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Biomarkers in cancer

Cod: M194

THE DIAGNOSTIC UTILITY OF PIVKA-II AND ALPHA-FETOPROTEIN (AFP) IN THE DIAGNOSIS OF HEPATOCELLULAR CARCINOMA

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INTRODUCTION
Hepatocellular carcinoma (HCC) is a liver malignant tumor with high mortality rate. Because HCC usually develops in the context of cirrhosis and as diagnostic imaging in early stages is difficult, it is important to find biomarkers that can help diagnose the onset of the disease. Alpha fetoprotein has been used so far, however, it is known that this tumor marker has a low sensitivity and specificity when used on its own..
A novel tumor marker, PIVKA-II or DCP is an immature form of the coagulation factor prothrombin synthetized in the liver and can be used to diagnose HCC.

OBJECTIVE
Analysis of the diagnostic utility of AFP and PIVKA-II in the study of hepatocellular carcinoma enabling differentiation from other pathologies.

MATERIALS AND METHODS
Prospective observational cohort study in which 101 patients were recruited from the Gastroenterology Service. In these patients, serum AFP and PIVKA-II were determined, both by the technique CLEIA (chemiluminescent enzyme immunoassay) in the analyzer LUMIPULSE® G600II (Fujirebio Europe NV, Gent, Belgium). Patients were grouped into the diseases listed in Table-1.
The diagnosis of hepatocellular carcinoma was performed in the service of Pathology by core needle biopsy (BAG). The results were included in a database and analyzed using SPSS 21.0 statistical program. Nonparametric Kruskal-Wallis tests were used, considering $p < 0.05$ as significant.

RESULTS
For two biomarkers, PIVKA-II and AFP, statistically significant differences between the group with newly diagnosed hepatocellular carcinoma and the other groups ($p < 0.001$) were obtained.
The area under the ROC curve for PIVKA-II was 0.946 ($p < 0.001$) , while for the AFP of 0.844 ($p < 0.001$) , which indicates that the marker PIVKA-II has greater diagnostic accuracy.

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ENORMOUS DISCREPANCY BETWEEN TWO CA 19-9 ASSAYS; CASE REPORT

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Interferences in quantitative immunoassays are quite possible in practice, so laboratory personnel should be aware of that challenge in everyday practice. We report the case of a woman with enormous discrepancy in CA 19-9 results measured by two immunochemistry analyzers.
The patient was 47 years old woman with initial diagnosis of Urticaria. First tests were performed in General Hospital laboratory in her hometown and the result for CA19-9 was >1200 U/mL (dilutions were not performed). CEA, AFP and CA15-3 were within their reference ranges. All markers were measured on Architect i2000, Abbott, USA and the method used was CLIA. After gastroenterologist exam she also underwent US exam of abdomen, MSCT, EGD, MRI of abdomen and colonoscopy. All findings were within physiological limits. She repeated measurements in our laboratory and definitive result for CA 19-9 was 5199 U/mL. We repeated analysis for all tumor markers using ECLIA method on Cobas e601, Roche, Germany. Values of other tumor markers were comparable, except for CA19-9 whose concentration was 8.25 U/mL. Patient had normal liver function tests, negative serum immunofixation and negative HBsAg and HCV. IgM was slightly elevated, RF in reference range and no prove of autoimmune disease was found in that moment. Enormous discrepancy in CA 19-9 results constrained us to exclude, as a first step, potential hook effect. Serial double dilutions were performed and possible hook effect was eliminated. In addition, we precipitated sera of the patients with PEG by mixing the identical volumes of 25% PEG (M.W.6000) and patient sera. After centrifugation, CA19-9 levels in supernatant were obtained on both systems. The difference after PEG precipitation was enormous for CA19-9 measured on Architect i2000 (5.8 U/mL; recovery 0.22%), contrary to Cobas e601 (5.2 U/mL, recovery 126.06%). We concluded that discrepancy was probably caused by protein interference, most likely due to complex formation between immunoglobulins and CA19-9 which leaded to falsely high values measured on one platform.
Presence of unacceptable results, disagreement of the laboratory test results with clinical data, different results obtained for the same analyte by two or more different immunoassays and non-linear dilutions should always draw attention of laboratory medicine specialist and cause adequate actions.

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Biomarkers in cancer

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THE LOSS OF ΔNP63 EXPRESSION PREDICTS THE SHORT-TERM RELAPSE AND PROGRESSION OF BLADDER CANCER AND PATIENTS' OUTCOME FOLLOWING TREATMENT

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Background: Bladder cancer (BlCa) represent the forth most commonly diagnosed malignancy of the Western male population. Accurate prognosis is a key-factor in establishing optimal therapeutic decisions; Patients' prognosis is mainly assessed by tumor stage and grade, however, disease heterogeneity is responsible for the highly variable clinical outcome of the patients of the same risk group. ΔNp63 (p40) is one of at least 6 isoforms of the TP63 gene, a member of the TP53 tumor suppressor gene family, and its role in cancer is poorly understood. The aim of the present study is the analysis of ΔNp63 expression regulation in bladder tumors and its clinical significance for the prediction of disease outcome following treatment

Methods: Total RNA was extracted following pulverization of bladder tumors and matched adjacent normal urothelium from 143 bladder cancer patients that underwent TURBT (Ta, T1) or radical cystectomy (T2-T4). Total RNA concentration and purity determined spectrophotometricaly. First strand cDNA synthesis performed by the reverse transcription of 1μg of total RNA using oligo(dT) primer and MMLV. ΔNp63 expression levels were thereafter quantified by an in-house developed and validated SYBR Green-based qPCR assay using HPRT1 as endogenous reference gene for normalization purposes and T24 bladder cancer cells as calibrator. Finally, extensive statistical analysis was performed for the study of ΔNp63 clinical value for prognosis of urothelial carcinoma patients.

Results: ΔNp63 expression was significantly increased in bladder tumors compared to normal urothelium ($p=0.002$). However, reduced ΔNp63 expression levels revealed in muscle-invasive (T2-T4) and high grade tumors compared to superficial (Ta, T1) ($p<0.001$) and low grade ($p<0.001$) tumors. Moreover, the lower ΔNp63 expression was also associated with high grade non muscle-invasive bladder cancer (NMIBC) patients ($p=0.004$) and higher EORTC-risk stratification ($p=0.003$) of the TaT1 patients. The survival analysis, using Kaplan-Meier curves and Cox regression models, clearly highlighted that the loss of ΔNp63 expression correlates with the stronger risk for short-term relapse and progression to muscle-invasive stage of the TaT1 patients, as well as the worse overall survival of the muscle-invasive (T2-T4) patients following tumor resection.

Conclusions: Our study highlights the correlation of ΔNp63 expression loss with significantly worse progression of bladder cancer patients following treatment and the clinical ability of ΔNp63 to predict disease outcome.

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Biomarkers in cancer

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THE EFFECTS TOXIC METALS EXPOSURE ON ORGAN FUNCTIONS, EXPRESSIONS OF 8-HYDROXY 21-DEOXYGUANOSINE, CYTOKERATIN – 19 FRAGMENTS (CYFRA 21 -1 PROTEIN) AND RISK OF LUNG CANCER IN SOME WELDERS.

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Introduction
The welding process has been known to generate fumes which contain toxic metals, therefore in the absence of best practices, welders are at high risk of adverse health effect due to inhalation of these metals.
Method
To investigate this line of thought, fifty welders (50) and forty (40) healthy volunteers were recruited as controls after ethical approval. Interviewer styled questionnaire was administered to all participants for demographic and occupational features. Lung function was assessed by spirometry, 10mls of urine was obtained from all subjects for protein urinalysis, 8-hydroxy-21deoxyguanosine concenrations and estimation of some heavy metals including iron, manganese, lead, chromium, zinc, nickel and cadmium using atomic absorption flame spectrophotometer (AAS). Blood obtained from venepuncture was used for plasma creatinine, troponin I ALT, AST, alkaline phosphatase, CK-MB, albumin, iron, transferring, ferritin, IgG, IgM, IgA, C3 and cytokeratin -19 fragments. Results were input into the computer system and statistical analysis were carried out using statistical package for social sciences software, student T -test, Mann- Whitney U test for degree of significance and Pearson correlation for dose- effect relationships.
Result
The mean ages of the welders and controls respectively were 42.97± 8.75, 40.36±7.51 $P = 0.100$.There were statistically significant differences in the values of urinary iron, lead, zinc, nickel, manganese and chromium $P<0.05$,but not in cadmium .There were also significant differences in plasma creatinine, unconjugated bilirubin, plasma iron, ferritin, transferrin, troponin I, total iron binding capacity, transferrin saturation, IgG, and C3, $P <0.05$. But not in ALT, AST, Alkaline phosphatase, CK-MB, IgA, and total bilirubin,. Furthermore iron concentrations were observed to be positively correlated with 8-hydroxy21 -deoxyguanosine and cytokeratin-19 fragments expressions.
Conclusion
Bad occupational practices in welders predispose them to exposure to higher levels of heavy metals which may lead to dysfunction of lungs, cardiac, kidneys, liver, immune system and risk of lung cancer which may be mediated by DNA oxidative damage by iron radicals. This underscores the necessity for health education, and periodic bio- monitoring for heavy metals exposure and effects in such welders.

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Biomarkers in cancer

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EFFECTS OF TUMOR-MICROENVIRONMENT CROSS-TALK ON INVASION AND MIGRATION OF OSTEOSARCOMA CELLS IN 2D AND 3D CO-CULTURE

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BACKGROUND: Tumor tissue is not a bulk of cancerous cells, it consist of cancer cells, fibroblasts, immune cells, blood vessels and non-cellular components. This structure is called tumor microenvironment. . Microenvironment plays an active role in tumor angiogenesis, invasion and migration, drug resistance and tumor progression. The aim of this study was to invesigate the effects of osteosarcoma- mesenchymal stem cell cross-talk on tumor behavior in 3-dimensional (3D) in vitro cancer model under normoxic and hypoxic conditions.
METHODS: Osteosarcoma cells and bone marrow-derived mesenhymal stem cells (BMSCs) were co-cultured under normoxic and hipoxic conditions and their invasion and migrationn rates were measured with xCELLigence DP (ACEA, Biosciences, San Diego, CA) real-time cell analysis system.
RESULTS: The invasion and migration rate of cells in 3D co-culture was found to increase significantly compared to the 2D. In addition, it has been shown that hypoxic conditions increase the migration and invasion of osteosarcoma cells compered to normoxic conditions, and this situation is triggered especially in 3D culture.
CONCLUSION: Prognostic evaluation of patients with osteosarcoma is limited to clinical parameters and molecular markers for tumor agressiveness effecting metastasis can't be defined accurately. At this point, 3D co-culture studies has been shown to reflect in vivo tumor structure and microenvironment relationships better. However, dynamism and components of tumor microenvironment should be analysed for detection of critical parameters independent of osteosarcoma subtypes.

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Biomarkers in cancer

Cod: M199

IMMUNE RELATED GENE EXPRESSION PROFILING DETERMINED DIRECTLY ON LIVER TISSUE PREDICTS PROGNOSIS OF HEPATOCELLULAR CARCINOMA PATIENTS.

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Background and aims: Human hepatocellular carcinoma (HCC) is the third cause of cancer-related mortality because of poor-prognosis due to recurrence after surgery. Literature data show alterations of the immune system leading to tumour tolerance and support the relevance of immune response for the clinical outcome of HCC. In the present study we evaluated the possible prognostic impact of immune gene expression profile determined directly on liver tissue samples.
Methods: RNA was extracted from frozen liver tissue samples of HCCs (T, n. 24) and non-tumorous tissues (NT, n. 27) obtained from 32 patients using the Norgen's Total RNA Purification Kit.
The immune gene expression profile was performed by the nCounter® GX Human Immunology v2 system (NanoString Technologies) that allows to detect the expression levels of 579 immune response related genes simultaneously. Inter-assay variability was assessed running a commercial normal liver RNA sample in each experimental session. Hierarchical clustering and survival analysis were carried out by nSolver® Analysis, TIGR MeV and Graph Pad Prism softwares.
Results: Median inter-assay CV% was 13% for both low and high-expression housekeeping genes, while mean signal (mean of low-level positive controls) to background (mean of negative controls) ratio was 4.7.
Unsupervised clustering of immune gene expression profiles identified two main clusters of expression both in T and in NT liver samples. Each expression cluster was associated with different median time to HCC recurrence (TTR; T: 18.5 vs 127 months; NT: 18.5 vs 46 months) but only T sample profiles were correlated to significantly different TTR (p=0.0048). No significant association was observed between liver immune gene profile and overall survival.
Preliminary results showed that worse prognosis was apparently related to lower expression of immune activation-related genes in T tissue.
Conclusions: The nCounter® Human Immunology v2 system appears to be a reproducible and reliable assay for the evaluation of immune gene expression directly on liver tissue without the need of isolating infiltrating cells. Moreover, immune gene expression profiles predictive of TTR can be identified in HCC tissues.

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Biomarkers in cancer

Cod: M200

HUMAN EPIDIDYMIS PROTEIN 4 IN COLORECTAL CANCER. A POTENTIAL MARKER.

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Introduction
Human Epididymis Protein 4 (HE4) is a secretory protein originally identified in the distal human epididymis. Serum levels of HE4 have been widely investigated in patients with ovarian and endometrial cancer as well as in lung cancer. A few studies have been carried out to evaluate the potential role in gastrointestinal cancer.

Objectives
The aim of this study is to evaluate the usefulness and the potential role of HE4 in colorectal tumor patients comparing with Ca19.9 and CEA.

Material and methods
89 healthy individuals (37 men and 52 women) and 75 diagnosed colorectal cancer patients (42 men and 33 women) were selected. No kidney disease, gynecological pathologies and other tumoral affections were found in both control and case groups. Case group was already under treatment.
Ca19.9 and CEA were performed using ADVIA Centaur XP Immunoassay System (Siemens®). HE4 was analyzed in the Cobas Elecsys E411 (Roche®).
Statistical analysis was performed with SPSS. Marker comparison test between case and control groups were made using U of Mann-Whitney Test. Student's t-test was made for Age comparison and Chi-squared test for gender comparison.
Receiving Operating Curve (ROC) was constructed carrying out a comparison between groups. Sensitivity (S), specificity (E) and cut-off points (COP) were calculated.

Results
HE4 median was 85.5 (46,2 IQR) pmol/L in case group and 50.1 (19 IQR) pmol/L in control group; Ca19,9 median was 17,0 (73,5) and 9,23 (11,0) respectively and CEA median was 2,80 (7,10) and 0,76 (0,93) respectively.
Statistically significant differences were found in Ca19.9, CEA and HE4 between case and control group ($p<0.001$ in all of them). But it was found statistically significant differences between age ($p<0.001$) so it was made an age standardization and the differences we found were still significants ($p<0.001$ in HE4; 0,015 in Ca19.9 and 0,007 in CEA).
ROC analysis showed an AUC of 0.9 with a COP of 64.9 pmol/ml (S=81.3% and E=87.6%) in HE4; 0.67 in Ca19.9 (S=36%, E=98.8%, COP= 32.9U/ml) and 0.76 in CEA (S=49.3%, E=95.1%, COP= 3.2U/ml) .

Conclusion
There are a few studies in which HE4 is evaluated in colorectal patients. In our study, HE4 shows a high prediction value comparing with Ca19.9 and CEA. HE4 has a high potential value as a tumor marker in these patients. More studies are needed with a high population in order to evaluate its potential role in diagnosis, treatment and management of colorectal cancer.

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Biomarkers in cancer

Cod: M201

PANCREATIC CANCER AND HUMAN EPIDIDYMIS PROTEIN 4

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Introduction
Human Epididymis Protein 4 (HE4) is a secretory protein originally identified in the distal human epididymis. It is related to ovarian and endometrial cancer. There are only a few studies in which HE4 has been investigated in pancreatic cancer mainly in tissue samples. The aim of this study is to elucidate the role of serum HE4 in pancreatic cancer.

Material and methods
89 healthy individuals (37 men and 52 women) and 41 diagnosed pancreatic cancer patients (13 men and 28 women) were selected. No kidney disease, gynecological pathologies and other tumoral affections were found in both control and case groups. Case group was already under treatment.
Ca19.9 and CEA were performed using ADVIA Centaur XP Immunoassay System (Siemens®). HE4 was analyzed in the Cobas Elecsys E411 (Roche®).
Statistical analysis was performed with SPSS. Marker comparison test between case and control groups were made using U of Mann-Whitney Test. Student's t-test was made for Age comparison and Chi-squared test for gender comparison.
Receiving Operating Curve (ROC) was constructed carrying out a comparison between groups. Sensitivity (S), specificity (E) and cut-off points (COP) were calculated.

Results
HE4 median was 97.2 (55.1) pmol/L in case group and 50.1 (19) pmol/L in control group; Ca19,9 median was 101U/ml (2247) and 9,23U/ml (11,0) respectively and CEA median was 2,80U/ml (3.9) and 0,76U/ml (0,93) respectively.
Statistically significant differences were found in Ca19.9, CEA and HE4 between case and control group ($p<0.001$ in all of them). But it was found statistically significant differences between age ($p<0.001$) so it was made an age standardization and the differences we found were still significants ($p<0.001$ in HE4; 0,025 in Ca19.9 and 0,001 in CEA).
ROC analysis showed an AUC of 0.94 with a COP of 63.2 pmol/L (S 87.8% and E 86.5%) in HE4; 0.75 in Ca19.9 (S=61,5%, E=100%, COP=35.5U/ml) and 0.84 in CEA (S=88.2%, E=72.8%, COP=1.3U/ml).

Conclusion
In our study, HE4 shows a high prediction value comparing with Ca19.9 and CEA. AUC of HE4 is better than Ca19.9 and HE4 with a excelent sensitivity and specificity. More studies are needed with a high population in order to evaluate its potential role as a clinical tumor marker.

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Biomarkers in cancer

Cod: M202

ROLE OF HUMAN EPIDIDYMIS PROTEIN 4 IN GASTRIC CANCER

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Introduction
Gastric cancer is the seventh more frequent cancer in spanish population with a 3.62% of the total of tumor. Ca19.9 and CEA are the tumor markers used in patients with gastric cancer. Human Epididymis Protein 4 (HE4) is a secretory protein originally identified in the distal human epididymis. There are only a few studies in which HE4 has been investigated as a gastric tumor marker. The aim of this study is to know the relationship between HE4 and gastric cancer.

Material and methods
89 healthy individuals (37 men and 52 women) and 26 diagnosed gastric cancer patients (14 men and 12 women) were selected. No kidney disease, gynecological pathologies and other tumoral affections were found in both control and case groups. Case group was already under treatment.
Ca19.9 and CEA were performed using ADVIA Centaur XP Immunoassay System (Siemens®). HE4 was analyzed in the Cobas Elecsys E411 (Roche®).
Statistical analysis was performed with SPSS. Marker comparison test between case and control groups were made using U of Mann-Whitney Test. Student's t-test was made for Age comparison and Chi-squared test for gender comparison.
Receiving Operating Curve (ROC) was constructed carrying out a comparison between groups. Sensitivity (S), specificity (E) and cut-off points (COP) were calculated.

