now be extended to a larger patient collective, and further glycosylation markers could be included in the analysis. The simultaneous analysis of the samples by a gold standard method would continue to refine the whole methodology to make it applicable to clinical use in diagnosis and prognosis.

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ENDOMETRIAL ADENOCARCINOMA: DETECTION OF CIRCULATING TUMOUR CELLS BY RT-QPCR

J.N. Mumm, R. Wellens, A.C. Kölbl, K. Friese, U. Jeschke, U. Andergassen

Department of Obstetrics and Gynecology, Ludwig-Maximilians University of Munich Maistrasse 11, 80337 Munich, Germany

Background: Endometrial Adenocarcinoma is a frequently occurring cancer entity in women. About 42.000 women die of this disease or its consequences every year. Up to now, patients are still treated with standard therapy, including surgery and irradiation and, if necessary chemotherapy. Nevertheless, the occurrence of remote metastases and local recurrences is very high. By the help of RT-qPCR minimal residual diseases (MRD), which are considered to be the origin of disease recurrence, can be detected and characterized, facilitating therapeutic decision making.

Methods: To save precious patient material, a number of marker genes were tested on blood samples of healthy donors, to which cells of endometrial carcinoma cell lines HEC-1A or RL-95-2 were added. From these spiked blood samples, leucocyte cell fraction, which also contains tumor cells, was enriched by density gradient centrifugation, RNA was isolated from the harvested cells and reverse transcribed to cDNA. This cDNA was then used for Real-Time PCR analysis of a set of potential marker genes. Those genes, which performed best, were then used for the examination of 13 blood samples from endometrial carcinoma patients.

Results: Cytokeratin 19 (CK19) and MIG7 were the two marker genes, which gave the best results in the artificial tumor cell detection system, so they were chosen for the analysis of patient samples. An upregulation of the expression of these two genes could be seen especially in small tumors, which are in an initial state of tumor formation and in one large tumor, which already showed strong dedifferentiation. On the other hand, no statistical correlations could be revealed between expression levels of these two genes and tumor characteristics like size, histology and grading.

Conclusion: Here we show, that there seems to be a coherence between gene expression of these two genes and the stage of tumorigenesis, but the number of sampling is still too small, to be able to obtain statistical significant differences. A higher number of patient samples and additionally more marker genes could help to get over this obstacle. A simultaneous analysis of the samples by gold standard methods for MRD-detection could be of great use.

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NEW MARKER GENES FOR REAL-TIME PCR BASED DETECTION OF CIRCULATING TUMOUR CELLS FROM BLOOD OF BREAST CANCER PATIENTS

J.N. Mumm, D. Bräuer, A.C. Kölbl, K. Friese, U. Jeschke, U. Andergassen

Department of Obstetrics and Gynecology, Ludwig-Maximilians University of Munich Maistrasse 11, 80337 Munich, Germany

Background: Circulating tumor cells (CTCs) can be detected by Real-Time PCR approaches, using the different gene expression of mesenchymal blood cells and epithelial tumor cells. Up to now, mostly Cytokeratin genes had been used for this PCR based detection, but it became clear, that a set of marker genes would refine detection and simultaneously open ways for a characterisation of such tumor cells. Here we present some new marker genes, which could improve CTC detection.

Methods: Blood samples of healthy donors were spiked with different numbers of Cama-1 breast cancer cell line cells and the increase in gene expression was measured by RT-qPCR. The genes which had the best performance in the artificial system were then used on blood samples of 20 adjuvant breast cancer patients. Gene expression was normalized to control samples from healthy donors. Gene expression levels between breast cancer patients and healthy individuals were compared and related to different tumor characteristics.

Results: In the artificial system Bcl2, ER, PR, CatL2, Her-2 and Ki67 were analysed for their performance in tumor cell detection. The best results were obtained for CatL2, ER, Ki67 and Her-2, so they were chosen for application in patient samples. The mean of gene expression levels was elevated for all four genes in the adjuvant breast cancer patient group in comparison to the samples of the group of healthy donors, but statistically no correlation between gene expression and tumor characteristics could be detected.

Conclusion: The results show, that CatL2, ER, Ki67 and Her-2 can be useful marker genes for CTC detection from blood samples of breast cancer patients by RT-qPCR, but the method needs to be refined. A good starting point could be a simultaneous analysis of some samples by RT-qPCR and a gold standard method like the CellSearch® system. Thus gene expression values could be more stringently related to the appearance of CTCs. Furthermore the genes would have to be tested on a greater number of patient samples and the addition of some more markers could help to improve CTC-detection, leading to new strategies in tumor cell characterisation.

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RADIOCHEMICAL EFFECTS ON F-18 FDG-UPTAKE, HEXOKINASE- AND MITOCHONDRIAL ACTIVITY IN RADIOSENSITIVE CELLS COMPARED TO THEIR WILD-TYPE

P. Oehr, N. Gilbert

University Bonn, Germany

Subject of our investigation was to find out whether and how radio- and/or chemotherapy might change the cell metabolism and glucose uptake, at hand of F-18 FDG PET-imaging as a tumor marker, and determination of the metabolic activities in radiosensitive (repair defective) cells compared to non-sensitive cells in tissue cultures. Hexokinase - and mitochondrial activities were selected in comparison to F-18-FDG-uptake, because they were supposed to be related to glucose uptake in cells.

Results: 1. Cellular stress induced a time- and dose dependent increased hexokinase- and mitochondrial activity and led to augmentation of protein concentrations in the cells, as well as increased F-18 FDG-uptake, while cell numbers decreased. 2. There was an increase in cell volume after cell irradiation, which seems to be in consequence to higher protein contents caused by induced protein-, DNA/RNA- and ATP- synthesis during coincidentally inhibited cell division. 3. Since the F-18 FDG-uptake correlated with increase of the radiation dose during the first 72 hours, it is concluded that this is according to the metabolic cellular reactions, not being related to the cellular damage. As a consequence the effectiveness of radiation according to damage of cells cannot immediately recorded (*e.g.* by decreased F-18 FDG-uptake). 4. There was a simultaneous increase of hexokinase- and mitochondrial activity during the first 48 hours, which might suggest a coupling of these reactions, in order to induce an increased glucose uptake in the cells for energy production (*e.g.* for repair). 5. Inhibition of cell growth and enhancement of metabolic activity were much lower when chemotherapy was applied, and an increase of cell volume could not be observed. It is assumed that in this case repair is not main subject of the cell, and hexokinase was used from the pool of the cell. 6. In case of simultaneous irradiation and chemotherapy, the increase of metabolic activity seems to be induced by irradiation. However, simultaneously applied chemotherapy could reduce the kinetics of metabolic increase and stimulation of F-18 FDG-uptake. 7. There was a significant difference in induction of f-18 FDG-uptake and the level of metabolic changes with respect ot the genotype of the cells. The repair defective cells which are radiation sensitive showed a higher increase of uptake and metabolic changes.

Conclusion: The tissue culture cell approach including PET (cc-PET) can give insight into changes and mechanisms responsible for the resulting changes of F-18 FDG-uptake in case of chemo- and/or radiotherapy, including the differences depending on differences in genetic disposition responsible for radiosensitivity. Regarding F-18-FDG as a tumor marker in patients, the reported results are the base to interprete the cell kinetics of glucose uptake in patients under radio- and/or chemotherapy.

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PROGNOSTIC VALUE OF BONE MARKER BETA-CROSSLAPS IN PATIENTS WITH BREAST CARCINOMA

N. Zulauf[1], I. Marzi[2], G.M. Oremek[1]

Departments of [1]Laboratory Medicine, [2]Internal Medicine, Hospital of the Johann Wolfgang Goethe University, Frankfurt/Main, Germany

Background: We investigated the usefulness of bone markers, Alkaline Phosphatase (AP) and Beta-Crosslaps (β-CTx) for diagnosis, treatment and monitoring of patients with breast carcinoma. AP is a marker of bone formation, while β-CTx is a marker of bone resorption. β-CTx is expressed as a degradation product of collagen type I and can be measured in blood and urine.

Objectives: The main objective of the present study was the evaluation of the significance of the bone markers β-CTx and AP regarding their usage for early diagnostics of bone metastasis and pathological bone metabolism in pre- and post-menopausal patients with diagnosed breast cancer.

Materials and Methods: Peripheral blood samples of patients with mammarial diseases (benign and malign) were collected and the bone markers AP and β-CTx were determined. A total number of 110 patients had no malignant disease (benign mammarial disease, BMD): 30 fibroadenoma, 50 mastopathy and 30 hypertrophy patients. 30 patients were suffering from a malignant breast cancer without bone metastasis. 50 patients had known bone metastasis. The determination of β-CTx was conducted based on the Immunoassay "ECLIA" and the analysis approach Elecsys 2010 and cobas by Roche Diagnostics (Mannheim, Germany).

Results: The sensitivity of AP in our study to detect bone metastasis was 94.0%, the specificity was 86.67%. β-CTx showed a sensitivity and specificity of 100.0%. An elevated β-CTx activity is significantly associated with bone metastasis in our study groups (p=0.000000). Furthermore there are significant differences (p=0.000000) due to the menopausal status of patients.

Conclusion: In conclusion β-CTx is more sensitive and specific to detect bone metastasis and bone turnover than AP. In patients with multimorbidity the origin of AP is not clear due to its multiorganic appearance. β-CTx might be helpful in the diagnostic procedure of breast cancer-patients to detect bone metastasis. Moreover β-CTx seems to be helpful to indicate patients with early dysfunctions in bone metabolism and helps initiate early treatment to them. β-CTx as indicators for bone resorption are capable of providing a significant differentiation between mamma-carcinoma patients with and without bone affection.

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CIRCULATING TUMOR CELLS: DETECTION, BIOLOGY AND CLINICAL IMPLICATIONS

K. Pantel

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

Sensitive methods have been developed to detect circulating tumor cells (CTC) in the peripheral blood at the single cell level. CTC can be distinguished and enriched from the surrounding leukocytes by either physical properties (*e.g.*, density and size) or biological properties (*e.g.*, expression of epithelial proteins such as EpCAM or cytokeratins) (Pantel *et al.*, Nat Rev Cancer 2008; Parkinson *et al.*, J Transl Med 2012). CTC/DTC are usually detected by immunostaining or RT-PCR assays, and more recently by the EPISPOT assay which measures the number of cells releasing/secreting tumor-associated marker proteins. Interestingly, detection of cell-free nucleic acids released by tumor cells into the blood might become an indirect way to detect micrometastatic disease (Schwarzenbach *et al.*, Nat Rev Cancer 2011). At present, most CTC assays rely on epithelial markers and miss CTCs undergoing an epithelial-mesenchymal transition (Alix-Panabieres & Pantel, Nature Rev. Cancer, 2014). New markers such as the actin bundling protein plastin-3 (Yokobori *et al.*, Cancer Res. 2013) are not downregulated during EMT and not expressed in normal blood cells might overcome this important limitation and, therefore, increase the sensitivity of CTC assays. Recently, *in vivo* capture of CTCs with an antibody-coated wire placed into the peripheral arm vein has become feasible and allows now the "fishing" for CTCs from approx. 1.5 liters of blood within 30 minutes (Saucedo-Zeni, Int. J. Oncol. 2012). CTC enumeration and characterization with certified systems provides reliable information on prognosis and may serve as liquid biopsy (Alix-Panabieres & Pantel, Clin. Chem. 2013; Pantel & Alix-Panabieres, Cancer Res., 2013). Recent studies

in breast cancer showed that CTCs are superior to serum tumor markers to predict outcome in patients with metastatic disease undergoing chemotherapy (Bidard *et al*., Lancet Oncology, 2014) and CTC counts predict prognosis in early breast cancer patients without overt metastasis (Rack *et al*., JNCI, 2014). CTCs are detected in various types of solid tumors (Alix-Panabieres & Pantel, Nature Rev. Cancer, 2014) including patients with glioblastomas (Müller *et al*., ScienceTM, 2014). Ongoing research is aimed to identify the CTCs that home in secondary organs and give rise to overt metastases. Our recent work indicates that the subset of EpCAMlow, $CD44^{high}$, $CD47^{+}$, c-Met^{+} CTCs obtained from breast cancer patients might represent potential metastasis-initiator cells (Baccelli *et al*., Nature Biotech. 2013). Moreover, monitoring and characterization of CTCs before, during and after systemic therapy (*e.g*., chemotherapy, hormonal therapy, antibody therapy) might provide unique information for the future clinical management of the individual cancer patient. This "liquid biopsy" information can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells.

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CO-DELETION OF 1P/19Q AS PROGNOSTIC AND PREDICTIVE BIOMARKER IN THE LONG-TERM FOLLOW UP OF ANAPLASTIC OLIGODENDROGLIOMA PATIENTS

J. Polivka Jr.[1,2], J. Polivka[3,4], V. Rohan[3,4], T. Repik[3,4], O. Hes[5], O. Topolcan[6]

[1]Department of Histology and Embryology and [2]Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague, Czech Republic; [3]Department of Neurology, Faculty of Medicine in Plzen, Charles University in Prague and [4]Faculty Hospital Plzen, Czech Republic; [5]Department of Pathology, Faculty of Medicine in Plzen, Charles University in Prague, Czech Republic; [6]Central Imunoanalytical Laboratory, Faculty Hospital Plzen, Czech Republic

Specific objective: Anaplastic oligodendrogliomas (AO) are rare tumors. AO are characteristic for the frequent co-deletion of chromosome 1p and 19q. Recently two phase III clinical trials (RTOG 9402 and EORTC 26951) proved favorable effects of radiotherapy (RT) with chemotherapy (procarbazine, lomustine, vincristine - PCV) in patients with AO carried co-deletion 1p/19q. We assessed 1p/19q co-deletion as a prognostic and predictive biomarker for our AO patients. Methods: We performed a retrospective study of 23 patients with a diagnosis of WHO grade III oligodendroglioma – anaplastic oligodendroglioma (n=23; 13 males and 10 females) who were treated (neurosurgery plus RT alone; n=10 or neurosurgery plus RT and PCV; n=13) with the standard protocol in the Faculty Hospital Plzen. The 1p/19q co-deletion was assessed by FISH (Fluorescent in Situ Hybridization) and was correlated with the progression free survival (PFS) and overall survival (OS) for the entire cohort and for the subgroups of patients with different treatment (neurosurgery plus RT alone *vs*. RT + PCV).

Results: The 1p/19q co-deletion was identified in 12 of 23 tumors (52.2%). Patients with co-deletion had longer OS – 587 *vs*. 132 weeks (p=0.012) and trend to longer PFS – 321 *vs*. 43 weeks (p=0.075) than patients without the mutation. Patients with 1p/19q co-deletion treated with neurosurgery and RT+PCV *vs*. RT alone had longer OS – 706 *vs*. 423 weeks (p=0.008). There was no survival difference for patients without 1p/19q co-deletion in relation to the treatment.

Conclusion: The prognostic value of 1p/19q co-deletion in our AO patients was observed. Also the strong positive predictive value of this biomarker was proven for OS and patients with co-deletion in relation to the PCV treatment regimen.

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EFFECT OF BSA COATED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES ON GRANULOSA CELLS

M. Pöttler[1], S. Hofman[2], S. Dürr[1,3], I. Wiest[2], J. Zaloga[1], U. Jeschke[2], C. Janko[1]

[1]Department of Otorhinolaryngology, Head and Neck Surgery, Section for Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftungsprofessorship, University Hospital Erlangen, Germany; [2]Department of Obstetrics and Gynaecology, Campus Innenstadt, Ludwig-Maximilians-University Hospital, Munich, Germany; [3]Department of Phoniatrics and Pediatric Audiology, University Hospital Erlangen, Germany

Introduction: Nanomaterials, especially iron oxide nanoparticles are nowadays regularly used in medicine. Particularly, with the purpose to target cancer cells and save healthy tissue they provide a very attractive platform *e.g*. for hypothermia or as carriers of chemotherapeutics. According

to literature, there are heterogeneous effects of nanoparticles described on mammalian cells with regard to reproductive tissue. To address nanoparticle impact on cells which play a key role in ovarian function, health and female fertility we examined the effect of superparamagnetic iron oxide nanoparticles (SPION) on granulosa cells. These cells are protectively located around the oocyte, producing estrogen as well as progesterone. An experimental study was accomplished to show effects on both steroid hormone receptor expression and viability of ovarian granulosa cells.

Materials and Methods: Human primary granulosa cells as well as cells of the HLG-5 granulosa cell line were cultured *in vitro* and incubated with SPIONs coated with bovine serum albumin (BSA). Different concentrations were tested and 25 μg/ml SPIONs were used for experiments, as this concentration is significantly lower compared to respective *in vivo* application dose. After 48h, steroid receptor expression as well as viability were evaluated and matched to untreated cells. Viable cells were assessed by life-dead staining. Estrogen receptor β1 and progesterone receptor A were constituted by immunocytological quantification after treatment with SPIONs.

Results: Granulosa cells treated with SPIONBSA revealed no changes in estrogen receptor ß1 or progesterone receptor A expression compared to untreated cells. SPIONBSA uptake had no notable effect on viability after different time points.

Conclusion and Discussion: For the first time we were able to show that nanoparticles pre coated with protein - SPIONBSA -do not affect granulosa cell viability and steroid receptor expression, whereas referred to literature gold -, as well as calcium phosphate - nanoparticles and quantum dots transferrin bioconjugates interfere with granulosa cells thereby inducing apoptosis and decreasing expression of steroid receptors. Our data indicate better outcome for SPIONBSA overall, since BSA coating of nanoparticles rescues the severe effect of a missing protein corona on interacting cells and tissue. This study proposes that the effects of nanoparticles on ovarian function should be intensively investigated, since alterations in the microenvironment of oocytes are a very potent platform to explore the influence of nanomaterial on reproductive tissue. This work was supported by the Bavarian State Ministry of the Environment and Consumer Protection (74-U8793-2012/7-35), Germany.