Results
HE4 median was 86,7 (40,3) pmol/L in case group and 50.1 (19) pmol/L in control group; Ca19,9 median was 13,0U/ml (11,0) and 9,23U/ml (11,0) respectively and CEA median was 1,60U/ml (1,20) and 0,76U/ml (0,93) respectively.
Statistically significant differences were found in CEA and HE4 but it wasn't in Ca19.9 between case and control group ($p<0,001$ in HE4 and $p=0,013$ in CEA). But it was found statistically significant differences between age ($p<0.001$) so it was made an age standardization and we only found differences in He4 ($p<0.001$).
ROC analysis showed poor value in Ca19.9 and CEA with an AUC of 0.6 (S=24%, E=100%, COP= 40.5 U/ml) and 0.66 (S=60%, E=74.1%, COP= 1.4 U/ml) respectively. AUC of He4 was 0.94 with a COP of 62.7 pmol/L (S=92.3% and E=85.4%).

Conclusion
Since HE4 shows an excelent AUC with a good sensitivity and specificity, better than Ca19.9 and CEA it suggests that there is a relationship between HE4 and gastric cance but bue to the design of this study and the low population, more studies are needed with a prospective double-blinded and with high population to assess the usefullness of HE4 as a gastric tumor marker.

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Biomarkers in cancer

Cod: M204

URINARY BIOMARKER FOR PROSTATE CANCER

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The early diagnosis of prostate cancer and the identification of new prognostic factors are the most important targets in the prostate cancer research.
This study tests a panel of cancer-specific markers in urine samples in order to have an early PC (Prostate Cancer) diagnosis.
120 urine samples have been collected right after DRE (Digital Rectal Exam) of the 120 candidates for biopsy and tested.
Using the Real-Time PCR (Polymerase Chain Reaction) technique the PCA3 (Prostate Cancer gene 3) expression and the methylation stutus of GSTP1 (Glutathione S-transferase P) are evaluated.
The PSA (Prostate Specific Antigene) has been studied in every patient. The results show that PCA3 is over-expressed in the prostate cancer cells while it is not discriminatory in patients with PIN (Prostatic Intraepithelial Neoplasia), PIA (Proliferative Inflammatory Atrophy) and PBH (Prostatic Benign Hypertrophy).
As GSTP1 in PC is hypo-methylated in PIA and PIN 40-80% is methylated.
The PSA is not significant for PC, PIN and PIA but it is significant for PBI at 80%.
The patients showing an over-expression PCA3 will show a PC positive biopsy.
GSTP1's methylation is correlated with carcinogenesis process and, more specifically, with PC due to its role in cellular cycle regulation.

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Biomarkers in cancer

Cod: M205

ASSESSMENT OF SERUM TUMOR MARKER CHROMOGRANIN A CONCENTRATIOS IN PATIENTS WITH NEUROENDOCRINE TUMORS

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BACKGROUND
Chromogranin A (CgA) is a 48 kDa glycoprotein, member of the granin family, exists within all type of neurons. CgA was a reable diagnostic biomarker for neuroendocrine tumors (NETs).
METHODS
Measurements of CgA were performed by ELISA method (DRG Instruments GmbH, Germany). CgA levels were measured in serum obtained from 24 healthy subjects and 98 patients with NETs. Median age of patients with NETs was 56 year and cohort included 50 females and 48 males.
REZULTS
The inter-assay coefficient of variation (CV) for ELISA assay was 7.2% and 6.8% at 60 µg/L and 96 µg/L and the intra-assay CV was 5.3% and 2.7% at 200 µg/L and 296 µg/L, respectively. Median values for CgA levels were significantly higher in patients with NETs compered to control group (32.3 vs. 212.2 µg/L, $p<0,0001$). Serum levels CgA were elevated in 75% (74/98) NETs patients.
CONCLUSIONS
The presented results performed by ELISA method for determination of human chromogranin A in serum showed an acceptable precision. Serum CgA levels were specifically elevated in NETs patients.

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Biomarkers in cancer

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DIAGNOSTIC CHALLENGES IN THE STUDY OF OLIGOSECRETOR COMPONENT

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Background: The detection of a monoclonal protein is fundamental in the diagnosis of patients with monoclonal gammopathies such as Multiple Myeloma (MM), Primary Amyloidosis and Light Chains MM. When the monoclonal protein is presented in low concentrations, it may be difficult to detect using conventional methods based on electrophoretic techniques like serum protein electrophoresis (SPE) and serum immunofixation (IFE). However, the quantification of serum free light chains (sFLC) is more sensitive than conventional methods. A algorithm based in the combination of sFLC and SPE presents a high sensitivity in the diagnostic study of monoclonal gammopathies.

Methods: study of five patients with suspect of monoclonal gammopathies where the algorithm (sFLC+SPE) was applied. Serum FLC were quantified by the assay Freelite (The Binding Site) and SPE were performed on Capillarys 2 (Sebia).

Results: The results are shown in the table.

Case 1 (Man, 68 years)
Clinical findings: Macrocytic anemia (9.0 g/dl hemoglobin), rouleaux formation of erythrocytes, discrete pancytopenia.
Algorithm (sFLC+SPE): Small peak in SPE (0.10 g/dL), sFLC ratio very abnormal (kappa=14450 mg/L, lambda=4.9 mg/L, ratio=2949) and immunoparesis of the immunoglobulins.
Diagnosis: Light Chain Kappa MM

Case 2 (Female, 75 years)
Clinical findings: Acute kidney injury, edema and proteinuria
Algorithm (sFLC+SPE): SPE negative, sFLC ratio very abnormal (kappa=17.7 mg/L, lambda=1800 mg/L, ratio=0.009) and immunoparesis of the immunoglobulins.
Diagnosis: Primary Amyloidosis

Case 3 (Male, 57 years)
Clinical findings: Intense back pain
Algorithm (sFLC+SPE): SPE negative, altered sFLC ratio (kappa=31.6 mg/L, lambda=15.4 mg/L, ratio=2.05)
Diagnosis: Non Secretory MM

Case 4 (Male, 57 years)
Clinical findings: Pathological fracture
Algorithm (sFLC+SPE): SPE negative, altered sFLC ratio (kappa=148 mg/L, lambda=5.6 mg/L, ratio=26.3)
Diagnosis: Light Chain Kappa MM

Case 5 (Male, 54 years)
Clinical findings: Severe bone pain in the chest, anemia and thrombocytopenia
Algorithm (sFLC+SPE): Small peak in SPE negative (1.17 g/dL), altered sFLC ratio (kappa=3.22 mg/L, lambda 4025 mg/L, ratio=0.0008)
Diagnosis: IgD Lambda MM

Conclusions: Quantification of sFLC it allows us to detect the presence of small amounts of monoclonal proteins in Light Chain MM, amyloidosis and Non Secretory MM that couldn´t have been detected by conventional methods.

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Biomarkers in cancer

Cod: M207

ASSOCIATION OF ANTI-HU ANTIBODIES WITH TUMOURS OF UNKNOWN ORIGIN

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Background: The ANNA-1 or anti-Hu antibodies are directed against an antigen localized in the nucleus of all neurons. They are directed against a family of RNA binding proteins with a molecular size of 35-40 kDa. They are expressed in the nuclei of neurons of the central and peripheral nervous system. Paraneoplastic syndromes associated with this antibody are sensory neuropathy, encephalomyelitis, cerebellar degeneration with autonomic dysfunction and limbic encephalitis. The tumours associated with the presence of this antibody are small cell lung cancer, prostate cancer, breast cancer, neuroblastoma and sarcoma.

Methods: We report five patients with paraneoplastic syndromes and the presence of anti-Hu antibodies were detected. Onconeural antibodies were identified in serum sample by indirect immunofluorescence (Euroimmun) and the positive results were confirmed on immunoblot assay (Euroimmun).

Results:

Case 1 (Male, 79 years)
Paraneoplastic syndromes: Limbic encephalitis
Antibody title: Anti-Hu 1/100
Diagnosis of the patient after study: Squamous cell lung cancer
Survival: Deceased (2 months)

Case 2 (Male, 67 years)
Paraneoplastic syndromes: Paraneoplastic encephalitis
Antibody title: Anti-Hu 1/1000
Diagnosis of the patient after study: Lung adenocarcinoma
Survival: Deceased (19 months)

Case 3 (Female, 50 years)
Paraneoplastic syndromes: Paraneoplastic encephalitis
Antibody title: Anti-Hu 1/1000
Diagnosis of the patient after study: Small cell lung cancer
Survival: Deceased (7 months)

Case 4 (Female, 76 years)
Paraneoplastic syndromes: Paraneoplastic encephalitis
Antibody title: Anti-Hu 1/100 + anti-SOX 1/100
Diagnosis of the patient after study: Squamous cell carcinoma of the tonsil
Survival: Deceased (9 months)

Case 5 (Female, 52 years)
Paraneoplastic syndromes: Sensory neuropathy
Antibody title: Anti-Hu 1/100
Diagnosis of the patient after study: hidden tumour
Survival: Alive

Conclusion: The presence of anti-Hu antibodies was associated to cancer in four patients while in the remaining patient was not found a tumour. In these four patient, the presence of anti-Hu antibodies was associated with a poor prognosis with short survival time. In summary, the presence of this antibody should help the clinician towards finding a hidden tumour, foremost among them, small cell lung cancer presents in 80% of cases of positivity for this antibody.

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Biomarkers in cancer

Cod: M208

EVALUATION OF HCG AS A MARKER OF PARATHYROID CARCINOMA

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Introduction
Parathyroid carcinoma (PCa) is a rare disease which accounts for less than 1% of primary hyperparathyroidism (PHP) and is associated with more severe clinical features. Differentiating PCa from PHP is challenging. We showed that an inverted third-generation to second-generation PTH ratio occurred in the majority of patients with advanced PCa and was absent in all 245 controls. Human chorionic gonadotropin (hCG) is a tumor marker in trophoblastic and nontrophoblastic cancers and hyperglycosylated hCG is increased in hCG-secreting malignancies. A study has shown that hCG might have the potential to become a marker of disease progression in malignant parathyroid disease. In this study, we investigated whether the hCG +β kit from Roche Diagnostics could distinguish PCa patients from PHP and add potentially prognostic information.
Material and methods
We used the leftover samples of 8 patients suffering from advanced PCa that came to the CHU de Liege for immunotherapy treatment. Most of these patients died a few weeks after the initiation of this last-chance therapy, but one is still alive after more than 10 years. 6 out of 8 patients presented an inversion of the PTH3/PTH2 ratio. We used a group of 20 PHP patients as comparative. hCG+β kit on Cobas (Roche Diagnostics) uses 2 monoclonal antibodies that recognize holo-hCG, nicked hCG, β-core fragment and free β-subunit. Limits of detection and quantification are <0.1 and <0.6 mUI/mL.
In nonpregnant and postmeopausal women and in men hCG (p95) is <1 (5.3), <7 mUI/mL (8.3) and <2 (2.6) mUI/mL, respectively.
Results.
The 8 PCa patients (3 women) presented positive hCG values at 1.29, 3.46, 5.7, 24.2, 31.2, 34.1, 36.5 and 164 mUI/mL, respectively. Values at 1.29 and 3.46 were obtained in 2 postmenopausal women. The lowest value was presented by the only still alive patient. There was a significant correlation (0.786; ρ <0.05) between HCG and PTH and a borderline correlation (0.750; ρ =0.05) between HCG and Ca concentrations. One patient presenting an inverted PTH3/PTH2 ratio had a positive HCG value (24.2) whether the second one was one of the postmenopausal women presenting HCG value at 3.46 mUI/mL. All the patients from the PHP group presented undetectable HCG values.
Conclusions
We confirm that hCG could be an interesting marker in the diagnosis of PCa. Since the only patient still alive presents the lowest values, hCG could thus be predicitive of the severity of the disease.

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Biomarkers in cancer

Cod: M209

PROSTATE HEALTH INDEX (PHI): THE IMPLEMENTATION OF NEW SOLUTIONS IN PRE-ANALYTICAL STAGE

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Background
The prostate health index (phi) is a combination of PSA, freePSA and [-2]proPSA results. The clinical performance of phi is strongly dependent on the accurate measurements of the serum biomarkers used to calculate it. The pre-analytical conditions for blood collection, serum preparation and transportation are critically important. In this study, various pre-analytical conditions that could affect phi results accuracy were compared to identify the optimal procedures for routine clinical practice.
Materials and Methods 22 men with initial PSA level < 12 ng/mL were recruited. Blood was collected from each man and serum samples were prepared and stored in various conditions. As reference condition (n°1), the blood collected and serum prepared according to phi index manufacturer instructions. For condition n°2 and n°3, the blood was kept at 2–8°C for 10 hours with or without centrifugation before serum preparation - non-separated serum from the clot. For condition n°4 and n °5, Vacuette closed tube system was used to get serum aliquot from closed tube and serum refrigerated at 2–8°C or frozen at -20°C. The PSA, freePSA and [-2]proPSA were measured on immunochemistry analyzer Access2 (Beckman Coulter Inc.). The percentage of variation was calculated for all samples as compared to the reference condition (n°1), Wilcoxson test for paired data was used to determine the statistical significance of the difference between the results.
Results The percentage of variation analysis indicates small but significant changes in the PSA (96%) and phi (107 to 109%) results for non-separated conditions (2 and 3) as compared to the reference condition but no significant variation was observed for % free PSA. The variations observed for closed tube conditions (4 and 5) were minimal for PSA (97%), free PSA (103%) and for phi (97%). The variation of the phi results observed for closed tube condition with sample freezing was not statistically significant.
Conclusion Pre-analytical procedures including non-separated serum in primary tube could lead to an overestimation of phi results with potential reduction of clinical accuracy. The use of closed-tube with serum freezing stage can preserved the quality of the phi results ensuring optimal clinical performance while facilitating the sample collection for optimal implementation of the phi index in routine practice.

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Biomarkers in cancer

Cod: M210

URINARY EXOSOMAL miRNAS AS BIOMARKERS OF PROSTATE CANCER DETECTION AND PROGNOSIS

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Background: Prostate cancer (PCa) is a very heterogeneous disease, including patients with low-risk of progression, in which cancer-specific survival rates exceed 99% at 15-year follow-up. New biomarkers are required to distinguish PCa patients according to their prognosis. Active surveillance has been proposed for low-risk patients. Aberrant miRNAs expression has been demonstrated in PCa, playing a critical role in tumor initiation, development and progression. Recent research showed that exosomes could be a promising source of new biomarkers, including miRNAs, due to their implication in carcinogenesis. Our aim was to evaluate the usefulness of the urinary exosomal miR-21, miR-141, miR-214, miR-375 and let-7c in PCa as biomarkers for the detection and prognosis of PCa.

Methods: We collected freshly voided urine samples after a prostate massage from 55 patients with PCa and 13 healthy controls. PCa patients were classified according to the D'Amico risk criteria: 4 patients with low risk PCa (cT1–cT2a, Gleason < 7 and PSA ≤ 10 μg/L), 11 with intermediate risk PCa (cT2b or Gleason = 7 or PSA 10-20 μg/L) and 40 with high risk PCa (cT2c or PSA > 20 μg/L or Gleason >7). Exosomes were isolated by differential centrifugation and the presence of exosomes was confirmed by electronic microscopy. Total RNA was isolated from exosomes using the miRNeasy reagent (Qiagen®). We analyzed the miRNAs expression using qRT-PCR analysis (Abi Prism 7300) after a preamplification (TaqMan® PreAmp). The results were normalized using cel-miR-39. Relative expression was calculated for every miRNA by the method $\Delta\Delta$Ct.

Results: Significant differences between PCa patients and healthy controls were found for miR-21 (p=0.010), miR-141 (p=0.048), miR-375 (p=0.001) and let-7c (p=0.025). We studied the prognostic value of miRNAs comparing patients with intermediate and high-risk PCa against low-risk PCa patients and healthy subjects. MiR-21 (p=0.004), miR-375 (p=0.001) and let-7c (p=0.048) were overexpressed in PCa patients with intermediate and high-risk.

Conclusions: These preliminary results showed that urinary exosomal miRNAs can be useful biomarkers for the detection and prognosis of PCa, showing higher expression in intermediate and high-risk PCa patients for miR-21, miR-375 and let-7c.

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Biomarkers in cancer

Cod: M211

SERUM LEVELS OF [-2]proPSA IN PATIENT WITH DUTASTERIDE TREATMENT

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Backround:5α-Reductase inhibitors are widely used in the treatment of benign prostatic hyperplasia. However, randomized clinical trials have raised concerns that their use may be associated with an increased risk of high-grade prostate cancer tumors that would ultimately lead to worse prostate cancer outcomes. A doubling factor is effective for maintaining the sensitivity and specificity of PSA for prostate cancer detection but to date, no study was addressed new biomarkers used in early cancer detection such as proPSA and Prostate Health Index (PHI).
Objectives: Monitoring changes in the serum levels of biomarkers PSA, freePSA, [-2] proPSA and PHI during dutasteride treatment after 3, 6 and 12 months in patient with benign prostate hypertrophy.
Methods: The Immunoanalytical Laboratory of the University Hospital in Pilsen examined sera of 30 patients from the Urology department of the University Hospital. We assessed the levels of PSA, freePSA, [-2]proPSA and we calculated the Prostate Health Index (PHI). Serum biomarkers were measured using the chemiluminescent DxI 800 instrument (Beckman Coulter, USA). SAS 9.3 software was used for statistical analysis.
Results: The mean levels of PSA decreased from 6.28 to 2.85 ug/L, [-2]proPSA from 14.1 to 3.0 pg/mL and PHI from 29.9 to 12.9 after 12 months. The median of decrease of PSA after 12 months was 0.5094 (95%CI 0.4180, 0.5941), [-2]proPSA 0.4795 (95%CI 0.3138, 0.5421) and PHI 0.5696 (95%CI 0.5000, 0.6622).
Conclusion: If the decision for a biopsy is only based on a increase in the serum PSA values, a variable percentage of potentially aggressive tumors cannot be diagnosed. The use of PHI may be helpful in this regard, but more data and verification in a larger cohort of cases is needed.
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Biomarkers in cancer

Cod: M212

THE DIAGNOSTIC AND PREDICTIVE VALUES OF HUMAN EPIDIDYMIS PROTEIN 4, CA 125 AND ROMA INDEX IN ENDOMETRIUM CANCER

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Background: Endometrial carcinoma (EC) is one of the most widespread gynecologic malignancy. The reliability and performance studies of CA -125, and HE4 in EC have been evaluated in previous studies. In this study, our purpose was to evaluate the clinical performance and prognostic and predictive efficacy of serum HE4, CA 125, and the risk of malignancy algorithm (ROMA) index in different stages of EC.
Methods: The study group comprised 64 patients with EC (median age 58), 60 subjects with benign uterine diseases (median age 51) and 34 healthy subjects as the control. Fasting blood samples were collected prior to surgery for the determination HE4 and CA 125. These markers were studied by electrochemiluminescence method using E170 autoanalyzer from Roche. Patients were staged according to International Federation of Gynecology and Obstetrics (FIGO) surgical staging. Most patients presented with stage 1A (62.5%). Receiver operating characteristic (ROC) curves were performed to establish the thresholds in order to determine the predictive values of markers .
Results: HE4, CA 125 and ROMA 2 index in EC patients were relatively higher than those in controls but only HE4 and ROMA 2 were significantly different (p=0.000, for both). HE4 was also different with regard to benign disease (p=0.005).HE4 and ROMA2 levels were also high in patients with stage IB, stage II or higher compared to stage 1A patients and controls (p=0.000).However, Ca 125 levels were only higher in stage 1B and stage II and higher group compared to benign disease and stage 1A ($p<0.05$). The best cut-off points determined by ROC curve were 59.7pmol/L for HE4 ; 14.2 U/mL for CA 125. AUC for HE4 (83%) resulted higher in comparison to CA 125 (44%) and ROMA alone (77%) in all EC patients. HE4 displayed a sensitivity of 71.4% with a specificity of 64.2, and a negative predictive value (NPV) of 82.9%, and ROMA 2 index had a sensitivity of 42% with a specificity of 84.1%, and a NPV of 75% in stage 1A. Sensitivities of HE4 and ROMA 2 increased according to higher stages; 100 % for HE4 and 94.1% for ROMA2 in stage II and higher.
Conclusion: The preoperative determination of HE4 and ROMA index are valuable markers for discriminating the endometrial cancer from benign disease and also beneficial for the stratification of tumor depending on their association with the tumor stages.

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Biomarkers in cancer

Cod: M213

RASSF1A GENE PROMOTER METHYLATION IN PRIMARY TUMORS, ADJACENT MORPHOLOGICALLY NORMAL TISSUES AND PLASMA SAMPLES OF PATIENTS WITH HIGH GRADE SEROUS OVARIAN CANCER

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BACKGROUND: RASSF1A promoter methylation is frequent in high grade serous ovarian cancer (HGSC), the most common histological subtype. We examined RASSF1A promoter methylation in primary tumors, adjacent morphologically normal tissues and corresponding plasma samples of patients with HGSC, using real-time methylation specific PCR (real-time MSP) and methylation-sensitive high-resolution melting analysis (MS-HRMA) for the detection and semi-quantitative estimation of methylation, respectively.
METHODS: A training group of 67 primary HGSC FFPEs was first analyzed using both real-time MSP and MS-HRMA. Our validation group consisted of 61 primary HGSC FFPEs, 58 adjacent tissues (analyzed using both assays), and 59 corresponding plasma samples (analyzed using real-time MSP). The specificity of both assays was evaluated by analyzing a small group of 16 fallopian tube samples and a larger group of 51 plasma samples obtained from healthy women. OVCAR29 and IGROV1 ovarian cancer cell lines were used as positive controls.
RESULTS: In the training group, RASSF1A promoter methylation was detected in 27/67 (40.3%) by real-time MSP and in 27/67 (40.3%) by MS-HRMA (Agreement=94.0%, P<0.001, Cohen's kappa=0.876). In the validation group, the values were 25/61 samples (41.0%) and 28/61 samples (45.9%), respectively (Agreement=95.1%, P<0.001, k=0.900). In the group of adjacent morphologically normal tissues, 17/58 samples (29.3%) were found methylated by real-time MSP and 21/58 samples (36.2%) by MS-HRMA (Agreement=86.2%, P<0.001, k=0.689). According to the semi-quantitative MS-HRMA, in most positive cases, RASSF1A promoter methylation was detected at a lower percentage in the adjacent morphologically normal tissues, compared to the paired primary tumors. In corresponding plasma samples, RASSF1A promoter methylation was observed in 15/59 samples (25.4%) by real-time MSP whereas no RASSF1A promoter methylation was found in both groups of normal samples using these assays.
CONCLUSIONS: RASSF1A promoter is highly methylated in primary tumors and at lower percentages in the adjacent normal tissues. Interestingly, RASSF1A promoter methylation was also observed in cfDNA. In all cases, MS-HRMA gave comparable results with real-time MSP.

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Biomarkers in cancer

Cod: M214

NOVEL LECTIN-NANOPARTICLE CONCEPT TO SPECIFICALLY RECOGNIZE CANCEROUS ISOFORMS OF GLYCOPROTEINS BIOMARKERS OF DIFFERENT CANCERS

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BACKGROUND: The great majority of circulating cancer biomarkers are non-specific as substantial overlap in concentrations may be found with samples from healthy subjects and patients with benign diseases. Thus, most of them are used only for follow-up of the disease and monitoring the treatment. Cancer markers are mostly glycoproteins and altered glycosylation is a universal feature of cancer cells. Detection of cancer-related glycosylation changes in conventional biomarkers is highly attractive for early cancer detection. Lectins are carbohydrate-binding proteins and can be exploited for specific recognition of these changes.
METHODS: We have established a lectin library, where individual lectins with known glycostructure specificity are immobilized onto fluorescent Europium-chelate-doped 97 nm nanoparticles (Eu+3-NPs) making them multivalent and highly reactive toward the target. The library was used for screening of multiple glycoprotein markers isolated from benign and malignant sources.

RESULTS: Using lectin-coated Eu+3-NPs, analytically sensitive cancer associated glycoprotein-lectin assays were developed that specifically recognize the isoforms of cancer biomarkers produced by cancer cells, whereas the detection of glycoproteins from benign conditions was reduced. This approach has been applied for CA125, PSA, CA15-3 and CEA derived from ovarian, prostate, breast and colon cancer specific cell line, respectively.

CONCLUSIONS: The improved analytical specificity of this test approach is dependent on a discriminating lectin immobilized in large numbers on Eu+3-NPs, providing both an avidity effect and signal amplification. The novel Lectin-Nanoparticle concept could be a trend-setting opportunity for early diagnostics in parallel with other timely approaches: circulating nucleic acid, exosomes (liquid biopsy) and play a part in determining cancer glycomics for “big data” approaches in the field of cancer omics. Finally, using appropriate combinations of lectins and antibodies, applying the lectin NPs concept can also be explored for other diagnostic targets, where changes in glycosylation are indicative of an ongoing disease process.

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Biomarkers in cancer

Cod: M215

CHEMOKINES MEASUREMENT IN EXOSOMES PRESENTS CLINICAL UTILITY IN CANCER

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BACKGROUND
Exosomes are microvesicles with increasing relevance in cancer research as potential biomarkers. Tumor-derived exosomes can contain molecules immunosuppressive or stimulant of the immune response against the tumor. The aim of this study was to quantify different chemokines in both serum and exosomes and evaluate their utility in cancer.
METHODS
Patients with prostatic (10), renal (9) and lung cancer (11) not receiving chemotherapy were selected for the study. After informed consent, peripheral blood was drawn and serum was kept at -80ºC until analysis. Healthy volunteers (10) were also included as controls. Exosomes were isolated from serum with ExoQuick (System Biosciences), according to manufacturer's instructions. Chemokines were quantified in both serum and serum-derived exosomes using a Luminex assay (R&D Systems). Ratio between concentrations in exosomes and serum was calculated. Statistical analysis was performed with GraphPad software.
RESULTS
IL-8, CXCL5, GRO beta and MIF chemokines could be detected in serum in controls and in all types of cancer evaluated. Regarding their levels in exosomes, IL-8 was only detected in cancer patients (80% in prostatic, 33% in renal and 45% in lung cancer) but not in controls ($p=0.003$). Very interestingly, levels of MIF were increased in exosomes from patients with prostatic cancer when compared with exosomes from healthy volunteers ($p<0.05$), whereas no difference was detected in serum. This difference was also observed when comparing exosomes/serum ratio ($p<0.05$). GRO beta levels were higher in prostatic cancer when compared with control group both in serum ($p<0.05$) and in exosomes ($p<0.05$). Even more, ROC curves for GRO-beta in exosomes presented slightly higher area under curve than serum analysis (0.90 vs 0.86). Related to CXCL5, no difference was observed between controls and cancer patients neither in serum nor in exosomes.
CONCLUSIONS
IL-8 can be detected in exosomes from cancer patients but not in controls. Other chemokines such as GRO beta presented higher efficiency when measured in exosomes than when measured in serum and MIF only presented utility when measured in exosomes. For that reason, chemokines evaluation in cancer should not be limited to serum but should be also performed in exosomes.

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Biomarkers in cancer

Cod: M216

EVALUATION OF SIGNIFICANCE OF SERUM BETA 2 MICROGLOBULIN AS A PROGNOSTIC MARKER IN NON HODGKIN LYMPHOMA

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Background : Lymphoma has been described as the proliferation of lymphoid cells, which arise as discrete tissue masses. It has been broadly divided into non -Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma (HL). NHL contributes to about 85 % of all malignant lymphomas.
Beta 2 microglobulin (B2M) is a small (11,800-dalton) protein that is present in nearly all nucleated cells and biological fluids, including serum, urine, and synovial fluid. It forms the light chain subunit of the MHC class I antigen. The objective of this study was to determine the role of beta 2 microglobulin in the prognosis of patients with NHL to strengthen its potential role as a convenient non-invasive biomarker.

Methods: Thirty diagnosed cases of NHL and thirty age and sex matched healthy controls participated in the study. Serum levels of B2M were estimated in newly diagnosed patients before initiating treatment and in controls by enzyme linked immunosorbent assay (ELISA). The patients were treated with CHOP Regimen (cyclophosphamide, hydroxydaunomyicin, oncovin, and prednisolone). Serum B2M was estimated again upon completion of chemotherapy.

Results: Serum B2M levels were found to be significantly higher ($P< 0.01$) in NHL patients (4.60±2.24 μg/ml) than in controls (0.47± 0.30 μg/ml); they were also higher in patients in advanced stages (stage III and IV) (8.30±0.099 μg/ml) than those in early stages (stage I + II) ($P<0.01$). The levels significantly decreased after therapy ($P< 0.01$) and were lower in patients achieving remission (3.92 ±1.78 μg/ml) than in those who did not show remission (8.52 ± 1.58 μg/ml).

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Biomarkers in cancer

Cod: M217

ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE İNTRON4 VNTR POLYMORPHISM IN PATIENTS WITH BREAST AND GASTROINTESTINAL CANCERS.

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Background: Endothelial nitric oxide synthase (eNOS), also known as NOS3 is an enzyme that in humans is encoded by the NOS3 gene located in the 7q35-7q36 region of chromosome 7. Nitric oxide (NO) is synthesized from L-arginine by eNOS in the vascular endothelium and plays crucial roles in cellular proliferation. In the present study, it is hypothesized that polymorphisms of the Intron4b/a variable number of tandem repeat (VNTR) polymorphism Intron4b/a in the eNOS gene may be associated with an increased risk in the developing breast and gastrointestinal cancers. Methods: We included 38 (38 F/Female) patients with breast cancer, 56 (18F/38M) patients with gastrointestinal cancer, and 70 (44M/26F) healthy controls. The VNTR polymorphism in intron4 (intron4b/a) was analyzed by PCR. The results were statistically analyzed by calculating the odds ratios (OR) and 95% confidence intervals using the $\chi 2$ test. Results: The distributions of genotype and allele frequency was compared among the groups. The bb, ab and aa genotypes were observed in 29 [76.3%], 9 [23.7%], 0 [0%] patients with breast cancer, and in 41 [73.2%], 13 [23.2%], 2 [3.6%] patients with gastrointestinal cancer, and in 33 [47.1%], 15 [21.4%], 22[31.5%] healthy controls respectively. The b and a alleles were observed in 67 [88.2%], 9 [11.8%] patients with breast cancer, and in 95 [84.8%], 17 [15.2%] patients with gastrointestinal cancer, and in 81 [57.8%], 59 [42.2%] healthy controls respectively. The susceptibility to patients with breast and gastrointestinal cancer had significantly higher frequencies in bb genotype (p=0.004 OR=3.613 and p=0.004 OR=3.065 respectively). The patients with breast and gastrointestinal cancer had significantly lower frequencies in aa genotype (p=0.001,OR=1.458 and p=0.001, OR=12.375respectively). The frequency of the a allele was significantly lower in the patients with breast and gastrointestinal cancer (p=0.001, OR=4.070). Conclusion: We conclude that there was sensible correlation between eNOS gene intron 4b/a VNTR polymorphism and the risk of breast and gastrointestinal cancer and bb genotype frequency was significantly higher in these patients than healthy controls.

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Biomarkers in cancer

Cod: M218

REGULATION OF MONOCLONAL PROTEIN (MONOIG) AND NORMAL IMMUNOGLOBULIN SECRETION BY CYTOKINES (S-SYNDECAN-1, BLYS & TGF-BETA-1) IN PATIENTS WITH PARAPROTEINEMIA ON PRESENTATION. PROGNOSTIC AND DIAGNOSTIC IMPLICATIONS OF THE USE OF INNOVATIONS IN IMMUNOG

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Introduction: Multiple Myeloma (MM), Waldenstrom's Macroglobulinemia (WM) and Chronic Lymphocytic Leukemia (CLL), where 50% of the cases also present increased levels of serum free light chains (sFLC), are the most common lymhpoproliferative diseases with monoIg secretion. MonoIgs are secreted by plasmacytes infiltrating the bone marrow. Local factors of the niche such as sol syndecan-1 (sSynd1) and BLyS promote normal plasmacyte development and secretory function, whereas others like TGFβ1 inhibit it. Estimation of monoIg is mandatory in MM and WM for diagnosis and follow-up of patients. In CLL sFLC have prognostic value. Total secreted immunoglobulin does not accurately reflect disease burden. Heavy chain assays using "HevyliteTM" measure individual IgAκ, IgAλ, IgGκ, IgGλ, Igμκ, Igμλ molecules thus estimating more accurately the monoclonal fraction and the degree of repression of polyclonal immunoglobulins.

Aim: To investigate possible correlation of serum levels of sSynd1, BLyS and TGFbeta1 and the amount of the monoIg, polyIg and FLC and their impact on disease outcome.

Material and Methods: 269 patients were recruited. 105 of them diagnosed with MM (79 IgG, 26 IgA and 33%, 31% and 36% ISS stage 1, 2 and 3 respectively). 64 with MW (44%, 28%, 28% WM-IPSS stage: 1, 2 and 3 respectively) and 100 with CLL (67%, 23%, 10% Binet stage 1, 2, 3 respectively). Patients were followed until last visit or death (median follow-up 63mos). Fresh or frozen samples at the time of diagnosis were used. sFLC/sFLCR and HLC/HLCR were assayed with Freelite™ and Hevylite™ (Binding Site Birmingham, UK) in the SIEMENS nephelometer BNII. sSynd1, BLyS and TGFbeta1 were assayed by elisa kits. Statistical analysis was performed with SPSS v22.0.

Results:
In MM patients values of: iHLC are in correlation with sSynd1 p=0.0034.
In WM patients values of: iHLC & HLCR ic with sSynd1 p=0.04, iHLC ic BLyS p=0.001, iFLC & FLCR ic BLyS p=0.02, HLC-difference ic BLyS.
In MM patients values of: HLCR inverse correlation with TGFb p=0.005, FLCR ic TGFb p=0.035, HLC difference ic TGFb p=0.02, FLC difference ic TGFb p=0.02 (ic:in correlation, iHLC: involved monoclonal Ig, iFLC: involved monoclonal FLC, HLCR: Igxκ/Igxλ ratio, FLCR: FLCκ/FLCλ ratio)

Conclusions: sSynd1 in MM and BLyS in MW and CLL correlate with Ig production. By repressing the production of monoIg as well as polyIg. TGFb1 correlated with the ratios and differences of HLC and sFLC in MM where they are of utmost prognostic significance.

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Biomarkers in cancer

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CLINICAL CYLOGY AND MOLECULAR BIOLOGY IN THYROID LESIONS

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Objectives. Fine needle aspiration cytology (FNAC) plays an important role in assessment of thyroid nodules, giving the tool for planning the surgical treatment or avoiding the surgery. But it is almost impossible to make the unequivocal decision in some cases. Additional methods are used in management of such patients, although sometimes it is difficult to implement them because of a small amount of material obtained. The aim of the study was to assess the possibility of improving the results of FNAC by the usage of conventional and liquid based cytology and additional molecular markers.
Materials and methods. The results of FNAC of thyroid lesions from Moscow and Tomsk hospitals for more than 600 patients will be presented. Air-dried smears stained by MGG and liquid-based slides, stained by Papanicolaou were used for cytological assessment. Immunocytochemistry for β-catenin, E-cadherin, Ki-67 and EpCAM markers was performed in all cases. Archive MGG slides from 17 patients with papillary carcinoma proven by subsequent histology were chosen for BRAF mutation assessment. The system for MLPA-analysis (Multiplex Ligation-dependent Probe Amplification) was created for this purpose.
Results. A significant difference of the expression values of β-catenin ($p<0.001$), E-cadherin ($p<0.05$) and Ki-67 ($p<0.001$) was determined between benign and malignant lesions. Cell separation by epithelial marker EpCAM (CD326) allows to obtain more sufficient material for RT-PCR. Mutation of V600E in BRAF gene was detected in 12 cases from 17 patients with papillary carcinoma.
Conclusion. Combination of various morphological and molecular methods can play an important role in individual management and treatment of thyroid diseases.

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Biomarkers in cancer

Cod: M220

IMPROVED DISCRIMINATION OF CLINICALLY SIGNIFICANT PROSTATE CANCERS BY DETECTING SPECIFIC PSA GLYCOVARIANT WITH NOVEL LECTIN-NANOPARTICLE ASSAY

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Background: Prostate-specific antigen (PSA), a glycoprotein with a single N-oligosaccharide chain, is used for early detection of prostate cancer (PCa) as well as for monitoring the disease. However, the clinical specificity is not optimal and new approaches are needed to detect clinically significant PCa. As altered glycosylation is a universal feature of cancer cells, detecting the cancer related glycosylation pattern via glycan-binding proteins (lectins) could be viable diagnostic target for PCa and a way to achieve specificity in tumor detection. The objective of our study was to develop lectin-assisted PSA immunoassay to specifically detect the cancer-associated glycovariants in urine PSA.