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UPDATE ON TUMOR MARKERS IN LUNG CANCER

N. Reinmuth

Lungen Clinic Grossahnsdorf, Abteilung für Onkologie, Grosshansdorf, Germany

Lung cancer is one of the leading causes of death from malignancy worldwide. For decades, the use of non-invasive tools for screening and monitoring has gained increasing interest. Serum markers have been extensively studied. As a general tumor marker, high LDH levels in serum have been used as a reflection of the disease. Various other markers, like NSE, Cyfra21-1, CEA and SCC have been shown to harbor prognostic but limited diagnostic value. In the differential diagnosis between NSCLC and SCLC a combination of CYFRA 21-1 and NSE was claimed to be helpful. NSE was shown to be useful to distinguish SCLC from malignant lymphoma. Although rising SCC and NSE levels in the post therapeutic surveillance of patients with lung indicated tumor relapse, these results are of minor clinical utility due to the absence of curative therapy. Recently, due to their predictive value, specific biomarker testing has become standard of care for patients diagnosed with non-small cell lung carcinoma (NSCLC). Although it can be successfully performed in circulating tumor cells at present, the vast majority of investigations are carried out using direct tumor sampling. Consequently, a new proposal for the histologic classification of adenocarcinomas has been formulated and a discussion on correct tissue processing, methodology and marker interpretation has been initiated.

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PSA IN POPULATION BASED SCREENING FOR PROSTATE CANCER

A. Semjonow

Prostatazentrum am Universitätsklinikum Münster, Münster, Germany

The wide spread use of prostate specific antigen (PSA) results in a marked increase in the incidence of prostate cancer. So far there is no recommendation for the use of PSA in a population based screening program, despite the fact that first results of the large randomised European screening study (ERSPC) showed a reduction of prostate cancer mortality of 20% in men aged 55-69 years as compared to the control group (Schröder *et al*., NEJM 2009). After nine years of follow-up the number of men who had to be invited for screening in order to avoid one death due to prostate cancer was 1410 (NNI=number needed to invite), the number of men needed to be detected with prostate cancer was 48 (NND=number needed to detect). More recent data from the study show more advantageous results after 11 and 13 years of follow-up (Schröder *et al*. Lancet 2014):

Follow-up	NNI	NND
9 years	1410	48
11 years	979	35
13 years	781	27

Nevertheless the study also shows, that with population based PSA screening there will be many cancers detected, which will presumably not cause death or even symptoms during life time. It is estimated, that 40 to 50% of the cancers detected may be "insignificant" cancers, not requiring immediate invasive treatment. Currently there are several studies ongoing worldwide to avoid unnecessary treatment in selected men. The largest study for active surveillance (PRIAS=Prostate Cancer Research International Active Surveillance) currently involves over 4000 men. The aim is to identify selection- and follow-up criteria which allow for a safe counselling of men with presumably "insignificant" cancers, thus to avoid unwanted side effects of treatment as long as there is a low risk of progression

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STEREOTACTIC BODY RADIOTHERAPY (SBRT) FOR PANCREATIC CARCINOMA (PC)

T. Schneider, K. Piefel, M.H. Seegenschmiedt, F. Fehlauer

Strahlenzentrum Hamburg / Radiation Oncology Clinic, Hamburg, Germany

Objective: Most patients diagnosed with pancreatic carcinoma (PC) are unable to have a curative surgical resection. Chemoradiation (CRT) is a standard of care treatment for patients with locally advanced unresectable disease, but local failure rates are high with conventionally fractionated radiotherapy. Stereotactic body radiotherapy (SBRT) or stereotactic ablative radiotherapy offers an alternative type of radiation therapy, which allows high-doses, high focussed, conformal radiation in 1-3 treatment fractions. The high biologic doses and shorter overall treatment time with SBRT result in high local control and disease outcomes, good quality of life and low toxicity profile. Herein we review the technology and clinical outcome of SBRT for pancreatic malignancy and its future direction in the overall management of PC.

Methods: Stereotactic body radiation therapy, also known as stereotactic ablative radiotherapy has emerged as a treatment modality that allows the precise delivery of a large ablative radiation dose to tumor volumes while sparing surrounding organs and tissues. Phase I and II studies since 2000 have shown good rates of local control of the disease but rates of distant metastasis remain significant. First expertise in SBRT started in the mid of the 90ies from Sweden, Germany and Japan. RESULTS: Different fractionations and RT-techniques were tested (n=5-150 in more than 30 study groups – we sort into 4 fractionation groups: Group # A – C, fraction (Fx), dose per fraction (dpf), 1-year-local control (LC), toxicity profile (TP), ≥ grade 3 toxicities.

#A: Fx: 1/ dpf: 15-25/ LC: 81-100%/ TP: 5-16%; (*late tox grade 4 reported)

#B: Fx: 3/ dpf: 5-12/ LC: 50-92%/ TP: 0-16%

#C: Fx: 5/ dpf: 4-10/ LC: 40-86%/ TP: no acute grade 3; 5 – 7%: grade 3 (late)

Various gemcitabine-based chemotherapy (induction-GEM or post SBRT-GEM) regimens were included. SBRT has also been used in the neoadjuvant management of borderline resectable and locally-advanced pancreatic adenocarcinoma. After mean time to surgery after SBRT of 3.3 months: complete resection (R0) was performed in 92% of patients. No grade 3+ acute toxicities SBRT were found. 1-/ 2-/ and 3-year OS of 92%, 64% and 51% were reported. Strategies to combine novel systemic therapy and stereotactic body radiation therapy are to be explored.

Conclusion: The management of pancreatic cancer continues to be challenging. Despite surgical, genetic and molecular advances, its overall prognosis remains poor. This overview reports on the clinical data (high local control, LC of 80-100% and low toxicity-profile (TP) of stereotactic body radiotherapy (SBRT) for patients with pancreatic carcinoma. SBRT was used for primary or recurrent, even in palliative cases. SBRT for PC is effective with high LC and associated low acute and long-term toxicity-profile, less than 10% grade 3 side effects. High levels of quality assurance (QA) is garantied in modern SBRT. Technical efforts in soft- and hardware development reduced complications, made it a safe non-invasive radiotherapy option.

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NATURAL RESOURCES IN/FOR CANCER THERAPY

J. Spitz, A. Spitz

Institute for Medical Information and Prevention, Schlangenbad, Germany

Lifestyle factors have a definite effect on health and are believed to exert a marked influence on the risk for developing certain diseases, especially cancer and heart disease. The World Health Organization indicates that one third of all cancer deaths are preventable. A reductionist approach has been predominant to date in medical as well as

in preventive research and has unraveled some of the fundamental mechanisms at the basis of food nutrients, physical exercise and stress management. This has led to increased life expectancy. However, despite 40 y of research, epidemics of obesity, diabetes and cancer are growing each year worldwide, both in developed and developing countries, leading to a decrease in healthy life years. Yet, interactions between preventive health relations cannot be modeled on the basis of a linear cause-effect relation between 1 measure and 1 physiologic effect but rather from multi causal nonlinear relations. So it is not just diet or physical exercise or stress management that has to be looked at, but all aspects of our lifestyle in a holistic way. To overcome these problems we have to work with "systems-prevention" in analogy to "systems-biology" considering three different environmental systems: 1. The ancient environment of mother nature with an abundance of natural health resources, 2. The new man-made environment of civilization with an ever growing pollution by non-historical elements, 3. The mental environment of social bonding and culture. A multitude of factors from these environmental systems influence the organization of our body as a complex system itself, a fractal of the universe, a symbiont consisting of billions of cells, bacteria, viruses and fungi working and living together, cooperating with each other. Understanding these non-linear complex interactions leads to a fundamental change in the strategy to overcome cancer and other non-communicable diseases. The new concept will be validated on a large scale, preferably in the efficient setting of workplace health.

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SURGICAL THERAPY OF LIVER METASTASES

G.A. Stavrou, R.M. Jenner, K.J. Oldhafer

Chirurgische Abteilung, Asklepios-Klinik Barmbek, Hamburg, Germany

The modern arsenal of interdisciplinary therapeutic options has led to a significant survival benefit for patients suffering from liver metastases. Especially patients with colorectal metastases reach a 5 year survival in 40-50% of cases. Even patients diagnosed in UICC IV can reach a survival up to 25-30%. This became mainly possible through additional chemotherapeutic regimens (*e.g.* use of antibodies). Additionally surgical technique and perioperative management has reached a level where even extended liver resections are possible with a general mortality between 5 % and 1% in dedicated centers. Patients are nowadays treated with neoadjuvant chemotherapy before resection, in many cases chemotherapy is used to render irresectable patients resectable through a down sizing effect of chemotherapy. As tumors can respond to chemotherapy very well surgery becomes possible even in advanced cases. With the use of new surgical strategies such as the hypertrophy concept (PVE, two-stage operations) and the combination of various interventional therapies (RFA, DEBIRI-TACE, SIRT, chemosaturation) with surgery seemingly incurable cases are left with a therapeutic option sometimes. Resectability of liver metastases should always be judged by an experienced HPB Surgeon at the beginning of therapy and at restaging scans. Surgery also has a successful role in metastatic lesions of other primaries in a highly individualized interdisiplinary approach. We performed over 500 resections in our own collective between 2010 and 2013. Forty five resections were done in patients suffering from non-colorectal metastases (neuroendocrine 6, NCNNE 39 – including stomach 6, mamma 4, GIST 4, esophagus 3, pancreas 4). Patients with metastasized pancreas carcinoma usually do not undergo surgery, in selected cases however surgery can be an option, *e.g.* when a solitary metastasis is found during exploration. In our collective of over 300 pancreatic surgeries between 2010 and 2013 we had 4 cases in which we resected synchronous liver metastases in the course of a pancreatic resection. With perioperative chemotherapeutic and radiation therapy concepts in advanced situations just at the beginning probably a higher rate of surgical explorations aiming at an R0 resection even in metastasized situations is to be expected in the future.