Methods: PSA from PCa cell lines, seminal plasma and urine were captured on anti-PSA antibody immobilized on microtitration wells. Our lectin library, where individual lectins with known glycostructure specificity are immobilized onto fluorescent Eu^{+3}-chelate-dyed nanoparticles (Eu^{+3}-NPs), was tested for lectins capable of desired discrimination of PSA from cancerous and non-cancerous origin. For improved assay sensitivity we used a smaller and a more dense capture area by printing the biotinylated anti-PSA antibody onto streptavidin coated microtitration wells and analyzing urine samples from males with clinical suspicion of PCa (n=144) and healthy young males (n=11).

Results: Several lectins were capable of preferentially detecting cancerous PSA, whereas PSA from healthy individuals was not reactive or showing reduced reactivity. Using macrophage galactose-type lectin (MGL) immobilized to Eu^{+3}-NPs, an analytically sensitive PSA assay was achieved that specifically recognized the cancerous PSA isoform. The MGL PSA assay didn't recognize PSA in young male urine, and moreover, showed improved discrimination ($p<0.001$) of patients having clinically significant PCa (Gleason score ≥ 7, n=73) from patients with negative biopsy or Gleason 6 tumor (n=71) compared to the conventional total PSA ($p=0.786$) or % free PSA ($p=0.006$) plasma measurements.

Conclusions: This Eu^{+3}-NP-aided concept offers excellent practical possibilities to improve the usually low affinity lectin assay, enabling low concentrations of certain PSA glycovariants to be precisely and specifically measured and show great promise for required specificity in PCa detection.

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Biomarkers in cancer

Cod: M221

DEVELOPMENT OF A ROBUST ELISA FOR PERIOSTIN, A CIRCULATING BIOMARKER OF TUMOUR PROGRESSION

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Background. Periostin is an adhesion-type molecule secreted during diseases of chronic inflammation including cancer and asthma, where it activates and sustains inflammatory response and cell proliferation. Periostin is proposed as a possible companion diagnostic for response to novel anti-asthma drugs. Periostin also binds to integrins on target endothelial cells, facilitating tumour cell adhesion to the target organ. This indicates that periostin may be a circulating biomarker of tumour invasiveness in various cancers. The aim of this study was to develop a new ELISA for the detection of periostin in human serum to facilitate the investigation of this biomarker in clinical settings.
Methods. Sheep were immunized with recombinant periostin. Lymphocytes were collected and fused to form hybridomas. Clones showing strong reactivity to periostin and low cross-reactivity to related proteins were selected to produce stable cell lines. An optimal antibody pair was verified for recognition of periostin in ELISA. Total periostin levels were then analysed in serum sample sets from cancer patients (n=31) and healthy donors (n=15).
Results. The analytical evaluation of the ELISA showed a sensitivity value <10ng/mL (calibration range of 0-256ng/mL). The intra-assay precision (n=16), expressed as CV(%) was <15%. A pilot study to assess recovery of the periostin assay in disease cohorts revealed, when compared to controls (median 7.5ng/mL), elevated levels of periostin in serum samples from patients with pancreatic cancer and Non-Small Cell Lung cancer (NSCLC) with medians of 36.5ng/mL and 37.8ng/mL respectively, whereas this was not observed in patients with Small Cell lung cancer (SCLC), median 7ng/mL. Significant p-values (Mann-Whitney test) were observed for pancreatic cancer and NSCLC versus control, $p<0.0001$ and $p=0.004$ respectively, as well as for NSCLC versus SCLC, $p=0.0052$.
Conclusions. The data indicate optimal performance of the ELISA for detection of periostin in serum. The assay detects significantly elevated levels of periostin in samples from pancreatic cancer and NSCLC patients. This ELISA represents a suitable analytical tool for application to clinical settings.

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Biomarkers in cancer

Cod: M222

DEVELOPMENT OF A SENSITIVE AND SPECIFIC ELISA FOR FREE LIGHT CHAINS IN SERUM

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We have developed a monoclonal antibody based sequential sandwich ELISA system for the quantification of kappa (κ) and lambda (λ) free light chains (FLC) in human serum.

The Seralite®-FLC ELISA uses two 96-well plates, one for κ-FLC and one for λ-FLC. Monoclonal antibodies to the respective FLC are coated to the ELISA plates to act as capture antibodies for the FLC. The performance of these monoclonal antibodies has been well characterised by analysis of thousands of patient samples and are used in the Seralite®-FLC lateral flow rapid test. Following incubation and a wash, a second monoclonal antibody (anti-κ-FLC or anti-λ-FLC) labelled with horseradish peroxidase is added. After a second incubation and wash, TMB-peroxide substrate is added and following addition of H2SO4 (1mol/L) absorbance is read at 450nm.

Normal range was determined by analysis of 91 normal serum samples, to give normal range for κ-FLC of 5.2 to 22.7 mg/L; λ-FLC 4.0 to 25.1 mg/L and κ/λ FLC ratio of 0.5 to 2.5. Intra-assay and inter-assay precision were determined by analysis of three samples of each of κ-FLC and λ-FLC 20 times in one assay for intra-assay precision and 20 individual assays over three days for inter-assay precision. Both assays demonstrated %CV of less than 10% both inter and intra-assay across the range ~10 to 100 mg/L. The assays did not show interference from haemoglobin (4mg/mL), bilirubin (0.2mg/mL), cholesterol (2mg/mL) or triglyceride (10mg/mL). Linearity was tested by dilution of pooled serum across the range of the calibration curve (2 to 150 mg/L), and comparison of observed versus expected results, was good for both κ-FLC ($y = 0.9845x – 1.5198$, $R2 = 0.997$) and λ-FLC ($y = 1.0032x – 0.618$, $R2 = 0.999$). Analytical sensitivity (calculated as +2 standard deviations from zero signal) gave limits of detection for κ-FLC of 0.8mg/L and λ-FLC of 0.4mg/L.

In comparison to the established Seralite®-FLC LFD rapid test using 91 samples from normal patients and 99 samples from patients presenting with myeloma (50 x kappa and 49 x lambda) Seralite ELISA had sensitivity of 98% and a specificity of 100%.

The assays are suitable for the high volume automated (or manual) testing of samples as an aid to diagnosis and management of patients with myeloma.

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Biomarkers in cancer

Cod: M223

FIR HAPLOINSUFFICIENCY PROMOTES SPLICING TO PYRUVATE KINASE M2 IN MICE THYMIC LYMPHOMA REVEALED BY SIX-PLEX TANDEM MASS TAG QUANTITATIVE PROTEOMIC ANALYSIS.

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Introduction

FUSE-binding protein (FBP)-interacting repressor, FIR, is a transcriptional repressor of c-myc gene. FIR is also known as PUF60 that is a member of U2AF splicing factor family. An alternatively spliced form of FIR is activated as a dominant negative in several cancers. Previously, FIR hetero knockout mice ($FIR^{+/-}$) was prepared, as a dominant negative model of FIR, and showed c-Myc activation in peripheral lymphocytes. Moreover, $FIR^{+/-}TP53^{-/-}$ mice frequently developed thymic lymphoma and/or T cell type acute lymphoblastic lymphoma (T-ALL).

Methods

To examine the mechanism of FIR's role in tumor development, the quantitative protein profile was revealed by six-plex tandem mass tags (TMT) in the above mice thymic lymphoma model. Furthermore, for potential marker among identified proteins in this study, and some proteins which involved in it, the protein levels were confirmed by western blotting.

Results

TMT indicated that six hundred and forty-eight proteins were up- or down regulated in mice thymic lymphoma tissues including transcriptional factors, DNA damage repair proteins, DNA replication, T-cell activation/proliferation, apoptosis and so on. Notably, pyruvate kinase subtype M2 (PKM2) protein, but not PKM1, was activated two times more in mice thymic lymphoma than that of thymus in wild type mice. Western blotting analysis revealed that protein levels of PKM2, hnRNPA1 and some protein which regulates splicing of PKM2/PKM1 switch, were also overexpressed in the thymic lymphoma of $FIR^{+/-}TP53^{-/-}$ mice than in those of control.

Conclusion

These results indicated that FIR haplo-insufficiency switches alternative splicing of PKM1 to PKM2 in thymic lymphoma cells prior to T-ALL cells potentially via at least partly affecting hnRNPA1 expression. Since PKM2 activation is already observed even in thymic lymphoma tissues, alternative splicing switch from PKM1 to PKM2 is required but not sufficient for T-ALL progression in our mice model. Together, FIR and its related spliceosomes are vulnerable therapeutic targets for T-ALL and cancers.

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Biomarkers in cancer

Cod: M224

MICRORNA-24-3P OVEREXPRESSION PREDICTS RELAPSE AND POOR OVERALL SURVIVAL OF COLORECTAL ADENOCARCINOMA PATIENTS, INDEPENDENTLY OF OTHER ESTABLISHED PROGNOSTICATORS

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BACKGROUND: Many small non-coding RNAs, including microRNAs (miRNAs) are aberrantly expressed in cancer and leukemia. MicroRNA-24-3p (miR-24-3p) is involved in cancer-related cellular processes, including cell growth and cycle control, proliferation, and apoptosis. In this study, we examined the potential prognostic and diagnostic significance of miR-24-3p expression in colorectal adenocarcinoma. METHODS: Total RNA was extracted from 182 colorectal adenocarcinoma specimens and 86 paired non-cancerous colorectal mucosae. After polyadenylation of 2 μg total RNA and reverse transcription into first-strand cDNA using an oligo-dT-adapter primer, miR-24-3p expression was quantified using an in-house–developed reverse-transcription real-time quantitative PCR method, based on the SYBR Green chemistry. SNORD43 (RNU43) and SNORD48 (RNU48) were used as reference genes. RESULTS: miR-24-3p levels are not significantly different between colorectal adenocarcinoma and non-cancerous colorectal mucosae, and therefore miR-24-3p expression could not be used for diagnostic purposes (AUC=0.54, 95% CI=0.46–0.61, P=0.34). However, high miR-24-3p expression predicts poor disease-free survival (DFS) and overall survival (OS) of colorectal adenocarcinoma patients (P=0.019 and P=0.011, respectively). Multivariate Cox regression analysis confirmed that miR-24-3p overexpression is a significant predictor of poor prognosis in colorectal adenocarcinoma and that its prognostic significance is independent of other established prognostic factors and treatment of patients (HR=4.51, 95% CI=1.05–19.33, P=0.043). Of note, miR-24-3p overexpression retains its rather unfavorable prognostic value in the subgroup of patients with advanced yet locally restricted colorectal adenocarcinoma (T3) and in those without distant metastasis (M0) (P=0.023 in both cases). Moreover, miR-24-3p overexpression is a potentially unfavorable prognosticator for patients who were not treated with radiotherapy (regarding DFS: P=0.017; regarding OS: P=0.015). CONCLUSIONS: High expression levels of miR-24-3p predict poor DFS and OS of patients with colorectal adenocarcinoma, independently of other clinicopathological parameters that are currently used for prognostic purposes in this human malignancy and treatment of patients.

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Biomarkers in cancer

Cod: M225

HIGH TISSUE LEVELS OF mir-15A-5P: A NOVEL POTENTIAL BIOMARKER OF RECURRENCE IN COLORECTAL ADENOCARCINOMA

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BACKGROUND: Colorectal cancer is the fourth most common malignancy after lung, liver, and gastric cancer, and the second leading cause of cancer-associated deaths among adults. MicroRNA-15a-5p (miR-15a-5p) constitutes a post-transcriptional regulator of the proto-oncogene MYB, a transcriptional factor essential for prolonged cell proliferation and survival. The aim of this study was the evaluation of the clinical significance of miR-15a-5p expression in colorectal adenocarcinoma, with regard to its diagnostic and prognostic value. METHODS: Total RNA was extracted from 182 colorectal adenocarcinoma specimens and 86 non-cancerous colorectal mucosae. After polyadenylation by poly(A) polymerase and subsequent reverse transcription with an oligo-dT adapter primer, miR-15a-5p expression was assessed using an in-house–developed reverse transcription quantitative real-time PCR method, based on SYBR-Green chemistry, using SNORD43 (RNU43) as endogenous control. RESULTS: Extensive biostatistical analysis revealed that miR-15a-5p was significantly upregulated in colorectal tumors, compared to non-cancerous colorectal mucosae. Moreover, ROC and logistic regression analysis suggested the potential use of this miRNA for diagnostic purposes (AUC=0.61, 95% CI=0.54–0.68, P=0.003). Furthermore, miR-15a-5p overexpression predicts poor disease-free survival (DFS) and overall survival (OS) (P=0.001 and P=0.035, respectively). Multivariate Cox regression analysis confirmed that miR-15a-5p overexpression is a significant unfavorable prognosticator of DFS in colorectal adenocarcinoma, independent of other established prognostic factors plus treatment of patients (HR=5.40, 95% CI=1.93–15.09, P=0.001). Importantly, miR-15a-5p represents an unfavorable prognosticator among T3 patients (lower DFS and OS rates, P=0.003 and P<0.001, respectively) as well as those without distant metastasis (M0) (lower OS rates, P=0.022). More importantly, the cumulative DFS probability of patients with early-stage tumors overexpressing miR-15a-5p was significantly lower (P=0.004). CONCLUSIONS: In conclusion, elevated expression of the cancer-associated miR-15a-5p predicts poor DFS and OS of colorectal adenocarcinoma patients, independently of clinicopathological factors currently used for prognosis.

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Biomarkers in cancer

Cod: M226

TUMOR NECROSIS FACTOR-A (TNF-A) IN MULTIPLE MYELOMA PATIENTS DEPENDING ON THE STAGE OF THE DISEASE.

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INTRODUCTION AND AIM: In multiple myeloma (MM) tumor necrosis factor-α (TNF-α) is synthesized by stromal and plasma cells. It is recognized as an important survival factor for human myeloma cells. TNF-α together with IL-6 stimulate migration of endothelial cells. The aim of this study was the evaluation of TNF-α concentrations in MM patients depending on the stage of the disease and as compared to the control group.
METHODS: The study group consisted of 41 patients (mean age 67.7) with newly diagnosed MM prior to treatment and categorized depending on the Durie and Salmon staging system. The control group consisted of 30 healthy subjects (mean age 65.5). TNF-α concentrations were determined in the serum with the use of ELISA method. Differences were considered statistically significant for $P<0.05$. Receiver operator characteristic (ROC) curve was generated to calculate the area under the ROC curve (AUC).
RESULTS: Median TNF-α in MM patients was significantly higher (15.1 pg/mL) as compared to the controls (10.9 pg/mL) ($P=0.000$). Moreover, TNF-α medians were significantly increasing with the stage of the MM (14.5 pg/mL, 18.3 pg/mL, and 23.2 pg/mL for I, II, and III stage respectively). AUC for TNF-α was 0.888 and it was significantly higher than AUC=0.500.
CONCLUSION: The evaluation of TNF-α in MM subjects revealed its clinical significance. Increased TNF-α in MM patients as compared to the controls indicate that this protein may play a role in MM development. Additionally, protein tested significantly increasing with the stage of the disease, what may indicate that TNF-α takes part in the progression of MM.

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Biomarkers in cancer

Cod: M227

DEVELOPMENT OF NOVEL REAL-TIME QPCR METHODOLOGIES FOR QUANTIFICATION OF THE COL11A1 mRNA GENERAL AND C TRANSCRIPTS AND EVALUATION IN LUNG CANCER TISSUE SPECIMENS

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BACKGROUND: It has been shown that the expression of collagen changes in malignancies, especially that of collagen XI. This would affect the ability of cancer cells to invade the stroma and metastasize. The a1 chain of collagen XI is transcribed from COL11A1 gene by alternative splicing in at least four different transcripts (termed A, B, C and E). The corresponding protein isoforms differ in the proteolysis of the N-terminus and potentially, in the way that mediate invasion in the extracellular matrix. Collagen isoforms have not previously been studied in lung cancer. The purpose of the study was the development of new quantitative methodologies for the general (total) COL11A1 transcript and the C transcript (qPCR methods for A and E transcripts have already been developed by our group in a previous work), the quantification of all COL11A1 transcripts and the investigation of their potential association with histopathological prognostic factors in lung cancer.
PATIENTS AND METHODS: For the quantitative determination of the general COL11A1 and C transcripts, real-time qPCR methodologies with hybridization probes on the LightCycler 1.5 platform (Roche) were developed. In 27 cDNA samples from lung tissues of patients with known histopathological data (8 control and 19 cancer tissues) all COL11A1 transcripts were measured. Statistical analysis was performed with the IBM SPSS vs.21 program.
RESULTS AND CONCLUSIONS: The real-time qPCR methodologies were appropriately validated. All lung cancer samples were positive for the general COL11A1 transcript, while 5 of 8 control samples were negative. Regarding isoforms A and E, 13 of 19 tumor samples were positive for each one (68%) and 11 for both (58%). Isoform C was detected in only 3 samples (confirmed by agarose electrophoresis and DNA sequencing). Isoforms A and E were significantly correlated ($p<0.05$), which was further confirmed by linear regression [(isoformA)=0.737*log(isoformE)+1.010]. No other statistically significant association of the transcripts with histopathological data was observed due to the small number of samples. As the number of general COL11A1 transcripts/μl exceeds the sum of A+E+C transcripts in all samples, there is room for discovery of other transcripts as well. This study was the first to assess the differential expression of COL11A1 isoforms in lung cancer.

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Biomarkers in cancer

Cod: M228

NSE: DOSAGE IMPORTANCE AND PRACTICAL CONSTRAINTS

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Introduction
The Neuro Specific Enolase (NSE) is a marker of the neuroendocrine differentiation found in cancers of the lung with small cells.
This tumor marker is also found in the neuroblastomas and neuroendocrine tumors. In addition, many studies underline the interest of the NSE in the cerebral damage where its dosage is done at the CSF.
Objectives
- to achieve the development of the NSE dosage.
-Propose a kinetics to clinicians for them to follow-up the NSE assessment of the therapy effectiveness.
Materials and Method
- The sample should rather be realized on citrated tube.
-The dosage is achieved by the method based on the Time Resolved Amplified Cryptate Emission (TRACE)
-the apparatus used is the B.R.A.H.M.S Kryptor (Thermoscientific)
Results
-The calibration of the method is carried out at 2 points (cal1=9,37 ng/ml, cal2=79,20 ng/ml)
-The quality control is carried out by the intermediary of a level of normal control Tumor Marker Control 1TMC1 (Δ=9,27ng/ml) and a level of control pathological Tumor Marker Control 2 TMC2 (Δ=88,5ng/ml)
-The analysis of quality control shows that the development of the NSE is satisfactory.
Conclusion
The dosage of the NSE in our laboratory is currently being carried out. The only constraint of this marker lies in the rendering of the result.
In fact the Reagents Stability allows us collect the samples for a better quality of the RESULT and therefore of the Therapeutic monitoring.

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Biomarkers in cancer

Cod: M229

THE PLASMA LEVELS AND DIAGNOSTIC UTILITY OF MACROPHAGE COLONY-STIMULATING FACTOR (M-CSF) AND TISSUE INHIBITOR OF METALLOPROTEINASE-2 (TIMP-2) IN BREAST CANCER PATIENTS.

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Background: Breast cancer is the most frequently diagnosed cancer in women. Tumor markers have been used to help monitor the recurrence of breast cancer and to diagnose the patients who have a symptoms of disease. Macrophage colony-stimulating factor (M-CSF) and tissue inhibitor of metalloproteinase-2 (TIMP-2) may play a role in progression and metastasis of breast cancer. In the present study, we investigated the plasma levels of M-CSF and TIMP-2 in comparison to cancer antigen (CA 15-3) in breast cancer patients and in relation to the control groups.
Methods: The study included 80 women with breast cancer, 40 patients with benign breast tumor and 40 healthy women. The plasma levels of M-CSF and TIMP-2 were measured using ELISA method, while CA 15-3 with CMIA method. We defined the diagnostic criteria: sensitivity, specificity, the positive and the negative predictive values. Furthermore, we defined the receiver-operating characteristics (ROC) and the area under the ROC curve (AUC) for all tested parameters.
Results: Our results showed that levels of M-CSF, TIMP-2 and CA 15-3 were significantly higher in the breast cancer patients comparing to the both control groups. TIMP-2 was the only parameter which has statistically significantly higher levels at all analyzed stages in comparison to healthy women. TIMP-2 also demonstrated a highest values of the sensitivity (63%) and the predictive value of a negative test result (57%) in the total group of breast cancer patients, but lower than CA 15-3 (66%, 59%, respectively). The diagnostic specificities of M-CSF, TIMP-2 and CA 15-3 showed equally high values (95%). The TIMP-2 area under the ROC curve (AUC=0.785) was the largest from all tested parameters and was slightly lower than the AUC of CA 15-3 (0.850). The combined use of tested parameters with CA 15-3 resulted in the increase in the diagnostic values (sensitivity, NPV and the AUC).
Conclusions: Our findings suggest a potential usefulness of M-CSF and TIMP-2 in the diagnostics of breast cancer, especially in combination with CA 15-3.

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Biomarkers in cancer

Cod: M230

MULTIPLEX TRANSCRIPTOME PROFILING OF IN-VIVO ISOLATED CIRCULATING TUMOR CELLS IN HIGH-RISK PROSTATE CANCER PATIENTS

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Introduction: Circulating tumor cells (CTCs) have been verified as prognostic markers for disease progression in various cancer types. The main aim of the EU project "CTC-SCAN" is to validate the number of CTCs isolated from patient's blood as a prognostic marker for relapse in high-risk prostate cancer (PCA) patients treated with primary radical prostatectomy or radiotherapy. In this study, we present our first results on gene expression profiling of CTCs that were isolated, using the CellCollector® (GILUPI, GmbH), a novel clinical device designed for the in vivo isolation of EpCAM-positive CTCs.

Patients and methods: We first developed and validated 3 multiplex and 3 single-plex highly sensitive RT-qPCR assays amplifying: (a) Epithelial markers: CK-19, EpCAM, E-cadherin & PBGD (reference gene), b) Stem cell markers: PSCA, ALDH1, CD133 & HPRT (reference gene), c) EMT markers: TWIST, vimentin, N-cadherin and B2M (reference gene) and d) PSA, e) TMPRSS2-ERG fusion, f) Plastin-3 (EMT marker). 62 patients and 36 healthy volunteers (used as a control group, both in vivo and in vitro) participated in the study. After in vivo isolation, the Ab coated region of the CellCollectorsTM were washed in PBS , cut, and stored in Trizol reagent till analysis. Total RNA was extracted from captured cells, lysed in Trizol, followed by cDNA synthesis. RT-qPCR was performed for the molecular characterization of captured cells. In all cases, peripheral blood was also collected and used for CTC analysis by CellSearchTM and the EPISPOT assay.

Results: Briefly, in 13/15 (87%) samples, in which at least one cell was detected by CellSearch®, we detected the expression of at least one gene. In 28/47 (60%) samples, negative by CellSearch®, we detected the expression of several genes by the developed RT-qPCR assays. In 9/14 samples that were exclusively found to be positive by EPISPOT for PSA immunospots, at least one of the analyzed genes was also expressed.

Conclusions: Our results indicate that in-vivo isolation of CTCs in combination with downstream molecular analysis from high risk prostate cancer patients represents an innovative and promising approach in the clinical management.

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Biomarkers in cancer

Cod: M231

ESR1 METHYLATION IN CIRCULATING TUMOR CELLS OF PATIENTS WITH BREAST CANCER

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BACKGROUND: DNA methylation is an epigenetic alteration which plays a decisive role in the regulation of signal translation processes. In our lab, we have demonstrated for the first time the epigenetic silencing of tumor and metastasis suppressor genes in CTCs through their promoter methylation. Estrogen receptor (ER) is an important prognostic biomarker and is predictive of response to endocrine therapy in breast cancer. In this study, we evaluated for the first time ESR1 methylation in CTCs of breast cancer patients.

METHODS: We developed and validated a novel highly sensitive and specific qMSP assay for ESR1 methylation using commercially available DNA methylation controls and the MDA-MB-231 cell line. We further examined its performance in EpCAM-positive immune-magnetically isolated CTC fractions, followed by DNA isolation and sodium bisulfite (SB) treatment from: a) 74 operable, b) 48 metastasis- verified breast cancer patients and c) 30 healthy donors (control group).

RESULTS: The developed assay is highly specific and sensitive since it can detect 0.1% methylated ESR1 sequences in the presence of 99.9% un-methylated. ESR1 was found to be methylated in 16/74 (21.6%) operable breast cancer patients, in 10/48 (20.8%) patients with verified metastasis, but only in 1/30 (3.3%) healthy donors (EpCAM-positive CTC fraction).

CONCLUSIONS: The EpCAM-positive CTC fraction was found to be methylated for ESR1 in about 20% of patients with breast cancer. We will further evaluate these findings in respect to the clinical outcome of these patients, since the epigenetic silencing of ESR1 could be of important clinical significance especially for its impact on the efficacy of treatment.

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Biomarkers in cancer

Cod: M232

COMBINATION OF PROSTATE CANCER ANTIGEN 3 AND PROSTATE-SPECIFIC ANTIGEN IMPROVES DIAGNOSTIC ACCURACY IN MEN AT RISK OF PROSTATE CANCER

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BACKGROUND: Prostate cancer antigen 3 (PCA3) is a non-coding RNA that is highly overexpressed in prostate cancer (PCa) tissue and excreted in urine in PCa patients. This study was conducted to assess the clinical utility of urinary PCA3 in men at risk of PCa.
METHODS: Urinary PCA3 mRNA scores were assessed using the Progensa assay. Diagnosis of PCa was confirmed by prostate biopsy. Performance characteristics PCA3were evaluated. A multivariable logistic regression analysis was conducted to develop a model incorporating the PCA3, PSA, and clinical and demographic risk factors.
RESULTS: Prostate adenocarcinoma was diagnosed in 77 men (28.4%). PCA3 score but not PSA level was a significant predictor of prostate biopsy outcome ($p<0.001$). The urinary PCA3 assay outperformed the serum PSA test, with significantly greater AUC (0.72 vs. 0.57, $p=0.007$). A PCA3 score of 30 was the optimal cutoff for our study cohort, with a diagnostic sensitivity of 72.7%, specificity of 67.5%, positive predictive value of 47.1%, negative predictive value of 86.2%, positive likelihood ratio of 2.24, negative likelihood ratio of 0.40, and diagnostic odds ratio of 5.55. At this cutoff score, the PCA3 assay could avoid 57.4% of unnecessary invasive biopsies in the overall study cohort and 70.3% in the subgroup with PSA level in the "gray zone" (4-10 ng/mL). A logistic regression algorithm combining PCA3 with PSA increased the AUC from 0.571 for PSA-only to 0.729 ($p<0.001$). Incorporating prostate volume and repeat biopsy into the model further improved the performance accuracy of combined PCA3 and PSA (AUC=0.787; $p=0.016$).
CONCLUSIONS: Our data suggest that PCA3 improves the diagnostic sensitivity and specificity of PSA and that the combination of PCA3 with PSA gives better overall performance in identification of PCa than serum PSA alone in the high-risk population.

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Biomarkers in cancer

Cod: M233

CLINICAL SIGNIFICANCE OF ANTI-NEURAL ANTIBODIES IN MALIGNANT TUMOURS OF FEMALE REPRODUCTIVE ORGANS

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Background. Traditionally onconeuronal antibodies are recognized as biomarkers of neurological paraneoplastic syndromes. However, their effect on prognosis in long-term observation has not been extensively studied. The aim of this study was to evaluate the prognostic value of anti-neural antibodies in patients with malignant tumours of female reproductive organs.
Methods. The study included 77 patients with endometrial cancer and 55 ovarian cancer patients. Anti-neural antibodies (onconeural antibodies, anti-myelin, anti-MAG, anti-GAD, anti-neuroendothelium antibodies) were evaluated by means of indirect immunofluorescence at baseline and after 5-years follow-up. Line blot confirmed the presence of onconeuronal antibodies.

Results. Anti-CV2, anti-Ma/Ta, anti-Tr, anti-myelin, anti-MAG, anti-gangliosides, anti-neuroendothelium anti-nucleosome antibodies were found in endometrial cancer patients. Anti-nucleosome antibodies in 40% of cases were confirmed by means of Line blot (anti-RNP 70, anti-PM-Scl, anti-Scl 70, anti-ds DNA). In ovarian cancer patients anti-Yo, anti-amphiphysin, anti-Hu, anti-Ma/Ta, anti-Tr, anti-myelin, anti-MAG, anti-neuroendothelium and anti-nucleosome antibodies were identified. The relative risk (RR) of death in ovarian cancer patients with autoantibodies was higher (RR=5.46;95%CI:1.39-21.51;P=0.0152) than in endometrial cancer patients with autoantibodies. RR of death in ovarian cancer patients with anti-neural antibodies was higher (RR=3.79;95%CI:0.998-14.41;P=0.050) than in endometrial cancer patients with anti-neural antibodies. Kaplan–Meier survival curves showed worse (P=0.0143) prognosis in endometrial cancer patients with anti-nucleosome antibodies than in seronegative subjects.
Conclusions. In long-term, prospective observation onconeuronal antibodies and anti-neural autoantibodies have prognostic value in gynecological cancer patients. In malignant tumours of female reproductive organs anti-nucleosome antibodies are identified, which are associated with negative effect on survival time in endometrial cancer patients.

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Biomarkers in cancer

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THE EFFECT OF SIMVASTATIN UPON THE mRNA EXPRESSION LEVEL OF SOX7 AND SOX9 IN PC3 AND LNCAP PROSTATE CANCER CELL LINES

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Introduction: Prostate cancer is the second leading cause of cancer death and the most frequently detected non-skin cancer in men in the United States. Researchers are trying to find effective treatment of the disease. Recently theanticancer effect of statins have been interested.
Simvastatin is the first line drug for preventing cardiovascular events, Additionallydetermine the rate of tumor growth by its effects on cellular proliferation and inflammation.
Although much evidence proposes that statins reduce the risk of many cancers, there are some disagreements over the pro- and anticancer effects of statins. Transcription factors are key regulator of signaling pathways. Among thesetranscripts encoding a number of SOX proteins, such SRY, SOX7 and SOX9, have been reported in prostate
In this study we investigate the effects of simvastatin on the SOX9 and SOX7 expression in prostate cancer cells
Methods: Prostate cell lines LNCaP, PC3 (National Cell Bank of Iran Pasture Institute, Iran) were cultured .Appropriate concentrations of simvastatin was determined by MTT assay.The expression of SOX7, and SOX9 was assessed by using quantitative RT-PCR, in Prostate cancer cell lines.
Results: Time and dose-dependent effects of simvastatin on LNCaP (androgen-dependent) and PC3 (androgen-independent) cells indicate that treatment with simvastatin at concentration 0.7 μM after 24 h was sufficient to upregulate SOX7 expression in LNCaP cell line. In additional expression level of SOX9 mRNA increased at all doses and times of treatment with simvastatin in LNCaP and PC3 cell lines except at concentration 0.1 μM after 24 h in PC3.
Conclusion: Our findings suggest a relationship between simvastatin and SOX7 and SOX9 in prostate cancer cell lines.

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Biomarkers in cancer

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EVALUATION OF THE NEW SEBIA FLC ELISA ASSAY FOR THE QUANTIFICATION OF SERUM FREE LIGHT CHAINS

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BACKGROUND. The clinical utility of serum Free Light Chains (sFLC) quantification is now well established for the diagnosis, follow up and/or prognosis of patients with monoclonal gammopathies. It is also part of international guidelines and used to assess patients' response to treatment. We have evaluated the performances and suitability for routine use of a new sFLC assay based on ELISA technology (Sebia FLC, Sebia) in comparison to a commercially available nephelometry technique (Freelite, The Binding Site).
METHODS. A total of 142 routine samples from patients at various stages of several monoclonal gammopathies and controls (including patients with renal insufficiency) were analyzed in parallel on both assays. Correlation, diagnostic sensitivity and specificity, absolute values and percentage of out-of-range patients' retests were investigated.
RESULTS. Our results showed significantly lower values of the individual monoclonal FLC with Sebia FLC (around half of the Freelite values in median).We show a good linear correlation for the sFLC kappa, lambda and Kappa/Lambda ratio between the 2 techniques, with the exception of monoclonal L chains. Sebia FLC satisfactorily distinguished patients at diagnosis of Multiple Myeloma (MM), Light Chain MM and AL Amyloidosis from those who achieved complete remission and controls (similar specificity and sensitivity than Freelite). Technical performances were also compared: with Sebia FLC, we counted 4 times less retests than with Freelite for both Kappa and Lambda quantification.
CONCLUSION. We show here that the new Sebia FLC assay is suitable for FLC quantification in a context of laboratory routine. Its diagnosis performances compare well to Freelite and it generates less retests for out-of-range samples.

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Biomarkers in cancer

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INTEGRIN ALPHA4 EXPRESSION AND EPIGENETIC SILENCING IN HUMAN PROSTATE CANCER

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BACKGROUND: Prostate cancer (PCa) is the most common cancer in men, with the highest reported cases among African-Americans. Metastasis of prostate cancer requires invasion through the basement membrane that surrounds the epithelial cells, which must be breached by tumor cells invading into surrounding tissues. Integrins are cell adhesion molecules that are involved in maintaining normal tissue morphology and have been implicated in the behavior of certain malignancies. Epigenetic alterations such as DNA methylation and histone modification play an important role in tumor initiation and progression through cancer-related gene such as alpha4 integrin. It has also been demonstrated that the lack of alpha4 expression with undefined mechanism might be involved in cancers metastasis.

METHODS: Laser capture microdissection microscopy was used to obtain exclusively affected epithelial cells from prostate gland biopsies of 30 patients with prostate cancer and 40 with benign prostate hyperplasia. Expression of alpha4 integrin was determined by semi-quantitative reverse transcriptase-PCR. DNA bisulfite modifications followed by methylation-specific PCR were used to evaluate the promoter methylation status of alpha4 integrin gene in extracted DNA from patients and in prostate cancer cell lines, DU145 and PC3, before and after treatment with 5-aza-2-deoxycytidine and trichostatin A.

RESULTS: The alpha4 integrin promoter in DU145 was fully methylated, whereas in PC3 cells, partial methylation was detected. Combination treatment of either drugs or 5-aza-2-deoxycytidine alone increased expression of alpha4 integrin. Also Integrin alpha4 was hypermethylated in 66.6 % of prostate cancer cases. No hypermethylation was observed in patients with benign prostate hyperplasia.

CONCLUSIONS: The obtained results suggest CpG methylation as a major mechanism of integrin alpha4 inactivation in human prostate cancer which in turn elects it as a potential molecular tumor marker.

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Biomarkers in cancer

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proGRP AND SCC METHOD VERIFICATION WITH COBAS E411 ANALYZER

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Background
With the development of a test which makes it possible to determine ProGRP, the molecule of GRP's precursor, the question of the applicability of this tumor marker is topical once again. Research to date has indicated its high applicability in small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) differential diagnostics and high sensitivity in differentiating SCLC from benign lung diseases. In the case of SCLC, ProGRP levels are already increased in the early stage of the disease.
Similar results were obtained in studies of different types of squamous cell carcinoma, including lung cancer. In the case of NSCLC it turned out that SCCA is less sensitive than CYFRA 21-1, but more specific for squamous-cell-type NSCLC.
Methods
The Elecsys ProGRP and SCC methods are used to determine the concentrations of ProGRP and SCCA in samples of human serum or plasma. To determine the method accuracy within a series (repeatability), we carried out the measurements on the pool serum sample concentrations. At the same time, we carried out the measurements of the highly concentrated control sample. The results of the measurements obtained over the course of six days were used to calculate the intermediate precision. The acquire reproducible results were statistically processed and presented in an Excel table (Kallner, version 6.84), which already provides the equations for calculating the average values of measured concentrations, standard deviation (SD), and coefficient of variation (CV %) which is a statistical indicator for the assessment of repeatability within a series and intermediate precision.
Results
The Westgard base only specifies the variability of SCCA; we cite the potentially useful information about the biological variability of ProGRP from an article (Qi Z. et al, ClinChimActa, 2015).
SCC: biological v. CVi = 39.4 (within subject) CVg = 35.7 (between subject)
Desirable spec: I (%) = 19.7 imprecision, B (%) = 13.3. inaccuracy, TE (%) = 45.8 (allowable total error)
ProGRP: biological v. Cvi = 4.75 (within subject) CVg = 16.42 (between subject)
Conclusions
With a statistical processing of the data acquired, we have proven that in everyday laboratory work the methods for determining ProGRP and SCC do not deviate from the limits acceptable by the manufacturers. We conclude that both methods are appropriate for measuring ProGRP and SCC tumor markers in the serum samples of patients who are suspected of having lung cancer.

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Biomarkers in cancer

Cod: M238

OSTEOPONTIN (OPN) AS AN INSTANT, PREDICTIVE BIOMARKER OF TUMOR HYPOXIA AND DISTANT DISSEMINATION OF HNSCC IN PATIENTS TREATED BY RADIATION AND CHEMOTHERAPY.