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IMMUNOHISTOCHEMICAL EVALUATION OF E6/E7 HPV ONCOPROTEIN STAINING IN CERVICAL AND HEAD AND NECK CANCER SLIDES

A. Stiasny[1], C. Kuhn[1], D. Mayr[2], C. Alexiou[3], C. Janko[3], I. Wiest[3], U. Jeschke[1], B. Kost[1]

[1]LMU Munich, Department of Obstetrics and Gynaecology, Munich, Germany;
[2]LMU Munich, Department of Pathology, Munich, Germany;
[3]ENT-Clinic, Section for Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftung, University Hospital Erlangen, Germany

Background: High risk human papillomavirus (HPV) subtypes (16 and 18) lead to cervical cancer as well as HPV-positive oropharyngeal cancer (OSCC), a form of head and neck cancer. The induction of HPV induced cancer is driven by virus specific oncoproteins E6 and E7. E6 protein of HPV types 16 and 18 interacts with the E3 ubiquitin-protein

ligase, resulting in ubiquitination and proteolysis of tumor protein p53. E7 inactivates Retinoblastoma protein (Rb) by phosphorylation followed by an increase of free E2F in the cell. This leads to an increase of cyclin-dependent kinase inhibitor p16, which is used as an immunohistochemical marker of HPV associated OSCC. Unfortunately, p16 is increased not exclusively by E7 oncoprotein in carcinogenesis. Therefore the aim of this study was to develop an immunohistochemical approach for the direct detection of E6/E7 oncoproteins in cervical cancer as well as in OSCC.

Materials and Methods: Paraffin sections of 250 cases of cervical cancer and 130 cases of OSCC were analysed. Immunohistochemical staining protocols were evaluated with tissue slides from cervical dysplasia patients (CIN3) und squamous epithelial carcinoma tissue with HPV infection. Brain and placental tissues were used as negative controls. E6 specific antibody (Biorbyt) was used as primary antibody. The polymer staining method and diaminobenzidine were applied for further development. A panel of E7 specific antibodies were tested (Biorbyt, Invitrogen and Calbiochem). Again, the polymer staining method and diaminobenzidine were applied for further development.

Results: E6 specific antibody (dilution 1:300) revealed specific and intense staining after pre-incubation of tissue slides with citrate buffer solution. Only the E7 antibody obtained from Calbiochem showed intense and specific staining in cervical dysplasia patients (CIN3) und squamous epithelial carcinoma tissue; however, placental tissue as negative control showed also a positive immunohistochemical reaction. Pre-incubation with Proteinase K diminished unspecific reaction.

Discussion: Our results revealed a useful staining protocol for the immunohistochemical evaluation of E6/E7 oncoprotein expression in cervical cancer, as well as in HPV-positive oropharyngeal cancer (OSCC). Advantages of this method compared to mRNA in situ hybridisation of E6/E7 are the much lower costs as well as the broader applicability in the pathological practice.

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CYTOKERATIN 19 AND ITS PERSPECTIVE IN PERSONALIZED MEDICINE OF BREAST CANCER

O. Topolcan[1], S. Svobodova[1], J. Kinkorova[1], M. Pešta[1], O. Fiala[2], R. Fuchsova[1], V. Třeška[2]

[1]Immunoanalytical Laboratory, Department of Nuclear Medicine, University Hospital in Pilsen, Czech Republic; [2]Department of Surgery, Faculty of Medicine and University Hospital in Pilsen, Charles University in Prague, Czech Republic

Breast cancer is a complex and heterogeneous disease displaying considerable variation in its clinical and molecular characteristics and thus, this cancer remains challenging to manage. Recent development of high-throughput technologies enabled the study of serum, tissue and genetic markers. We will demonstrate our results with literature data related to cytokeratin 19 and to discuss its role for diagnostics, therapy choice and prognosis estimation. Serum tumor marker cytokeratin 19 (CYFRA 21-1) has been known and used in oncology for many years. Based on our own results and the literature data as well, it seems to be a more reliable marker than CEA or CA 19-9, particularly for disease recurrence or progression detection. Tissue expression of this marker has been already intensively studied recently. It has been confirmed that the higher expression of cytokeratin 19 has a significant influence on disease progression and thus can be used as a molecular tool to classify breast cancer patients. Cytokeratin 19 has been used for micro- and macro-metastases detection in sentinel lymph nodes (SLN) - using one-step nucleic acid amplification (OSNA) assay. Cytokeratin 19 is included in reverse transcription quantitative PCR RT-qPCRmultimarker (MM) assay based on the tumor cell mRNA markers - keratin 19 (KRT19), containing other markers - mammaglobin A (hMAM), and TWIST1. This marker can also be used for breast cancer micro-mestastases detection in bone marrow. Circulating tumor cells (CTCs) have also the potential to predict the effect of adjuvant treatment for detecting breast cancer CTCs in bone marrow (BM) from early breast cancer patiens. Cytokeratin 19 seems to be undoubtedly a very challenging biomarker these days. We can presume that it will be used for individualized diagnostics, not only of breast cancer but also of many other cancers.

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PROSTATE HEALTH INDEX (PHI) IN EARLY DETECTION OF PROSTATE CANCER

O. Topolcan[1], R. Fuchsova[1], M. Hora[2], J. Windrichova[1], R. Kucera[1], O. Dolejsova[2], L. Pecen[1], P. Kasik[1], S. Svobodova[1], J. Kinkorova[1]

[1]Immunoanalytical Laboratory, Department of Nuclear Medicine, University Hospital in Pilsen, Czech Republic; [2]Department of Urology, Faculty of Medicine and University Hospital in Pilsen, Charles University in Prague, Czech Republic

Aim: To evaluate biomarkers PSA, %freePSA, [-2]proPSA and calculation of Prostate Health Index (PHI) in the diagnostic algorithm of early prostate cancer.

Patients and Methods: 310 patients from the Urology department of the University Hospital with suspected prostate cancer who underwent TRUS biopsy and their PSA was within a range between 0 - 20 ng/mL were enrolled into the study. Serum levels of free PSA and [-2]proPSA were measured and %freePSA and PHI were calculated. Laboratory test results were correlated with Gleason score. The biomarkers were measured using the chemiluminescent DxI 800 instrument (Beckman Coulter, USA). SAS 9.2 software was used for all statistical analyses.

Results: We found highly statistically significant increased levels of [2]proPSA, PHI and %freePSA in patients with prostate cancer diagnosed by prostate biopsy *vs*. patients with benign prostate hypertrophy ([-2]proPSA median 16 *vs*. 21 pg/mL, PHI median 35 *vs*. 62 and %freePSA 16,7 *vs*. 11.7%). The best correlation with Gleason score was found for pro PSA and PHI.

Conclusion: The assessment of [-2]proPSA and the calculation of PHI appear to be of great benefit for a more accurate differential diagnosis of benign hyperplasia. PHI calculation leads to a biopsy reduction frequency and has a significant impact in the assessment of tumor aggressiveness prior to surgery.

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METHYLATION OF THE ER ALPHA PROMOTER IS INFLUENCED BY ITS LIGAND ESTROGEN IN OSTEOSARCOMA CELLS SAOS-2 *IN VITRO*

J. Tübel, C. Marthen, L. Kuntz, I. Wiest,
R. von Eisenhart-Rothe, U. Jeschke, R. Burgkart

Department of Orthopedics and Sportorthopedics,
Technical University Munich, Germany

Introduction: The aggressive fast-growing osteosarcoma is the most common primary malignant bone tumor. The relevance of estrogen as a key player in bone metabolism and bone tumor is well known. At the molecular level estrogen activates the estrogen receptor α (ERα) as natural ligand of this receptor. ERα acts as a transcription factor by binding to the "estrogen response element (ERE)" and regulates the expression of a various number of genes. Epigenetic processes, *e.g*. the methylation of the "cytosine-phosphatidyl-guanine (CpG) islands" can change the transcription of target genes and subsequently protein expression. As DNA methylation is generally associated with gene transcription repression, up to now little is known about the ERα methylation in osteosarcoma cells. The aim of the present pilot study was to evaluate the methylation status of ERα in osteosarcoma cells SAOS-2 and MG 63 after stimulation with estrogen.

Methods: SAOS-2 and MG 63 cells were cultured in DMEM. After treatment with 10 nmol estrogen (E2) for 24h the expression of ERα was detected by Immunocytochemistry (ICC). Untreated cells were used as control. Staining was evaluated semi quantitatively by the Immunoreactive score of Remmele and Stegner. To determine the mRNA gene expression, extracted RNA was transcribed into c-DNA and a qPCR was carried out. The semi quantitative evaluation of the ERα mRNA was based on the $2^{-\Delta\Delta ct}$ method using untreated cells as reference control. One microgram of each extracted genomic DNA sample was converted with bisulfite and a real-time methylation-specific PCR (rt-MSP) was carried out.

Results: The estrogen stimulated SAOS-2 cells showed a significantly increase of ERα expression. A 7-fold up regulation of ERα mRNA confirmed the results of the immunocytochemistry. Methylation of the ERα promoter was not detected in the treated cells. In contrast, we identified a methylation of the ERα promoters in the untreated cells. The staining of MG 63 cells showed a weak gain of ERα expression in the stimulated cells as well as a weak increase of the ER α mRNA (2-fold). Methylation of the ERα promoters was not detectable as well as in treated and untreated cells.

Conclusion: The aim of the pilot study was the evaluation of the methylation of the ERα promoters in osteosarcoma cells. These results reveal that the methylation status of ERα in osteosarcoma cells is affected by estrogen. These findings indicate that epigenetic changes of genomic DNA regulate the ERα synthesis. Taken together our results we suggest that SAOS-2 cells can be an interesting model for further investigations in the ERα synthesis. In addition, the evaluation of ERα methylation in osteosarcoma specimens is in preparation.

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BORON CONTAINING MAGNETIC NANOPARTICLES FOR NEUTRON CAPTURE THERAPY - AN INNOVATIVE APPROACH FOR SPECIFICALLY TARGETING TUMORS

H. Unterweger[1], R. Tietze[1], N. Taccardi[2], B. Weigel[1],
S. Lyer[1], C. Janko[1], S. Dürr[1], P. Kudejova[3],
F.M. Wagner[3], W. Petry[3], R. Turcu[4],
E. Tombacz[5], L. Vekas[6], C. Alexiou[1]

[1]ENT-Department, Section for Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftung-Professorship, University Hospital Erlangen, Germany;
[2]Chair of Chem. Engineering I (Reaction Engineering) University Erlangen-Nuremberg, Germany;
[3]Forschungs-Neutronenquelle Heinz Maier-Leibnitz (FRM II), TU-München, Garching, Germany;
[4]National Institute R&D of Isotopic and Molecular Technologies, Romania;
[5]Department of Physical Chemistry and Materials Science, University of Szeged, Hungary;
[6]Laboratory of Magnetic Fluids-Center for Fundamental and Advanced Technical Research, Romania

Introduction: The key for a highly efficient tumor therapy with minimal adverse side effects for the patient lies in the successful and specific delivery of the therapeutic agent to the tumor site. The combination of boron neutron capture therapy (BNCT) with Magnetic Drug Targeting (MDT) provides a very promising targeting approach. It involves the linkage of boron on superparamagnetic iron oxide nanoparticles (SPIONs), which – after intra-arterial application – can be focused to the region of interest by an external magnetic field. By this means the boron can be effectively enriched in the tumor region and the location of activity is confined by two mechanisms: Firstly, by the impinging neutron radiation and secondly, by the external magnet, which accumulates the boron-loaded SPIONs to a specific site of action.

Materials and Methods and Results: In this study, we launched different experiments to merge BNCT and MDT concepts. On the one hand, we developed boron-containing magnetic nanoparticles, prepared by precipitation of dextran coated SPIONs and subsequent surface modification using carboranes. These particles have a size of approx. 70 nm and possess a boron-to-iron-ratio of up to 14.6%, which was determined by ICP-AES and is sufficient for potential clinical applications. Flow cytometry measurements revealed that without irradiation, those particles do not show any sign of cytotoxicity (AnnexinV-PI staining) and do not induce DNA degradation (PIT staining). On the other hand, we investigated the neutron flux behavior by irradiating three-dimensional agarose gel (1.5%) cubes, mimicking biological tissue. Experiments of the neutron beam behavior were performed at the prompt gamma activation analysis (PGAA) facility of the Forschungs-Neutronenquelle Heinz Maier-Leibnitz (FRM II) research reactor in Munich, Germany. The thermal neutron flux equivalent chosen for the measurements was 2.35×10^9 n/cm^2 in air. This is appropriate to irradiate agarose gel cubes for evaluating the irradiation of physiological tissues. For neutron flux density determination and dose calculations, the neutron flux attenuation was measured in dependence on the depth, concentration of the boron-containing layer, co-presence of SPIONs in the boron-layer, co-presence of nitrogen and neutron beam attenuation behind bone material. Flux density was measured using the gold foil activation method.

Conclusion: Our experimental setting resulted in the attempted concentration of the radiation doses in the targeted area and opens the opportunity for a successful combination of MDT and BNCT. The high linear energy transfer of BNCT in our experiments still prevails, which is a precondition for further studies.

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INFLUENCE OF CIRCULATING TUMOUR CELLS ON PRODUCTION OF IL-1ALPHA, IL-1β AND IL-12 IN SERA OF PATIENTS WITH THE PRIMARY DIAGNOSIS OF BREAST CANCER BEFORE TREATMENT

T. Vilsmaier[1], B. Rack[1], A. König[1], K. Friese[1], W. Janni[2], U. Jeschke[1], T. Weissenbacher[1], SUCCESS Study Group

[1]Department of Gynecology and Obstetrics, Klinikum Ludwig-Maximilians-Universität, Munich, Germany;
[2]Department of Gynecology and Obstetrics, University Hospital Ulm, Ulm, Germany

Background: Circulating tumour cells (CTCs) have been found to be a prognostic marker for reduced disease free survival (DFS), distant DFS, breast cancer–specific survival, and overall survival (OS) before the start of systemic treatment. Determination of CTCs with the CellSearch System (Veridex, Raritan, NJ) is a valuable but time consuming and costly method. Therefore the aim of this study was the evaluation of cytokine profiles as marker for CTC involvement.

Methods: Patients chosen for this study were defined as women with breast cancer who agreed to participate in the phase I SUCCESS study. CTC analysis, the blood sampling time points, and the methodology were prospectively designed, and the prognostic value of the CTCs was defined as a scientific objective of the study protocol. A total of 100 patients being positive for circulating tumour cells and additional 100 patients being negative for circulating tumour cells were matched into pairs of two. Matching criteria were histo-pathological grading, lymph node status, hormone receptor type, TNM classification and survived breast cancer patients vs. deceased tumour associated patients. A novel multi cytokine/chemokine array (Meso Scale Discovery®,

Rockville, USA) was used to screen the blood serum samples for the TH1 cytokines: IFN-gamma, TNF-alpha, IL-12, IL-1, IL-2, IL-1β and IL-18. The cytokine levels correlation to the matching criteria listed above was analysed with the Spearman correlation coefficient and the Mann-Whitney-U rank-sum test.

Results: The CTC positive patient group indicated a significant difference in terms of lymph node involvement regarding IL-1α (p=0.043). The CTC negative collective exposed a significant correlation regarding progesterone receptor positive/negative patients in terms of IL-1β (p=0.029). Furthermore, the living patient collective established significant differences in IL-12p40 levels in association to lymph node involvement (p=0.041) and triple negative hormone receptor breast cancer (p=0.043). Deceased patients on the other hand presented significant results within oestrogen receptor positive/negative patients in terms of IL-1α (p=0.050) andIL-1β (p=0.034). Moreover, IL-1α levels also indicated a significant correlation regarding triple negative hormone receptor breast cancer (p=0.033) within the deceased collective. To continue, the collective graded G2 showed significant correlations amongst patients with Her2/neu association regarding IFN-gamma levels (p=0.031) and in terms of lymph node involvement concerning IL-1α levels (p=0.014). The patient group graded with G3 on the other hand revealed a significant correlation regarding progesterone receptor positive/negative patients in terms of IL12p70 Levels (p=0.048) and triple negative hormone receptor breast cancer with regard to IL12p40 levels (p=0.033).

Conclusion: Regarding CTC involvement, we found significant differences in IL-1α secretion in CTC positive patients. Increased values of this cytokine were found in sera of patients without lymph node involvement. Therefore we may speculate that IL-1α might be a marker for the release of tumour cells into the circulation and not in the lymphatic system. In addition, also Il-1β was found to be correlated to CTC release. Only CTC negative patients showed a correlation of the progesterone receptor expression and Il-1β release. Therefore we conclude that IL-1α, as well as IL-1β, are connected to CTC release of breast cancer patients.

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MANAGEMENT AND DIAGNOSTIC STRATEGIES FOR THE PERSONALIZED TREATMENT OF NON-SMALL CELL LUNG CANCER - CAN WE STILL CALL IT THE LUNG CANCER?