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Purpose: Plasma OPN to be a putative marker of tumor hypoxia in patients with head and neck squamous cell carcinomas (HNSCC). The aim of the study was to assess clinical utility of OPN as the biomarkers of treatment outcome of radiotherapy (RT) or radiochemotherapy (CHRT) in HNSCC.
Material and Methods: Between 01/2009 and 08/2013 251 patients in the mean age of 59 years with squamous cell carcinoma of oropharynx (39%), hypopharynx (13%), larynx (44%) or oral cavity (4%) were treated with RT alone (48%) or combined with chemotherapy (52%). The median duration of symptoms prior the treatment was 33 months (range: 1 – 70). The stage of disease was determined due to a TNM scale. There were 15 (6%), 112 (45%), 74 (29%), and 50 (20%) patients with T1, T2, T3 and T4 tumor stage, respectively, and 99 (40%), 26 (10%), 105 (42%), and 21 (8%) patients with N0, N1, N2 and N3 nodal stage of disease, respectively (no patients with distant metastases were included). OPN was indicated in plasma before treatment and immediately after treatment completion.
In statistic overview of the results STATISTICA 9.1 (StatSoft) program was used. While interpreting the results median value was used. U Mann-Whitney test was used for analysis of correlation between protein concentration and the stage of the disease. Multivariate Cox analysis of factors related to OS was carried on. Log-rank test was used to compare OPN as categorized value acc. to median respectively.
Results: Pretreatment OPN levels were higher in patients with advanced T stage compared with early stage (p=0.024). There was no correlation between N stage and OPN (p=0.58). Median plasma levels of OPN measured before (67.9 ng/ml) and after (97.8 ng/ml) treatment differed (p=0.0001).
OPN levels before treatment were significantly related to overall survival (OS) ratio in both, univariate (p=0.019) and multivariate analysis (0.001). Posttreatment OPN levels (97.8 ng/ml) were also associated with survival time in univariate analysis (p=0.04). Additionally, OPN after treatment was significantly higher in patients with distant metastasis (p=0.015).
Conclusions: High levels of OPN before therapy have been associated with advanced stage and adverse prognosis. OPN after therapy may play important role in the process of tumor development and metastasis. OPN concentrations increase during treatment may reflect acute mucosal reaction after radiotherapy. Pretreatment OPN is an independent prognostic determinant of survival.

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Biomarkers in cancer

Cod: M239

THE SERUM LEVELS OF SPECIFIC RECEPTOR FOR INTERLEUKIN-8 IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA PATIENTS

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Background. A specific receptor for interleukin-8, known as CXC chemokine type-2 receptor (CXCR-2), may facilitate the progression of many malignancies, including esophageal squamous cell carcinoma (ESCC). The aim of present study was to investigate the clinical usefulness of serum CXCR-2 in ESCC patients in relation to classical tumor marker for ESCC (squamous cell cancer antigen, SCC-Ag) and marker of inflammatory states – C-reactive protein (CRP).
Methods. The study comprised on 27 patients with ESCC and 30 healthy controls. The concentrations of proteins tested were measured in serum of patients using immunoenzyme assays.
Results. The serum CXCR-2 concentrations were significantly higher in ESCC patients compared to healthy controls ($p=0.029$). Similar results were revealed for SCC-Ag ($p=0.006$) and CRP ($p<0.001$) levels. The percentage of elevated concentrations of CXCR-2 (56%) was higher than SCC-Ag (30%) but lower when compared to CRP (70%) levels. However, the highest diagnostic sensitivity (85%) was assessed for the combined use of CXCR-2 with CRP.
Conclusions. Present findings suggest the potential role of CXCR-2 in ESCC.
Acknowledgement. The study was conducted with the use of equipment purchased by Medical University of Białystok as part of the RPOWP 2007-2013 funding, Priority I, Axis 1.1, contract No. UDA-RPPD.01.01.00-20-001/15-00 dated 26.06.2015.

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Biomarkers in cancer

Cod: M240

THE DIAGNOSTIC VALUE OF SERUM TOTAL ALCOHOL DEHYDROGENASE AND ITS ISOENZYMES AND ALDEHYDE DEHYDROGENASE ACTIVITY IN URINARY BLADDER CANCER

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Background: In the previous experiments, we have found an increased activity of total alcohol dehydrogenase (ADH) and its isoenzyme class III in urinary bladder cancer cells. ADH activity in the serum may reflect changes in cancer cells and could thus be helpful for diagnostics of bladder cancer. The aim of this study was to investigate a potential role of ADH and ALDH (aldehyde dehydrogenase) as tumor markers for urinary bladder cancer.

Methods: Serum samples were taken from 41 patients with urothelial cell carcinoma and 60 healthy subjects. 16 patients were classified as low-grade and 25 as a high-grade (mostly metastatic) bladder cancer. For the measurement of class I and II ADH and ALDH activity, the fluorometric method was employed. Class III and IV of ADH and total ADH activity were measured by the photometric method.

Results: The total activity of ADH in the sera of patients with bladder cancer was significantly higher (7.92±7.75 IU/l) in comparison to healthy subjects (0.95±0.48 IU/l). The activity of ADH class I was also significantly elevated in the total bladder cancer group (5.88±7.54 mIU/l vs. 1.89±0.92 mIU/l). The activity of ADH class I was significantly higher only in high-grade bladder cancer group (6.37±9.78 mIU/L) compared to the controls. Significantly increased total activity of ADH in comparison to control group, was found in the sera of both, low-grade (7.89±7.72 IU/l) and high-grade (8.72±7.84) bladder cancer. ALDH activity did not significantly differ between all tested groups. The highest diagnostic value for bladder cancer had total ADH activity. The diagnostic sensitivity for total ADH activity was 81.5%, specificity 98.1%, PPV and NPV were 97.4% and 92.3% respectively. Area under ROC curve for total ADH activity reached the value of 0.848.

Conclusions: The increased activity of ADH I isoenzyme in the sera of cancer patients was probably caused by metastatic tumors. The increase of total ADH activity in the sera of bladder cancer patients can be a result of enzymatic disturbances in cancerous cells and isoenzymes being released from cancerous cells. The results suggest a potential role of ADH activity as a marker for bladder cancer.

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Biomarkers in cancer

Cod: M241

DETERMINATION OF LACTATE DEHYDROGENASE (LDH) IN SERUM OF PATIENTS WITH NASOPHARYNGEAL CARCINOMA (NPC): CASE-CONTROL STUDY

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Background: Nasopharyngeal carcinoma (NPC) is the sixth most common cancer in the world, men are more frequently affected than women. Its incidence varies according to ethnic and geographical origin.
In Algeria, it is the first head and neck cancer in women and the second after laryngeal cancer in men.
The NPC is characterized by its late diagnosis.
In addition to the involvement of oxidative stress in the development of tumors, overexpression of glycolytic enzyme LDH involved in energy metabolism is associated with distant metastases events.
The objective of our study was to evaluate the serum LDH found in a population of patients with NPC and compare this rate with that of a control population.

Materials and Methods: Our study was performed on 31 patients with NPC and 31 healthy controls.
Patients were recruited from the ENT Department of the Central University Hospital Mustapha Pasha. Algiers- Algeria.
The assay of LDH was performed on heparinized plasmas, by the standardized method by the IFCC, on the analyser Dimension® RXL (SIEMENS).
Statistical analysis of the results was performed using the Graph Pad Prism software 6.

Results:
Plasma levels of LDH found in NPC patients 155.78 ± 82,44U / L.
The LDH values found in the controls are 112.94 ± 48,45U / L.
Statistical analysis shows a significant difference $p = 0.0238$.

Conclusion: Our results show that patients with NPC have higher LDH levels than controls.
This work will subsequently involve a larger cohort and will be completed by the measurement of glycolysis products in particular, lactate which the increase would impact on the pH, which would reduce the adhesive properties of tumor cells , thus to improve the spread of the tumor.

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Biomarkers in cancer

Cod: M242

BLEOMYCIN INCREASES THE SENSITIVITY OF HUMAN TESTICULAR CANCER CELLS TO TRAIL/APO-2L-INDUCED APOPTOSIS THROUGH UPREGULATION OF DEATH RECEPTORS

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Background: The most common solid tumor is testicular cancer among young men. Bleomycin is an antitumor antibiotic used for the treatment of testicular cancer. TRAIL, is a proapoptotic cytokine inducing apoptosis in cancer cells. Killing cancer cells selectively via apoptosis induction is a encouraging therapeutic strategy in clinical settings. Combination of TRAIL with chemotherapeutics has been reported to enhance TRAIL-mediated apoptosis in cancer. The sensitization of tumour cells to TRAIL by chemotherapeutics might involve upregulation of TRAIL receptors or activation of proapoptotic proteins including caspases. The curative potential of TRAIL in testicular cancer has not been studied before. Therefore, we investigated effects of bleomycin, TRAIL, and their combined application in NTera-2 and NCCIT testicular cancer cell lines on caspase3 levels, and TRAIL receptor expression using flow cytometry.

Materials and Methods: We measured caspase3 levels, and TRAIL receptor expression using flow cytometry.

Results: NTera-2 and NCCIT cells were resistant to TRAIL's apoptotic effect as indicated by higher IC50 doses compared to other cancer cells. Incubation with bleomycin alone caused a significant increase in caspase3 activity in NCCIT cells, while incubation with TRAIL and bleomycin together did not increase apoptosis in NCCIT cells as indicated by low caspase 3 level. In contrast to NCCIT cells, incubation with TRAIL and bleomycin together increased caspase 3 activity in NTera-2 cell line. Incubation with bleomycin for 72h increased TR-1, TR-2, and TR-3 cell-surface expressions in NTera-2 cells. 24h incubation with bleomycin elevated TR-1 cell-surface expression in NCCIT cells.

Conclusion: Both cells were resistant to TRAIL's apoptotic effect as indicated by higher IC50 doses compared to other cancer cells. The higher rate of TR-4 expression in both cell lines might be responsible for TRAIL resistance, since high expression of decoy receptors, rather than low expression of death receptors are known to be more effective in TRAIL resistance. Bleomycin's increasing effect on TR-1 and TR-2 receptor levels in NTera-2 cells reflects increased sensitivity of this cell line to TRAIL-mediated apoptosis. The characteristics of the cancer cell type obviously play a very important role in the success of a therapeutic regimen in general cancer treatment approaches including TRAIL.

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Biomarkers in cancer

Cod: M243

PROTEOMIC VALIDATION OF BIOMARKERS FOR DISCRIMINATION OF BENIGN AND MALIGN PROSTATIC HYPERPLASIA

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Background: Serum protein profiles were analyzed in order to discriminate between prostate cancer (PCa) and benign prostatic hyperplasia (BPH).

Methods: Histological specimens were obtained performing trans-rectal ultrasound guided prostate biopsy (TRUS) in the patients in order to identify PCa, BPH and inflammation. Surface Enhanced Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (SELDI-ToF-MS) and two-dimensional gel electrophoresis (2-DE) coupled with Liquid Chromatography-MS/MS (LC-MS/MS) were used to analyze serum samples from patients with PCa and BPH.

Results: SELDI-ToF-MS analysis of serum samples did not show differences in protein profiles between PCa and BPH. Differences became evident when the presence of inflammation was taken into consideration. When samples with histological sign of inflammation were excluded, 20 significantly different protein peaks were detected. Subsequent comparisons (comparison of PCa with and without inflammation, and BPH with and without inflammation) showed that 16 proteins were differently expressed in the presence of inflammation, while 4 protein peaks were not modified. With 2-DE analysis, comparing PCa without inflammation vs PCa with inflammation, and BPH without inflammation vs BPH with inflammation, 29 and 25 differentially expressed protein spots were identified, respectively. Excluding samples with inflammation, the comparison between PCa vs BPH showed 9 unique PCa proteins, 4 of which overlapped with those previously identified in the presence of inflammation, while other 2 were proteins, not identified.

Conclusions: This study indicates that inflammation might be a confounding parameter for proteomic biomarkers of PCa. The results indicate that only a well-selected protein pattern should be considered as a potential biomarker of PCa.

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Biomarkers in cancer

Cod: M244

DIAGNOSTIC PERFORMANCES OF SERUM HE4, CA125, AND RISK OF OVARIAN MALIGNANCY ALGORITHM (ROMA) IN THE DIFFERENTIAL DIAGNOSIS OF OVARIAN CANCER FROM BENIGN GYNECOLOGICAL DISEASES

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BACKGROUND:
Detection of ovarian cancer at early stage is crucial for improvement of survival of patients. Human epididymis protein 4 (HE4) is a promising biomarker to diagnose ovarian cancer. HE4 has a protease inhibitor activity and highly expressed in a number of tumor cell lines including ovarian cancer. We aim to determine the diagnostic performance of HE4, CA125, and the risk of ovarian malignancy algorithm (ROMA) in the differential diagnosis of ovarian cancer from benign gynecological diseases.
METHODS:
During June 2014 to May 2016, a total of 218 women visited at our institution for evaluation of ovary mass were included in this study. Preoperative serum CA125 and HE4 levels were determined using the Abbott ARCHITECT i2000SR analyzer (Abbott Diagnostics, Chicago, IL, USA) with the ARCHITECT CA125II assay and ARCHITECT HE4 assay. The ROMA predictive index (PI) and ROMA value were calculated using the following equations:premenopausal PI =-12.0+2.38×ln(HE4)+0.00626×ln(CA125), postmenopausal PI =-8.09+1.04×ln(HE4)+0.732×ln(CA125), ROMA value (%)=(Exp(PI)/([1+Exp(PI)])×100. Cut-off values of CA125 was 35 U/mL and those of HE4 and ROMA were 70 pmol/L and 7.4% for premenopausal and 140 pmol/L and 25.3% for postmenopausal women, respectively. The statistical analysis was performed using SPSS Statistics version 24.0.0 (IBM Corp., Armonk, NY, USA).
RESULTS:
158 patients (72.5%) were in premenopausal state and 30 patients (13.8%) were diagnosed as ovarian cancer. Using the manufacturer-recommended cut-off values, the sensitivity, specificity, positive predictive value, and negative predictive value were 63.3%, 71.3%, 26.0%, and 92.4% for CA125, 40.0%, 94.7%, 54.5%, and 90.8% for HE4, and 71.0%, 89.3%, 52.4%, and 94.9% for ROMA. In the ROC analysis, CA125, HE4, and ROMA showed fair (0.769), good (0.834), and excellent (0.906) AUC values, respectively. In addition, serum HE4 concentrations were elevated in various benign conditions, such as, pelvic inflammatory disease, endometriosis, etc.
CONCLUSIONS:
HE4 was more specific than CA125. Overall, ROMA showed more balanced diagnostic performances than CA125 and HE4 in discriminating ovarian cancer from benign gynecological diseases.

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Biomarkers in cancer

Cod: M245

COMBINING CLASSIC AND NEW TUMORAL MARKERS IN DIAGNOSIS: A VIEW FROM THE CLINICAL DEMAND

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Background
Tumoral marker HE4 (Human epididymal protein 4) is expressed in the respiratory epithelium and in the female genital tract in normal circumstances; HE4 has the highest sensibility to detect an ovarian carcinoma, especially in early stages. Combination of HE4 with CA125 offers the higher sensibility (76, 4%) with a specificity of 95% to indicate if a pelvic mass is benign or malignant in women of any age.
HE4 also relates to clinical response to treatment and recurrence of the disease. CA125 has been the elective marker in ovarían carcinomas, and its sensibility is related with stage (I 50-70%, II 70-90%, III and IV >90%) as with the histological type.
Objetive
To determine the clinical acceptance of HE4, a novel tumoral biomarker, in a referral hospital.
Material and methods
We collected 357 specimens' results from January 2015 to April 2016 of HE4 and CA125 combined, and we distributed them by months and the clinical service where the patient came from.
Results
We have noticed a growing demand of combined HE4 and CA125 since HE4 his being measured in our Laboratory. 72,26% of the total of sample came from Obstetrics and Gynecology while the remaining Services in which these markers can play a significant role were Neurology, Family Medicine and Internal Medicine with its related areas (mainly Oncology, Gastroenterology and Neumology).
Discussion
HE 4 was introduced in our Hospital in september 2014, while our experience with CA125 traces back to more than two decades. Thanks to the combined used of these two tumoral markers, we can diagnose patients suffering from ovarian carcinomas by a method that is sensible and specific. Clinicians are getting used to diagnosing cancers with the combined help of both biomarkers, more specifically various types of ovarian and endometrial carcinomas, but some lung adenocarcinomas can also be detected by HE4 and CA125 elevation. Ovarian and lung cancers may sometimes provoke cerebral dissemination in advanced stages, so measuring their levels in undiagnosed oncological patients who consult for neurological symptoms can unveil a primary tumor in many occassions.

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Biomarkers in cancer

Cod: M246

NGAL: A NEW BIOMARKER FOR HUMAN CANCER

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Background: Recent researches highlight how NGAL is involved in the development, proliferation and invasiveness of human cancers. In fact, elevated levels of this protein have been detected in serum or urine of patients affected by different type of neoplasm. In cancer cells, NGAL function ranges from inhibiting apoptosis (thyroid), invasion and angiogenesis (pancreas), to increasing proliferation and metastasis (breast, colon). Therefore, we evaluate NGAL as both a prognostic and diagnostic marker for different types of human cances.
Methods: We searched electronic database Medline and Embase and selected studies evaluating NGAL as a prognostic or diagnostic marker for human cancers. Two authors, independently, screeneed the full text to select studies and extracted pertinent data. We analysed data using the random effects models for the meta-analyses. All analyses were performed using Meta-disc and RevMan5 softwares.
Results: We analysed data about 4388 patients affected by colorectal, pancreas, breast, thyroid, gastric, kidney, endometrial, brain, liver, lung, oesophageal, oral or ovarian cancers. In patients with colorectal, positive NGAL expression was associated with a decrease of disease free survival (HR 2.27, 95%CI 1.54-3.36). NGAL was a negative prognostic marker of overall survival in colorectal (HR 2.37, 95%CI 1.68-3.34) and endometrial (HR 4.38, 95%CI 1.9-10.12) cancers. Discriminative power of NGAL between cancer patients and control was moderate in colorectal cancer (AUC 0.6; pooled Sensitivity was 0.56; pooled Specificity was 0.72), acceptable in pancreatic cancer (AUC 0.8; pooled Sensitivity was 0.6; pooled Specificity was 0.8) and good in thyroid cancer (AUC 0.9; pooled Sensitivity was 0.85; pooled Specificity was 0.96).
Conclusions: NGAL is differently expressed in several human cancers. Its determination in plasma and urine could be useful in the prognosis of colorectal and breast cancer, but its prognostic accuracy remains uncertain for other human tumours.

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Biomarkers in cancer

Cod: M247

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AS POTENTIAL TUMOR MARKER FOR PANCREATIC CARCINOMA

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Tumors induce blood vessel growth and this process is known as tumor angiogenesis. The tumor cells secrete various factors, and one which is considered as to have the greatest role is signaling protein vascular endothelial growth factor (VEGF) also known as Vascular Permeability Factor. In various neoplastic diseases it is impossible for tumors to grow and create metastasis without forming of new blood vessels. Here also belongs Pancreatic Carcinoma. The aim of this study is to investigate plasma levels in Pancreatic Carcinoma and to see if it has any possible connection with tumor growth, progression and metastasis.We collect plasma samples of 26 patients with pancreatic cancer. All samples were not frozen and were processed routinely on site. We use Human VEGF Assay kit from Immuno-Biological Laboratories Co. Ltd (www.iblJapan.co.jp).
Out of 26 patients with pancreatic cancers, 19 have tumors larger than 3mm, all 19 of them were in stage III and IV by TNM classification and were very progressive. 15 out of 19 patients have higher levels than 120 pg/mL and the 7 other patients with smaller tumors were negative respectively.
In conclusion, blood levels of VEGF in general have large potential as diagnostic but also as prognostic marker.

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Biomarkers in cancer

Cod: M248

EXPECTED VALUES FOR HUMAN EPIDIDYMIS PROTEIN 4 (HE4) WITH FEMALE POPULATION IN REPUBLIC OF SRPSKA.

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Background: Ovarian cancer is the fourth most common cause of cancer-related death in women worldwide. Ovarian cancer,the most lethal form of gynecological cancer, is potentially curable if diagnosed early. 70-75% of ovarian cancers are detected at a late stage. However, when the disease is diagnosed earlier, the survival rate increases to 94%. CA 125 has been the tumor marker of choice in ovarian cancer with a poor diagnostic specificity of 50% in the early stages. Human epididymis protein 4 (HE4), a relatively new marker for ovarian carcinoma, is the product of the WFDC2 (HE4) gene that is overexpressed in patients with ovarian carcinoma. As a single tumor marker, HE4 had the highest sensitivity for detecting ovarian cancer, especially in stage I disease, the early non-symptomatic stage. The aim of this study is to determine the reference intervals of HE4 in population from Republic of Srpska.
Methods: 181 women participated in the study. They were distributed in the five age groups. All women were subjected to a pelvic ultrasonography screening and confirmed to be free from ovarian patology on recruitment. Serum HE4 levels were determined by electrochemiluminescence immunoassay "ECLIA", intended for use on cobas e 601.
Results: Patients were distributed according to their age in the five groups. For each group 95th percentile is calculated: <40 years (53,9 pmol/l), 40-49 years (72,04 pmol/l), 50-59 years (77,2 pmol/l), 60-69 years (94,2 pmol/l) and >70 years (104,3 pmol/l). Also, for each group median is calculated: <40 years (39,0 pmol/l), 40-49 years (46,0 pmol/l), 50-59 years (45,4 pmol/l), 60-69 years (51,1 pmol/l) and >70 years (57,1 pmol/l). The concentration of HE4 was noted to be increasing with age especially in women who were more than 60 years.
Conclusions: Given results do not differ significantly from the results presented by the manufacturer, ie. from their expected values for each group.

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Biomarkers in cancer

Cod: M251

A ENT- KAURENOID DERIVATIVE OF STEVIOSIDE; CPUK02, RESTORE ERA GENE METHYLATION PATTERN VIA ALTERATION IN mRNA LEVELS OF DNMT3A AND DNMT3B GENES IN MDA-MB 231 CELL LINE

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Background: CPUK02 (15-Oxosteviol benzyl ester) is a new ent-kaurenoid derivative of stevioside and exhibits strong anti-cancer activity in vitro and in vivo research models. Nowadays, the role of epigenetics in cancer has been the subject of intensive study and DNA methylation targeting represents a relevant strategy for cancer treatment. Since, no study conducted to this mechanism, we attempt to evaluate whether CPUK02 induce its anti-cancer effects via alteration the level of mRNA DNMT3b, DNMT3a expression and ERα methylation pattern in breast cancer cell lines.
Methods and Results: MCF-7 (ER +) and MDA-MB231 (ER-) cell lines were treated for 24, 48 hours with 1 μM CPUK02 and 5-AZA-CdR (DNA methyltransferase inhibitor). Quantitative expression of DNMT3b and DNMT3a genes and ERα promoter methylation was assessed by Real-Time PCR and MS-PCR, respectively. Treatment of MDA-MB 231cells with CPUK02 restored unmethylated allele. In addition, the intensity of band in cells treated with CPUK02 was more than 5-aza-2- deoxycytidine. These results suggest that CPUK02 was even more powerful than 5-aza-2- deoxycytidine in restoring of ERα promoter unmethylation status of MDA-MB 231 cell lines. Herein, treatment with CPUK02 decreased the expression of both DNMT3a and DNMT3b genes like 5-AZA. The expression of DNMT genes were diminished by half compared with control cells following treatment with CPUK02.
Conclusions: These results showed that treatment of MDA-MB231 cell lines with CPUK02 could return ERα gene unmethylation phenotype with demethylating property and diminishing of DNMT3a and DNMT3b genes expression level.

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Biomarkers in cancer

Cod: M252

PROGNOSTIC MODEL FOR ASSESSING THE RISK OF TUMOR PROGRESSION IN OVARIAN CANCER PATIENTS

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BACKGROUND: The aim of this work was to develop a prognostic model based on available laboratory tests that reflect clinical course of ovarian cancer and determine the probability of metastases and relapse after complex therapy.
METHODS: The study comprised 85 ovarian cancer patients (T1-3N0-2M0-1), all of whom underwent surgery and 6 cycles of adjuvant chemotherapy (CT). CA125 and HE 4 tumor markers, vascular endothelial growth factor (VEGF), tumor necrosis factor, interleukin-8 and ROMA index (Risk of Ovarian Malignancy Algorithm) were measured in the serum using immunoassay at several time points during treatment, which allowed to identify two tests (CA125, VEGF) that can be used to assess the risk of tumor progression.
RESULTS: Analysis of the association between these indices and the effect of therapy (57 patients with tumor progression and 23 without) revealed that percentage change in their serum concentrations after 3 CT cycles was of greater prognostic value than their respective absolute values. ROC analysis was performed to select the most informative tests. $_{\Delta}$CA125 (AUC=0,793; p<0,003) and $_{\Delta}$VEGF (AUC=0,774; p<0,006) were used to build a model that allows to assess the risk of tumor progression after 3 CT cycles. Model performance was evaluated on a validation set, which comprised 26 ovarian cancer patients. Applying the developed model during therapy allowed to correctly predict tumor progression in 80.8% of cases (21 out of 26 patients). False negative results were obtained in 11.5% (3/26) of cases and 7.7% (2/26) of patients were false positives.
CONCLUSIONS: The newly developed prognostic model for assessing the risk of tumor progression provides clinically significant information on the effectiveness of therapy early in the treatment, which allows to make timely adjustments to CT regimens and thus to improve treatment quality.

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Biomarkers in cancer

Cod: M253

HEMOSTATIC STATUS IN RENAL CANCER

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BACKGROUND: In malignancy, activation of the hemostatic system influences tumor growth, thereby causing tumor progression. Endothelial dysfunction in cancer patients is directly linked to platelet hemostasis, as fibrinolytic factors and factors of platelet aggregation are synthesized and inhibited by vessel endothelium. As such, endothelial dysfunction can be viewed as a marker of relapse and tumor progression.
METHODS: Endothelial growth factor, platelets and plasma components of hemostatic system were tested by immunoassay, latex agglutination assay, clotting, immunoturbidimetry and photometry. The study comprised 40 patients with verified stage I-IV renal cancer and 30 clinically healthy individuals. Data were analyzed using nonparametric statistics (STATISTICA 8.0). Differences were considered significant at $p<0.05$.
RESULTS: Investigation of platelet aggregation in cancer patients revealed a statistically significant increase in spontaneous aggregation 4.5 (3.5; 6.5)%, ($p<0.0001$) as well as in ADP-mediated aggregation 30.1 (19.5; 47.0)%, $p=0.03$ and aggregation velocity 31.4 (18.2; 34.8)%/min, $p=0.02$, as compared to healthy individuals. According to coagulogram data, hemostatic balance was shifted towards hypercoagulbility in 48.0% of patients. Fibrinogen concentration was 4.0 g/l or higher in 36.0% of patients. Activity of von Willebrand factor exceeded 160.0% in 48.0% of patients. D-dimer concentration was 0.5 µg/ml or higher in 45.0% of patients. Activity of prothrombin complex factors was found to be lower in cancer patients as compared to the control group, at the same time, cancer patients had a higher INR ($p<0.0001$). Fibrinolytic system of cancer patients was characterized by a lower antiplasmin activity 98.0 (93.0; 106.0)%, ($p=0.0003$). Concentration of endothelial growth factor exceeded the reference interval in about 40% of patients, reaching a maximum of 325.3 ng/l.
CONCLUSIONS: At diagnosis, platelet-endothelial imbalance may serve as a potential marker of tumor progression in renal cancer patients.

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Biomarkers in cancer

Cod: M254

PATIENTS` SELF ORDERING OF TUMOR MARKERS TESTS – GOOD OR NOT?

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BACKGROUND
In Poland every patient is able to self order every laboratory test. The authorities in laboratory diagnostics suggest that unlimited access to these test should not be recommended due to patients` lack of specific knowledge to interpret results of tumor markers test. What is more, choosing of tumor markers is probably based on medial/internet popular science publications without proper cognizance of organ specificity of such a tests and potential false positive and false negative results. Therefore the aim of this study was to analyze the range of this phenomenon.
METHODS
We compare the quantity of doctors` orders and patients` self orders of chosen tumor markers as PSA, CA 125, CEA, CA 15-3 and CA 19-9 in medium size, multi profile Medical Diagnostic Laboratory in Nowy Sącz, Poland.
RESULTS
Our laboratory perform more than 350000 tests annually, therein number of tumor markers tests increase annually, make up 0.41% in 2011 and 0.99%, 0.95%,1.02%, 1.30% in subsequent years. Usually the number of self ordered was twice doctors ordered test. Beside this, profile of ordered tests was similar in both cases – around 50% belong to PSA and 31% to CEA. The shocking is rate of results over reference range in both groups. In CEA and CA19-9 test results over reference range were found only in the group of patients` self ordered tests. In the case of remaining tumor markers also the majority of positive results were in the group of patients self ordered tests.
CONCLUSIONS
Self ordering of tumor markers tests become year to year more popular. Lack of sufficient knowledge about the limitations of these tests may lead to a deterioration of the situation of patients self ordered such a tests because of false negative results, typical f.e. in the early stages of the disease. On the other hand we have lack of organ specificity and false positive results. A high rate of positive results in patients self ordered tumor markers tests suggests the necessity of improvement in the screening programs of early detecting tumors and is against the thesis that only doctors should order these tests. However patient should not be left alone – laboratory staff does not know patient`s medical records, and cannot be persuaded to interpret the results of markers. The solution to the situation of pathological results of self ordered tumor markers tests is compulsory inform patients that they should consult these results with the doctor.

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Biomarkers in cancer

Cod: M255

DEVELOPMENT OF A NEW sFLC ELISA ASSAY FOR THE QUANTIFICATION OF SERUM FREE LIGHT CHAINS

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BACKGROUND. In the last decade, the serum-free light-chain (sFLC) assay has been shown to be important in the diagnosis and management of plasma cell dyscrasias. Commercial nephelometric and turbidimetric assays are available to detect sFLC. Since its availability in 2001, there have been several publications discussing the clinical utility but also the numerous analytical limitations of this technology for sFLC testing. We describe herein the validation of a new generation of sFLC assays based on ELISA technology to overcome these very well known limitations.
METHODS. We industrialized a home-made ELISA assay (Sebia FLC, Sebia, France) developed at the University of Nijmegen (The Netherlands) for both free kappa (K) and free lambda (L) quantification. This assay is a sandwich ELISA and uses polyclonal anti-FLC capture antibodies. This test was compared to the Freelite assay (The Binding Site, UK) on 130 samples. Sensitivity, linearity, measurement range, reproducibility, interference assessment and coherence to Serum Protein Electrophoresis (SPE) peak quantification were performed during validation. All experiments above were carried out in parallel on a manual ELISA and on an ELISA processor (AP22 Elite, das, Italy) using a specific validated program.
RESULTS. We first established the normal reference ranges of the Sebia FLC test using healthy donors. We show good correlation for sFLC K, L and K/L ratio between the 2 tested techniques for the tested patients. The Sebia FLC assay has a sensitivity of 0.5 mg/L for both K and L. We show that the assay has a very good reproducibility (intra-assay, inter-assay and lot-to-lot) and linear on the measuring range. Sebia FLC has a measurement range 5 times broader that the average measurement range of the nephelometric/turbidimetric techniques on the market and this allows obtaining 4 to 5 times less out-of-range samples in a batch of analysis leading to significantly decreased reagents' consumption. In addition to the above, this new test brings more coherent results with SPE results. All the above were proven on both the manual technique and on AP22 Elite.
CONCLUSION. We describe here a new generation of commercial FLC assay based on ELISA. This assay has been fully validated in comparison to existing methods. Moreover, it brings more coherence with electrophoresis results and generates less retest for out-of-range samples.

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Biomarkers in cancer

Cod: M256

ROLE OF THE PIVKA-II CONCENTRATION IN PERIPHERAL BLOOD IN HEPATOCELLULAR CARCINOMA LIVER TRANSPLANT PATIENTS.

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Background
The orthotopic liver transplant (OLT) appears as the most reliable option for the treatment of patients with Hepatocellular carcinoma (HCC) (with Milan criteria). PIVKA-II is a Factor II precursor that increases specifically in HCC. The objective of this study was to determine the role of PIVKA-II in the management of the patients candidate to OLT.

Methods
31 HCC patients included in waiting list for OLT were evaluated. PIVKA-II and alfa-fetoprotein (AFP) serum levels were performed using the LUMIPULSE G1200 system (Fujirebio Europe N.V. Gent, Belgium). Spearman Rho and Mann-Whitney tests were used to evaluate the relationship between these markers and time on the transplant list, time from diagnosis, number of tumors, number of transcatheter arterial chemoembolization and Standard uptake value. To assess the role of these markers in 23 patients post-transplant, t-Student test was performed (SPSS 18.0).

Results
PIVKA-II and AFP median concentrations were respectively 98 mAU/mL (Range: 19-20660 mAU/mL) and 7.8 ng/mL (Range: 1.9-3638 ng/mL). A statistically significant positive association was found between PIVKA-II and the number of tumors (Rho=0.410; P<0.05). The mean concentrations and standard deviations from 23 patients for AFP pre-transplantation and 1, 6 and 12 months after transplantation were respectively: 10.6±8.4 ng/mL, 3.7±2.2 ng/mL, 12±27 and 3.3±1.7 ng/mL. For PIVKA-II, the results were: 154±192 mAU/mL, 28.5±23.3 mAU/mL, 47.2±66 mAU/mL and 25.1±5mAU/mL. Statistically significant differences in PIVKA-II concentrations were found at all time points post-transplantation (1 month t= 2.9 p=0.008, 6 months t= 3.3 p=0.005 and 12 months t= 2.2 p=0.05). For AFP 6 months after transplantation, no statistically significant differences were found (1 month t= 3.8 p=0.001, 6 months t=-0.413 p=0.68 and 12 months t= 3.2 p=0.008).

Conclusions
In contrast to AFP, PIVKA-II values correlate with the number of tumors and show significant differences at OLT after 1, 6 and 12 months in patients that do not present tumors by imaging tests. From these results it can be stated that PIVKA-II could be useful in the management of these patients as a tumor marker, playing an important role in the prioritization criteria and evolution after transplantation.

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Biomarkers in cancer

Cod: M257

ONCOLOGICAL STUDY USING MICROFLUIDIC LIQUID BIOPSY IN PERIPHERAL BLOOD IN HEPATOCELLULAR CARCINOMA LIVER TRANSPLANT PATIENTS

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Background
Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. The orthotopic liver transplant (OLT) is a relevant curative alternative for patients in early stages. Detection of circulating tumor cells (CTCs) in peripheral blood indicates progression of neoplastic disease. We aimed to determine the role of CTCs as a complementary test to imaging, in the management of the patients candidate to OLT, in regard to their prioritization and treatment monitoring.
Methods
29 HCC patients included in the transplant waiting list for OLT (with Milan Criteria) were evaluated. Isolation and count of CTCs were performed by IsoFlux™ system (Fluxion) and fluorescence microscopy. Spearman Rho test was used to evaluate the relation between CTCs and time on the transplant list, time from diagnosis, number of tumors, number of transcatheter arterial chemoembolization (TACE) and Standard uptake value. To assess the role of CTCs in 21 patients post-transplant and their relation with vascular invasion, Wilcoxon and Mann-Whitney tests were performed (SPSS 18.0).
Results
For the 29 patients, the median of CTCs was 16 CTCs/10 mL (Range: 0-539). A statistically significant positive association was found between CTCs and wait list time (Rho=0.41; P=0.027) with a median of 112 days (Range: 7-645) and CTCs and TACE´s number (Rho=0,419; P=0,024) with a median of 1 (Range: 0-3). For 21 patients, the median and range for CTCs before and 1, 6 and 12 months after transplantation were respectively: 27, 6, 5 and 1.5 CTCs/10 mL (Ranges: 0-1768, 0-1150, 0-214 and 0-104). Statistically significant differences in CTCs levels were found using Wilcoxon test at 1 (P=0.03) and 12 months (P=0.02) post-transplantation. The CTCs median of patients without and with vascular invasion were 13 and 539 CTCs/10 mL (ranges: 0-188 and 448-1768) respectively. A statistically significant difference was found between both groups (P=0.006).
Conclusions
High numbers of CTCs are related to a longer time in waiting list, to the number of TACEs and with vascular invasion after OLT. The number of CTCs diminishes significantly 12 months after transplant. We can state that CTCs are useful in the management of these patients, regarding the prioritization criteria and monitor after transplant.

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Biomarkers in cancer

Cod: M258

ARE THERE DIFFERENCES BETWEEN CIRCULATING TUMOR CELLS COUNT IN DIFFERENT ETIOLOGIES OF LIVER CANCER? A PRELIMINARY STUDY.

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BACKGROUND
Hepatocellular carcinoma (HCC) usually occurs as a consequence of an already established cirrhosis. Furthermore, some risk factors such as chronic hepatitis virus infection, environmental toxins, alcoholism, and other chronic liver diseases, may be responsible for this pathology. Althought liver transplantation is considered as a potential cure for HCC, 10% of patients approximately have recurrence during the first year after surgery due to the presence of circulating tumor cells (CTCs). These cells derived from primary tumor lesion.
We aimed to compare the levels of CTCs in patients with different etiologies of HCC, before transplantation, a month, 6 months and a year or longer after surgery.

METHODS
39 HCC patients waiting liver transplantation according to Milan criteria, 20 liver transplant patients after a month of surgery, 13 liver transplant patients after 6 months of surgery and 15 liver transplant patients after a year or longer of surgery, were included. CTCs were isolated using Isoflux System™ with immunomagnetic beads coated with antiEpCAM antibodies. Cell count was performed in a fluorescence microscope.
CTCs levels are compared among 10 different etiologies of HCC in all patient groups.
Kruskal-Wallis test was estimated to compare levels of CTCs in patients with different etiologies of HCC (SPSS 22.0).