C.l. Wesseler

Department of Pneumonology, Asklepios Klinikum Harburg, Hamburg-Harburg, Germany

In contrast to the binary classification of lung cancer in non-small and small cell lung cancer, different findings in pathology, molecular oncology and genomics led to an altered view on this disease. New approaches in molecular pathology conducted a novel understanding of the cellular mechanisms of tumor initiation, proliferation, angiogenesis and have led to personalized and targeted therapy strategies. The treatment of patients with advanced-stage non-small cell lung cancer is changing from choosing therapy based on histopathology and cytology to the use of biomarker-driven treatment algorithms based on the molecular profile of each individual tumor. Guidelines recommend tyrosine kinase inhibitor therapy as first line setting for patients with positive EGF-receptor mutations. In the meantime there are three drugs approved for this indication. Newer findings have shown, that within the group of EGF-receptor mutation positive tumors, response to the targeted therapy differs depending on the mutation subtype (activating or resistant). Even between the subgroups of activating mutations (Del 19, L858R) different response to targeted therapy is seen. Other individualized therapy strategies aim for EML4-ALK-translocations. Crizotinib is a highly potent kinase-inhibitor and has been approved but only for second-line treatment in this indication. Ongoing research focusses on ROS-1 rearrangements, KRAS, MET-pathway and resistance mechanisms of the therapies mentioned above. In the meantime 50-75% of the adenocarcinomas and 20-40% of the squamous cell lung cancer can be subtyped by molecular based diagnostics. Unfortunately, for many of those new subtypes there is no specific treatment available yet, and benefit in survival and quality of life could not be proven. Some factors may even be seen as indicators for worse prognosis. The advantages of targeted therapy of tumors with positive EGFR-Mutation or EML4-ALK-Translocation are improved progression free survival, better quality of life, less symptoms like cough, dyspnea or pain. Side effects seen under TKI therapy (rash, skin disorders) are entirely different to those seen under standard chemotherapy. In the future routine, diagnostics will (have to) contain molecular characterization and next generation sequencing, so that early detection of genetic alterations will enable optimized and individualized tumor therapy for every patient, resulting in maximum efficacy and minimum toxicity at the same time.

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EXPRESSION OF THE TUMOR-ASSOCIATED (TA) MUCIN 1-EPITOPE ANALYSED WITH THE HUMANISED PANKOMAB-GEX ANTIBODY IN MALIGN AND NORMAL TISSUE OF HEAD & NECK

I. Wiest[1], C. Alexiou [2], K. Friese [1], A. Agaimy[3], P. Betz[4], J. Tübel[5], S. Goletz[6], D. Dian[1], U. Jeschke[1]

[1]Department of Obstetrics and Gynecology – Innenstadt, LMU Munich;
[2]Department of Oto-rhino-laryngology, Head and Neck Surgery, FAU Erlangen, Section of Experimental Oncology and Nanomedicine, Else-Kröner-Fresenius-Stiftung-Professorship;
[3]Department of Pathology, FAU Erlangen;
[4]Department of Legal Medicine, FAU Erlangen;
[5]Department of Orthopedics, Technical University Munich,
[6]Glycotope, Berlin, Germany

Introduction: During genesis of neoplasia glycosylation is changed. In these setting mucins, especially Mucin 1 (MUC1), becomes a carrier for oncofetal carbohydrates and relieves invasive growth. Some epitopes of MUC1, which are shed to the serum, are already used as tumor markers with a high clinical relevance for diagnosis and follow-up treatment. The recently described MUC1 epitope TA-MUC1 is primarily restricted to malignancies and also over-expressed in these tissues. The humanized monoclonal antibody PankoMab-GEX recognizes specifically TA-MUC1. In comparison to all other established anti-MUC1 antibodies it shows the highest dependency on glycosylation, the greatest binding-capacity and therefore the highest tumor specificity.

Materials and Methods: Laryngeal cancer specimens (n=125) and normal tissue of head & neck (n=7) were used for the study. Paraffin-embedded sections were incubated with the humanized anti-Mucin1 antibody PankoMab-GEX. Staining reaction was done by POD-labeling and DAB. Breast cancer tissue was used as positive control, unspecific mouse IgM as negative control. Semi-quantitative evaluation was done double-blinded by two independent investigators, including a pathologist, by using the immunoreactive score of Remmele and Stegner (IRS).

Results: A total of 31 from 125 laryngeal cancer specimens were classified as G1. Therefrom 22 (71%) were completely negative, the remaining 9 showed a very weak staining with an IRS of 2. 94 cases of the cancer specimen collective were classified as G2 and G3. 34 of them were also negative, but 60 reached an distinct positive IRS up to 9. All of the investigated normal tissue of the upper aero digestive tract remained completely negative.

Conclusion: Summing up, all G1 tumors are completely negative or do not reach an IRS relevant range. This finding can be helpful for histopathological examination, especially concerning the grading. Beside the already known facts, that the TA-MUC1 stays adherent to the cell membrane and is primarily restricted to malignancies, and being aware of our former results with the PankoMab-GEX pertaining its affects *in vitro* and its immunohistochemical features in the gynecological scope, this antibody is holding a great potential capacity to be used as a therapeutic antibody in laryngeal cancer as well.

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FUTURE OF MAMMOGRAPHY SCREENING

I. Witzel

University Medical Center Hamburg-Eppendorf
Department of Gynecology, Martinistr. 52, 20246
Hamburg, Germany

Screening mammography can help reduce the number of deaths from breast cancer among women aging between 40 and 74 years. In Germany, screening occurs biennially from ages of 50 to 69 years. Screening in Germany has been criticized recently. Potential limitations of screening mammography include false-positive results, overdiagnosis and overtreatment, false-negative results, and radiation exposure. The question whether improvements in breast cancer mortality have been achieved due to multimodal adjuvant treatment regimens or due to earlier detection of breast cancer cannot be answered. New screening strategies should be based on stratification in risk-groups and addition of new techniques.

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BIOMARKERS IN SCREENING FOR CERVICAL AND VULVOVAGINAL CANCER

L. Woelber

University Medical Center Hamburg-Eppendorf and
University Cancer Center Hamburg-Eppendorf (UCCH),
Hamburg, Germany

Background: Despite the relative success of the cytology-based screening programs for cervical cancer, there has recently been an emotional debate on the further organisation and course of screening in face of human papilloma virus (HPV) testing. For vulvovaginal cancer no screening exists, nationally or internationally.

Methods: Available biomarkers and cytological methods are discussed regarding their value for screening in different populations.

Results: The current screening strategy for cervical cancer in Germany is a cervical cytology taken once yearly (Pap testing). The sensitivity of cytology is known to be variable and dependent on many interfering factors. As HPV is the main risk factor for the development of cervical cancer, implementation of HPV testing in primary screening seems promising to improve detection of cancer and high grade precursor lesions. There is however also evidence that such a strategy might put patients at risk for overdiagnosis dependent on the population screened. With regard to unclear

screening results there are several markers available to potentially clarify the picture (p16/ki67, L1, HPV typisation), their indication is however poorly defined.

Conclusion: In the current situation it is very difficult to receive objective information regarding the potential benefit of different screening strategies. There is a variety of biomarkers suited for certain clinical situations, however, their indications have yet to be clearly defined for physicians involved in primary screening.

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L1CAM AS PROGNOSTIC FACTOR IN TYPE-I ENDOMETRIAL CARCINOMA

A.G. Zeimet, D. Reimer, B. Winterhoff, U. Puistola, A. Ben-Arie, L.C. van Kempen, E. Petru, L.F. Massuger, F. Amant, S. Polterauer, W. Hackl, M. Netzer, P. Altevogt, C. Marth, M. Fogel

Department of Obstetrics and Gynecology, Innsbruck Medical University, Innsbruck, Austria

Endometrioid (type-I) endometrial cancers diagnosed in FIGO stage I have an excellent prognosis. Nonetheless, a significant number of patients experience recurrence and die from this disease. In a retrospective study on 1021 type-I endometrial cancers we used imunohistochemical L1CAM expression to identify these patients at high risk for poor clinical outcome. 181 of these cancers (17.7%) were rated positive for L1CAM expression (*i.e.* ≥10% positive staining cancer cells). During follow-up 51.4% of L1CAM positive cancers recurred compared to only 2.9% of the L1CAM-negative tumors. Accordingly, patients with L1CAM-positive cancers exhibited a significant poorer disease-free and overall survival; (p=0.0001). Independency of these results was confirmed in multivariate analyses, which revealed an increase in the likelihood of recurrence (HR: 15.80; 95%-CI: 10.36-24.10) and death (HR: 13.61; 95%-CI: 8.65-21.39). Of special note is that in L1CAM-negative cancers FIGO stage I subdivision, grading and the classical multifactor risk assessment lost their predictive power for DFS and OS. The prognostic relevance of these parameters was strictly confined to cancers showing L1CAM expression. CRT-decision-tree identified L1CAM as the best variable for predicting recurrence (sensitivity: 0.74; specificity: 0.91) and death (sensitivity: 0.77; specificity: 0.89). In conclusion, L1CAM expression in type-I endometrial cancers appears to indicate the need for adjuvant treatment. This adhesion molecule might itself serve as a treatment target for the fully humanized anti-L1CAM antibody currently under development for clinical use.

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HOW GOOD IS ENDORECTAL MAGNETIC RESONANCE IMAGING IN THE PREOPERATIVE STAGING OF PELVIC LYMPH NODES IN PATIENTS WITH PROSTATE CANCER?

V. Zugor[1], S. Poth[1], R. Kühn[2], A.P. Labanaris[3]

[1]Department of Urology, St. Antonius Medical Center, Gronau, Germany;
[2]Department of Urology, Martha Maria Medical Center, Nurnberg, Germany;
[3]Department of Urology, Interbalkan Medical Center, Thessaloniki, Greece

Introduction and Objective: The objective of this study was to evaluate the diagnostic sensitivity, specificity, and accuracy of endorectal magnetic resonance imaging (e-MRI) of the prostate as a preoperative staging modality in the diagnosis of lymph node metastasis (LNM) in patients with biopsy proven prostate cancer (PCa).

Methods: A retrospective review of the records of patients (N=168) with biopsy proven PCa, who subsequently underwent radical prostatectomy (RP) between April 2004 and April 2013 at two tertiary medical centers was conducted. Prior to RP all patients underwent an e-MRI of the prostate. Inclusion criteria for the study were PSA levels >20 ng/ml or Gleason biopsy score >7. The presence of one or more nodes with a short axis diameter >1 cm was considered as LNM. Interpretation of the images was performed by highly experienced radiologists blinded to patient clinical data. The examinations were performed on a closed 1.0-T system combined with an endorectal body phased-array coil and imaging results were correlated with histopathology. T1-weighted axial-oriented sequences were applied from the prostate base up to the aorta bifurcation. Regional lymph node resection included external iliac, internal iliac and obturator nodes. The clinicopathological parameters of the patients included age, PSA levels, biopsy Gleason score, e-MRI findings, histologically proven LNM, amount of lymph nodes dissected, size of lymph nodes dissected, sensitivity, specificity and accuracy.

Results: The clinicopathological characteristics of the patients are listed in the Table. Of the 168 patients included N=18 patients (10,7%) had histologically proven LNM. e-MRI was true-positive in N=6 of 18 patients (33.3%) and false-negative in N=12 patients (66,6%). N=150 (89.3%) patients had no LNM. e-MRI was true-negative in N=144 of 150 patients (96%) and false-positive in N=6 (4%). The sensitivity was 96%, the specificity was 33% and the accuracy 64.5%. Conclusion: The results of the present study showed that although e-MRI can be considered as a useful preoperative staging modality in the diagnosis of LNM, it

has its limitations as seen through its specificity and accuracy.

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INTRADETRUSOR INJECTION OF BOTULINUM NEUROTOXIN TYPE A IN PATIENTS WITH IDIOPATHIC DETRUSOR OVERACTIVITY UNDERGOING RADICAL RETROPUBIC PROSTATECTOMY

V. Zugor[1], S. Poth[1], M. Addali[1], R. Kühn[2], A.P. Labanaris[3]

[1]Department of Urology, St. Antonius Medical Center, Gronau, Germany;
[2]Department of Urology, Martha Maria Medical Center, Nurnberg, Germany;
[3]Department of Urology, Interbalkan Medical Center, Thessaloniki, Greece

Introduction and Objective: To determine the clinical relevance of intradetrusor injection of botulinum neurotoxin type A (BoNTA) in patients with idiopathic detrusor overactivity (IDO) and prostate cancer (PCa) undergoing radical retropubic prostatectomy (RP).

Methods: From April 2004 to April 2010, N=18 patients with a histological conformation of PCa, an urodynamic diagnosis of IDO with or without incontinence, absence of an obstruction and of any associated or contributing neurological, hormonal, and infective pathology underwent RP. Urodynamic assessment of patients was carried out with cystometry, using a filling rate of 20 ml/min. During RP and before the vesicourethral anastomosis took place, all patients were injected 100 units of BoNTA (BOTOX®) with 10 ml of normal saline, intradetrusally at the rate of 0.5 ml at each site for 20 sites of the posterior wall, lateral wall and the dome of the bladder sparing the trigone and ureteric orifices. After discharge, all patients underwent rehabilitation for 3 weeks and at the end of their rehabilitation were evaluated in terms of urgency, frequency, nocturia and incontinence with the1-hour pad test. The functional bladder capacity (FBC) was evaluated preoperative and at the end of their rehabilitation.

Results: The median age of the patients was 61.1 years (54-72). N=9 patients (50%), of which N=6 had preoperative an IDO with incontinence, exhibited exceptional improvements in frequency, urgency and nocturia. Furthermore, they demonstrated an absence of urge incontinence and a 45% median increase of their FBC (median 241 ml to median 350 ml). Their 1-hour pad test was <1 g (median 0.8 g). N=6 patients (33.3%), of which N=3 had preoperative an IDO with incontinence, although exhibiting a 14% median increase of their FBC (median 278 ml to median 318 ml), did not exhibit improvements of their preoperative clinical symptoms. Their 1-hour pad test was 10-50 g (median 23.3 g). N=3 patients (16.6%) demonstrated a 35% median decrease of the FBC (median 251 ml to median 163 ml) as well as a worsening of their urge incontinence and clinical symptoms. They demonstrated a 1-hour pad test of > 50 g (median 66 g).

Table

Patients	N=168
Age	56-74 (median 65.6)
PSA Gleason score	
6	N=27 (16.1%)
7	N=36 (21.4%)
8	N=72 (42.8%)
9	N=24 (14.2%)
10	N=9 (5.5%)
e-MRI findings	
cN0	N=156
cN1	N=12
Histologically proven LNM	
pN0	N=150 (89.3%)
Amount	6-33 (median 17.3)
Size	0.2-1.6 cm (median 0.73)
pN1	N=18 (10.7%)
Amount	15-40 (median 21.7)
Size	0.6-2.1 cm (median 1.1)
Sensitivity	96%
Specificity	33%
Accuracy	64.5%

Conclusion: The results of the present study indicate that the simultaneous use of BoNTA in such patients could normalise micturition frequency, diminish urge incontinence and increase the functional capacity of the bladder in up to 50% of cases. However, due to the small amount of patients involved in this study a prospective trial with more patients is warranted to assess the impact of these results on clinical practice.

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IS AN EXTENDED PROSTATE BIOPSY SCHEMA ASSOCIATED WITH AN IMPROVEMENT IN THE ACCURACY BETWEEN THE BIOPSY GLEASON SCORE AND RADICAL PROSTATECTOMY PATHOLOGY? A MULTIVARIATE ANALYSIS

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

[1]Department of Urology, St. Antonius Medical Center, Gronau, Germany;

[2]Department of Urology, Martha Maria Medical Center, Nurnberg, Germany;
[3]Department of Urology, Interbalkan Medical Center, Thessaloniki, Greece

Introduction and Objectives: To examine whether an extended prostate biopsy (PB) schema is associated with an improvement in the accuracy between the PB Gleason score (GS) and radical prostatectomy (RP) pathology and to identify probable preoperative variables that stratified patients likely to harbor significant upgrading (SU).

Patients and Methods: A retrospective review of N=538 patientss diagnosed with PCa, who underwent RP and exhibited an SU at two tertiary medical centres was conducted. The patients were divided into 3 groups: N=194, who underwent a 6-core PB (36%), N=156 who underwent a 12-core PB (28.9%) and N=188 (34.9%) that underwent an 24-core PB. A multivariate analysis was conducted including the following parameters: PSA level, clinical stage, prostate size, and duration from PB to RP.

Results: The 6-core group exhibited a 42.7% SU, the 12 core group exhibited a 38,8% SU and the 24-core group exhibited a 14.1% SU. There was a significantly lower rate of SU in the 24-core than that in the 6-core (p<0.001) and 12-core PB group (p<0.001) but no significant difference in the rate of SU was noted between the 6-core and 12-core group (p=0.913). According to the multivariate analysis, only a prostate size of >35 g significantly elevated the probability of SU in the 6-core (p<0.025) and 12-core PB groups (p<0.025), respectively.

Conclusion: An extended PB scheme is associated with a significant improvement in the accuracy between the PB GS and RP pathology. Prostate size >35 g in patients that undergo a 6 or 12-core PB is the only preoperative variable that stratifies patients likely to harbor SU.

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THE VALUE OF ENDORECTAL MAGNETIC RESONANCE IMAGING OF THE PROSTATE IN IMPROVING THE DETECTION OF ANTERIOR PROSTATE CANCER

V. Zugor, M. Addali[1], R. Kühn[2], A.P. Labanaris[3]

[1]Department of Urology, St. Antonius Medical Center, Gronau, Germany;
[2]Department of Urology, Martha Maria Medical Center, Nurnberg, Germany;
[3]Department of Urology, Interbalkan Medical Center, Thessaloniki, Greece

Introduction and Objective: The diagnosis of anterior prostate cancer (APC) is troublesome due to its anatomical location. Patients with an APC often require multiple sets of biopsies until diagnosis is made. The objective of this study was to examine whether endorectal magnetic resonance imaging (e-MRI) of the prostate could improve the detection of APC.

Methods: A retrospective review of the records of patients (N=628) with a clinical suspicion of prostate cancer (PCa) (PSA levels >4 ng/ml or a suspicious finding on digital rectal examination), who underwent conventional (e-cMRI) and functional (e-fMRI) e-MRI of the prostate and subsequently prostate biopsy from April 2004 and April 2013 at two tertiary medical centres was conducted. All patients had a history of at least one prior negative set of prostate biopsy. N=408 (65%) patient images were considered to be suspicious for PCa of which N=80 (12.8%) cases were considered to be suspicious for APC (defined as the presence of PCa anterior to the urethra). All patients underwent an 18 core TRUS guided biopsy of the peripheral zone and an additional 3 core TRUS targeted biopsy anterior to the urethra. The examinations were performed on a closed 1.0-T system combined with an endorectal body phased-array coil. e-fMRI included contrast-enhanced e-MRI (e-dcMRI) and diffusion weighted imaging (DWI).

Results: The median age of the patients was 67.3 years old (54-72) and median PSA values was 13.1 ng/ml (6.4-23.5). A positive digital rectal examination was evident only in N=8 patients (10%). 37.3% of patients had undergone one prior negative biopsy set, 41.5% two prior negative sets and 21.2% three prior negative biopsy sets. APC was detected in 90% of patients (N=72).

Conclusion: e-MRI of the prostate has a high predictive value in detecting APC. Patients with a constant increase of PSA levels, negative DRE findings and prior negative sets of prostate biopsy are ideal candidates for e-MRI of the prostate and subsequently TRUS targeted biopsies of possible suspicious anterior gland sites.

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THE ROLE OF TRANSURETHRAL RESECTION OF THE PROSTATE IN THE DETECTION OF PROSTATE CANCER IN PATIENTS WITH PREVIOUS NEGATIVE SETS OF BIOPSY AND LOWER URINARY TRACT SYMPTOMS

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

[1]Department of Urology, St. Antonius Medical Center, Gronau, Germany;
[2]Department of Urology, Martha Maria Medical Center, Nurnberg, Germany;

[3]Department of Urology, Interbalkan Medical Center, Thessaloniki, Greece

Introduction and Objective: To determine the clinical relevance of transurethral resection of the prostate (TUR-P) in patients with a clinical suspicion of prostate cancer (PCa), at least 1 negative extended prostate biopsy and mild, moderate or severe lower urinary tract symptoms (LUTS).

Methods: From April 2004 to July 2013, patients (N=134) with elevated PSA levels (>4 ng/ml), no signs of prostate cancer (PCa) after at least 1 extended negative TRUS prostate biopsy (18-core) and mild, moderate or severe LUTS underwent TUR-P for diagnostic purposes. The clinicopathological parameters of the patients included age, PSA levels, number of negative sets of biopsies, IPSS, prostate size, prostate tissue resected, detection rate of benign prostatic hyperplasia (BPH) in the resected tissue and detection rate of PCa in the resected tissue.

Results: The median age was 66.8 years (52-76) and median PSA levels were 9.1 ng/ml (4.5-23.5 ng/ml). 33.3% of patients demonstrated 1 extended negative set of biopsy, 33.3% 2 sets, 28.1% 3 and 5.3% 4 sets. 20.8% of patients had minor LUTS (IPSS 0-7, median 5.4), 48.9% moderate LUTS (IPSS 8-19, median 13.1) and 29.4% severe LUTS (IPSS 20-35, median 24.7). The median prostate size was 52.1 g (20-90 g) and the median prostate tissue resected was 326 g (6-72 g). After TUR-P was performed, the histologic analysis of the resected specimen revealed that 17.1% of patients had PCa (pT1a in 64.7% of cases and pT1b in 35.3% of cases). PCa was detected in 20% of patients with minor LUTS, in 14.9% with moderate LUTS and in 20.6% with severe LUTS. According to the number of negative biopsies, 12.5% of patients were diagnosed with PCa after 1 negative set of biopsy, 15.6% after 2 negative sets, 22.2% after 3 negative sets and 0% after 4 negative sets. Regardless of the histological outcome, all patients with moderate or severe LUTS experienced a significant symptomatic benefit.

Conclusion: The results of this study indicate that TUR-P can be an important diagnostic tool in patients with rising PSA levels, prior sets of negative biopsy, as well as mild, moderate and severe LUTS. The presence of PCa in the resected tissue could lead to a potentially curative benefit. The absence of PCa in the resected tissue will result in a clear symptomatic benefit and the remaining smaller prostate will be easier to sample if PSA increases again.

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PREDICTORS OF SIGNIFICANT OR HIGH GRADE PROSTATE CANCER IN PATIENTS OVER 75 YEARS OLD UNDERGOING PROSTATIC BIOPSY

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

[1]Department of Urology, St. Antonius Medical Center, Gronau, Germany;
[2]Department of Urology, Martha Maria Medical Center, Nurnberg, Germany;
[3]Department of Urology, Interbalkan Medical Center, Thessaloniki, Greece

Introduction and Objective: Several health authorities advocate the cessation of routine prostate biopsy (PB) in men older than 75 because of their belief that most patients will have a clinically insignificant and low grade prostate cancer (PCa) and subsequently will not benefit from radical therapy. The objective of this study was to indentify possible preoperative variables that stratify patients over 75 years old to exhibit significant or high grade PCa on PB.

Patients and Methods: A retrospective review of the records of patients (N=1875) that underwent a PB between April 2004 and April 2013 at two tertiary medical centers was conducted. Inclusion criteria for the PB were PSA levels >4 ng/ml or a suspicious finding on digital rectal examination (DRE). Inclusion criteria for this study were patients aged over 75 years. A multivariate analysis was conducted in order to detect potential predictors of a high grade or significant PCa. The parameters analyzed included: age, prostate size, PSA levels, positive DRE findings, PCa detection rate, clinically significant PCa detection rate (according to the Epstein criteria; PCa found in at least 3 biopsy needle cores or present in >50% of any one biopsy needle core or Gleason score is >6 or PSA density >0.15 or free PSA <15 %) and high grade PCa detection rate (defined by the presence of any elements of Gleason grade 4 or 5 in the biopsy specimen).

Results: N=435 patients were aged over 75 years. PCa was detected in N=265 of patients (61%), with 86.1% of them being defined as clinically significant ($p<0.001$) and 63.4% ($p<0.001$) as high grade. On multivariate analysis a PSA level >10 mg/dl ($p<0.05$) and positive DRE findings ($p<0.05$) were independent predictors for PCa but only a PSA level >10 ng/ml with a simultaneous positive DRE finding was a predictor for high grade or significant PCa ($p<0.001$).

Conclusion: Our findings suggest that although the prevalence of significant and high grade PCa in the elderly population is higher than previously thought, PB should be performed only in patients that exhibit high PSA levels and positive DRE findings simultaneously. Screening and diagnostic recommendations should reflect the potential benefit of identifying patients with aggressive prostate cancer even after an age of 75.

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APPROPRIATE USE OF CA125 IN MONITORING OF OVARIAN CANCER- ADVANTAGE OR DISADVANTAGE FOR AN INDIVIDUAL PATIENT

M. Zwirner, K. Korte

Frauenklinik , Universität Tübingen Calwer Str. 7 D-72076 Tübingen, Germany

CA125 serodiagnostics play a prominent role in monitoring activity of disease during all stages of epithelial ovarian cancer. CA125 as a screening parameter is not recommended. The EGTM group recommends the marker as an alternative prognostic factor when it is difficult to estimate the amount of multiple intra-abdominal metastases by imaging techniques, as well as for monitoring activity of disease during treatment. CA125 is established in the surveillance of ovarian cancer patients with a base line concentration preoperatively, as well as during chemotherapy and control periods. A major challenge in monitoring patients is to define a change in CA125 concentrations that reliably correlates with a changing tumor burden in both clinical response and progression as well. Response criteria as well as progression criteria including the marker have been established by Gyn.Oncol. societies. As a high percentage of patients exhibit advanced stages of ovarian carcinoma (Stage III or IV) during primary diagnosis post surgery, levels as well as progression criteria have an important role for consequences in therapy. A consequence of the EORTC study, which investigated the time of treatment for relapsed ovarian cancer including CA125 concentration was a reassurement for ovarian cancer patients that there is no benefit from early detection of relapse by routine CA125 measurements and even if CA125 rises, early chemotherapy did not influence overall survival compared to late treatment indicated by clinical symptoms. The European Society of Gynecologic Oncologists (ESGO) therefore has recently advised against universally abounding CA125 in routine follow-up of all patients with ovarian cancer. Regarding disease-related issues within the EORTC study there are still many open questions due to the heterogeneity of therapy performed within the study. The EGTM has recognized the special challenge with conducting and reporting clinical tumor marker monitoring studies. The EGTM stated therefore the differentiated use of CA125 for monitoring patients during treatment and follow-up and established criteria for therapy response as well as for CA125 based progression. The sampling intervals are still undefined, but current practice suggests that CA125 should be measured every 24 months for 2 years and then less frequently if the marker is silent. The oral presentation includes the use of CA125 course reflecting advantages and disadvantages as well as limitations based on a case report that may indicate the wide spread of appropriate use of the tumor marker CA125 during monitoring. According to the recommendations above several clinical situations during follow up were exhibited to show advantage or disadvantage for the individual situation of the patient.

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