RESULTS
The median CTCs concentrations in pre-transplant, 1 month post-transplant, 6 months post-transplant and 1 year or longer post-transplant patient groups were 10±43 CTCs/10 mL, 6±21.2 CTCs/10 mL, 4±40.5 CTCs/10 mL mL and 1±4 CTCs/10 mL, respectively.
The comparison of CTCs levels between different etiologies in pre-transplant, 1 month post-transplant, 6 months post-transplant and 1 year or longer post-transplant patient groups using Kruskal-Wallis test shows the following values: χ^2=8.918 p=0.445; χ^2=3.443 p =0.751; χ^2=1.953 p=0.856 and χ^2=8.155 p=0.148, respectively.

CONCLUSIONS
The results showed that CTCs levels were not significantly different between different etiologies of HCC, either before or after surgery. We concluded that the levels of CTCs are independent of the kind of disease responsible for cancer. Thus, there is a risk of recurrence and metastasis due to the existence of CTCs, regardless of the etiology of neoplasia.

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Biomarkers in cancer

Cod: M259

ROLE OF GALECTIN 3 COMBINED WITH MULTI- DETECTOR CONTRAST ENHANCED COMPUTED TOMOGRAPHY IN PREDICTING DISEASE RECURRENCE IN PATIENTS WITH OVARIAN CANCER

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Galectin-3 (Gal-3) is an endogenous β-galactoside-binding lectin playing an important role in the pathogenesis of multiple malignancies.Aim of the study is to evaluate the role of Gal-3 combined with multi-detector contrast-enhanced computed tomography (CECT) as predictor of recurrence disease in a group of patients treated for ovarian cancer (EOC)
Seventeen follow-up women with recurrent ovarian cancer and 13 follow-up women with stable ovarian disease who performed CECT at one-year follow-up after cytoreductive treatment were enrolled. Serum Gal-3 concentrations were determined by using ELISA method. 20 healthy controls were included in the analysis. Two radiologist blinded to patients status independently reviewed CECT exams recording the following signs of disease recurrence: local tumor spread, enlarged lymph-nodes, carcinomatosis implants and metastases.
We calculated the respective threshold values of Gal- 3 identified by ROC curve analysis for each imaging findings related with disease recurrence : lymphoadenopathies 92.45 ng/ml (AUC :0.81, Se=91% Spe=73%) , carcinomatosis 85.95 ng/ml (AUC :0.93Se = 93.7%, Sp=92.8%), local tumor spread 99.05 (AUC:0.90 , Se=100% , Spe=73%) and metastasis 99.05ng/ml (AUC :0,78, Se=100% , Spe=70%). We found a significant correlation between high galectin 3 serum levels and presence of local tumor spread (n=11/17 ,p=0.001), carcinomatosis (n=16/17, p=0.00), lymphoadenopathies (n=15/17 p=0.00) and metastasis (n=11/17, p=0.003) related with recurrence disease.
Patients with recurrence of ovarian cancer presents higher Gal-3 values compared to women with stable diseases . Gal-3 combined to CECT should be used to improve the monitoring of EOC patients.

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Biomarkers in cancer

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SERUM VALUES OF CEA AND CA 19-9 IN COLORECTAL CANCER

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Background: Colorectal carcinoma is the thert on the incidence and the second cause of death in the world. About 95% of colorectal cancers are sporadic and 5% hereditary. It is recommended the determination of CEA and CA 19-9 in patients with colorectal cancer for the prognosis and monitoring the efficiency of the tretment.
Methods: The determination of CEA and CA 19-9 was done on the IMMULITE 1000 analyzer, SIEMENS company, chemiluminescence immunometric essay. The method consists of 2 phases, the first passes through the binding of antigens (serum) and antibodies to alkaline phosphatase (reagent), and in the second is added adamantyl dioksetan phosphatase which leads to the luminescence.
Results: A statistical analysis of the data led to conclusion that CEA and CA 19-9 was no significantly higher in patients whit B-stage in relation to A-stage Duke's (p=0,318 for CEA and p=0,803 for CA 19-9). The values of CEA and CA 19-9 were significantly higher in patients whit D-stage in relation to C-stage (p=0,010, p=0,003). Between the examined groups, the CEA values showed a statistically significant difference (p=0,001), while the value of CA 19-9 was no statistically significant difference (p=0,175). The sensitivity of CEA between the examined groups and the control group was 73,3% with the specificity of 63,4% while CA 19-9 has a sensitivity of 26,7% and specificity of 100%. Area under the curve (AUC) of the CEA was 0,763 and 0,588 for CA 19-9. For group 1 patients (Duke's A and B stages) CEA sensitivity was 60,0% and specificity of 33,3% and CA 19-9 was 13,3% and 36,7%. AUC for CEA was 0,222 and 0,400 for CA 19-9. For group 2 patiens (Duke's stage C and D) the sensitivity of CEA was 86,67% with the specificity of 40% and CA 19-9 36,67% with the specificity of 86,67%. AUC was 0,778 for CEA and 0,600 for CA 19-9.
Conclusions: As a result of our research, we found that the sensitivity and specificity of CEA and CA 19-9 increases with the progression of the disease, as well as their value. By analyzing the results, we concluded that the correlation is statistically significant among the respondents in all groups.

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Biomarkers in cancer

Cod: M261

THE NOVEL HUMAN GENE, UBE2Q1, AFFECTS P53 EXPRESSION VIA MDM2 IN COLORECTAL CANCER CELL LINES

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BACKGROUND: Recently, it was reported that Ubiquitin Conjugating Enzyme E2 Q1 (UBE2Q1) gene was highly expressed in human colorectal tumors but its role in colorectal cancer progression remains unexplored. Since p53 tumor suppressor protein is a well-known key molecule in carcinogenesis and also is a substrate for the ubiquitin-proteasome system; we speculated that UBE2Q1 might also participate in cancer development by p53 regulation. In the present study, the effect of UBE2Q1 overexpression on the level of p53 as well as on expression levels of MDM2 gene has been investigated in SW480 (expressing mutant p53) and LS180 (expressing wild type p53) colorectal cancer cell lines.
METHODS: Using lipofection method, SW480 and LS180 cell lines were transfected with pCMV6-AN-GFP vector containing UBE2Q1 open reading frame (ORF). SW480 and LS180 cells that express the green fluorescent protein (GFP) fusion proteins containing UBE2Q1 (GFP-UBE2Q1) were established. Western blot analysis was employed to verify the overexpression of UBE2Q1 in these cells and to evaluate the expression level of p53 before and after cell transfection.
RESULTS: Our study revealed that the levels of p53 were markedly lower in UBE2Q1 transfected SW480 cells as compared with control. Although, the decrease in the level of p53 protein was not considerable in transfected LS180 cells compared with the control. Quantitative real-time PCR was used to assay the mRNA expression level of MDM2 gene. The expression of MDM2 was in line with the results of p53 in both cell lines.
CONCLUSIONS: This repression of p53 may be due to its UBE2Q1 mediated ubiquitination and subsequent proteasome degradation, a process that may involve the direct interaction of UBE2Q1 with MDM2 and/ or p53. In addition to proteasome-mediated degradation, ubiquitination of p53 acts as signals for degradation-independent functions, such as nuclear export and repress the transcriptional activities of p53. Therefore, overexpression of UBE2Q1 may affect the p53-dependent transcriptional activities, and might also participate in cancer development by regulation of p53.

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Biomarkers in cancer

Cod: M262

REFERENCE RATES AND PREVALENCE OF TUMOUR MARKERS CA 15-3 AND CA 125 IN DIFFERENT GROUPS OF CANCER PATIENTS IN VOLGA FEDERAL DISTRICT, THE RUSSIAN FEDERATION

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Objectives. Cancer antigens 15-3 (CA 15-3) and 125 (CA 125) are the most frequently used serum markers of breast and ovarian cancer respectively. We aimed to evaluate the serum levels of these antigens in samples from healthy individuals and cancer patients in regional population.
Methods. CA 15-3 and CA 125 were measured using quantitative ELISA tests (RPC Diagnostic Systems). Serum samples from healthy blood donors (n=245) and oncology patients with confirmed diagnoses (n=560) were evaluated. The groups were compared using Mann–Whitney U test; the difference between groups was considered statistically significant if $p<0.05$. The studied population is representative for Eastern Europe (Volga Federal District, the Russian Federation).
Results. In the population of healthy donors the measured concentrations were defined within the range from 1.3 to 68.5 U/mL for CA 125 and from 2.6 to 54.1 IU/mL for CA 15-3. We calculated the upper reference limits with a nonparametric method, in which the upper reference limit is regarded as 97.5 percentile value. Thus, the normal values for the region of interest were established at the level 31.6 U/mL for CA 125 and 29.2 IU/mL for CA 15-3.
The prevalence of abnormal concentrations of the tumour markers was evaluated in groups of patients with different malignancies: gastrointestinal, prostate, ovarian, breast, cervical, and testicular cancer. The significant difference from the control group was observed in the groups with ovarian, breast, prostate and gastrointestinal cancer for CA 125 and with cervical, breast, ovarian and prostate cancer for CA 15-3. The highest prevalence rates were observed in the groups with ovarian cancer (53.5%) for CA 125 and cervical cancer (61.9%) for CA 15-3.
Conclusions. According to these data, the upper limit of normal range is 31.6 U/mL for CA 125 and 29.2 IU/mL for CA 15-3 in our region. The prevalence of CA 125 and CA 15-3 over normal value are higher in ovarian and cervical cancer groups, respectively.

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Biomarkers in cancer

Cod: M263

EVALUATION OF SOLUBLE HER2/NEU RECEPTOR LEVEL IN CA 27-29 NEGATIVE BREAST CANCER PATIENTS

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Background. HER2 status of a malignant breast tumor is crucial to determine diagnostics and treatment strategy. Development of serum tests is one of the foremost trends in biomarker research. Today, the basic markers for diagnosis and follow-up of breast cancer (BC) are CA 15-3 and CA 27-29. The aim of the present study was to evaluate the level of soluble HER2/neu receptor in CA 27-29 negative breast cancer patients.
Methods.73 newly diagnosed patients with stage I–IV BC, aged 35 to 85 years, were enrolled in the study. The control group was comparable to the study group in terms of age ($p>0.05$) and comprised 50 clinically healthy women with no cancer history. Serum levels of HER2/neu epidermal growth factor receptor and CA 27-29 carbohydrate antigen were measured by immunochemiluminescence. Data were analyzed using nonparametric statistics and presented as Me (Q1; Q3). All differences were considered significant at $p<0.05$.
Results. Serum HER2/neu (13,8 (10,6; 17,8) ng/ml) was found to be significantly higher ($p_{Mann\text{-}Whitney}<0.01$) in BC patients than in clinically healthy women (10,1 (7,4; 12,5) ng/ml). CA 27-29 values were in the normal range (0.0- 33.72 U/ml) and no difference was found between the two groups ($p_{Mann\text{-}Whitney}=0.17$). Patients with metastatic BC had a significantly higher concentration of HER2/neu than those with resectable tumors ($p_{Mann\text{-}Whitney}=0.022$). A correlation was revealed between tumor stage and serum HER2/neu ($R_{Spearman}=0.32$; $p=0.037$).
Conclusion. Our data suggest that soluble HER2/neu receptor might become an adequate non-invasive marker and complement the classic laboratory tests.

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Biomarkers in cancer

Cod: M264

PLATELET/LYMPHOCYTE RATIO IN PATIENTS WITH MULTIPLE MYELOMA

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BACKGROUND: Multiple myeloma (MM) is a malignant disorder of plasma cells. Increased platelet to lymphocyte ratio (PLR) was associated with poor prognosis in a various types of cancer. PLR which can be calculated from the whole blood count is a sensitive index presenting a systemic inflammatory response that combines prognostic values of a subjects's platelet and lymphocyte count. The aim of this study was to investigate the platelet/lymphocyte ratio in patients with multiple myeloma.
METHODS: This is a retrospective observational study; evaluating the PLR values of 50 healthy control and 50 patients with multiple myeloma. The mean age for controls and patients were 41.3±1.1 and 43.2±4.9 respectively. Patients with chronic disease and inflammatory disorders were excluded. Platelet and lymphocyte counts were analyzed with Abbott Cell Dyne heamotology analyzer. Statistical analysis was performed with IBM SPSS v20.
RESULTS: Platelet counts were higher but not statistically significant in patients with multiple myeloma compared to control group (270.8±75.6 vs 260±73.8) (p=0.473). Lymphocyte counts were lower in patients with multiple myeloma compared to control group (1.65±0.8 vs 2.47±0.6) (p=0.000). Platelet/Lymphocyte ratio was statistically higher in patients with multiple myeloma compared to control group (228.6±167.7 vs 112.6±42.3) (p=0.000).
CONCLUSIONS: PLR can be easily calculated and is universally available marker, which should be implemented into the clinical practice. This study suggested that PLR is a simple and reliable inflammatory prognostic factor.

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Biomarkers in cancer

Cod: M265

FAST IMMUNODIAGNOSTIC ASSAY FOR DETECTION OF INTACT FREE PSA USING A MUTANT FORM OF MONOCLONAL 4D4 ANTIBODY FRAGMENT

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BACKGROUND Intact free prostate specific antigen (iPSA) is one of the prostate cancer biomarkers with a high diagnostic value. A 2-step immunoassay utilizing a mutant form of recombinant Fab fragment of iPSA specific monoclonal antibody 4D4 (m-4D4-Fab) was published in 2015 where the mutant Fab exhibited improved characteristics over the wild-type monoclonal reference (wt-4D4-Mab). Diagnostic tests with high sensitivity and precision can be produced with inherently fluorescent lanthanide chelates as label molecules. In this study, using an inherently fluorescent label, an updated version of the iPSA immunoassay with m-4D4-Fab was developed to better answer the requirements set for point-of-care diagnostic tests.

METHODS A fast 1-step sandwich-type iPSA immunoassay was developed using recombinant proPSA as the calibrator analyte. Capture surface was created by attaching biotinylated capture antibodies m-4D4-Fab or wt-4D4-Mab to streptavidin microtiter wells. Total reaction volume was 40 µL, where 20 µL of sample was added simultaneously with 20 µL tracer antibody in MES assay buffer pH 6.75. After 15 minute incubation at +36 °C and 900 rpm shaking, the wells were washed and dried. Eu^{3+} luminescence of the tracer antibody, labeled with inherently fluorescent 9-dentate europium chelate, was measured from the dry surface using time-resolved measurement mode. Analytical sensitivity was calculated from a calibration curve (linear fit, cut off value 3xSD where SD was the average specific luminescence signal of blank with 10% CV).

RESULTS The previously reported analytical sensitivities of the 2-step iPSA assays with a (1+1)-hour incubation time with 150 µL reaction volume (50 µL sample) were 0.25 µg/L with wt-4D4-Mab and 0.07 µg/L with m-4D4-Fab. The preliminary analytical sensitivities with the developed 15-minute assays were 0.05 µg/L and 0.03 µg/L, respectively.

CONCLUSIONS The preliminary analytical sensitivity of the updated version of the iPSA immunoassay using the m-4D4-Fab as capture antibody was better than that of the 2-step assay. The assay sensitivity looks promising considering that the assay protocol was a simple 1-step assay and the sample incubation time was reduced from 2 hours to 15 minutes and sample amount from 50 to 20 µL. The reported results hold a great potential for point-of-care diagnostic applications where the tests need to be both rapid and cost-efficient.

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Biomarkers in cancer

Cod: M266

ASSOCIATION OF RESISTIN AND ITS RECEPTOR ADENYLATE CYCLASE-ASSOCIATED PROTEIN 1 WITH COLORECTAL CANCER

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Aim: Resistin is a proinflammatory cytokine produced mainly by peripheral blood mononuclear cells (PBMCs), macrophages and bone marrow cells. Its proinflammatory effects are exerted via adenylate cyclase-associated protein 1 (CAP1) that was recently described as bona fide receptor for human resistin. CAP1 is a multifunctional protein considered to have the ability of linking cell signalling with actin polymerisation, and have a fundamental role in cell growth and cytoskeletal organisation. Although, several studies have suggested that resistin is associated with colorectal cancer (CRC), the importance of CAP1 and resistin mRNA levels as a potential biomarkers in CRC diagnosis have not been thoroughly evaluated.
The aim of this study was to determine if peripheral blood mononuclear cells (PBMCs) CAP1 and resistin mRNA levels, as well as plasma resistin concentration differ between healthy subjects and patients with CRC.
Material and methods: This study included 103 patients with CRC and 114 healthy subjects (CG). Circulating resistin was measured by ELISA, while PBMCs CAP1 and resistin mRNA were determined by real-time PCR.
Results: Plasma resistin and CAP1 mRNA levels were significantly higher in CRC patients compared to CG ($P<0.001$, $P=0.043$, respectively), while resistin mRNA levels were significantly lower in patients compared to healthy subjects ($P<0.001$). A strong positive correlation was found between CAP1 and resistin mRNA ($\rho=0.324$, $P=0.002$), but only in CG.
Conclusion: Elevated plasma resistin together with upregulation of CAP1 and downregulation of resistin gene is associated with CRC. Circulating resistin is able to exert stronger effects on PBMCs due to overexpression of its receptor CAP1 thus leading these cells into more proinflammatory state. Downregulation of resistin in PBMCs could be compensatory reaction to its increased circulatory levels. While the contribution by PBMCs to the resistin plasma concentrations is not to be underestimated, it should be taken under the consideration that local production of resistin by macrophages in the sites of inflammation and its liberation into circulation influence its overall concentration in blood.

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Biomarkers in cancer

Cod: M267

NLR AND PLR IN COLORECTAL CANCER PATIENTS

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Background: Prognostic value of inflammatory factors, including CRP, leukocytosis, neutrophilia, lymphocytopenia, and calculated ratios as the NLR (neutrophil/lymphocyte) and PLR (platelet/lymphocyte) have been demonstrated in some tumors.
Aim: The purpose of our study was to evaluate the prognostic value of NLR, PLR and CEA in colorectal cancer (CRC) patients.
Material and Methods: Hematological parameters and serum CEA level were measured before surgery in 136 patients with CRC and in 50 healthy individuals.
Results: Colorectal cancer patients, in comparison to the reference group, had significantly higher concentrations of CEA ($P=0.00002$) and also significantly higher values of NLR ($P<0.000001$) and PLR ($P<0.000001$). In the studied group of patients there were no significant correlations between the concentrations of CEA vs. NLR and PLR values. Analysing concentrations of the determined factors in respect to tumor stage (I+II vs. III+IV), significantly higher CEA concentration ($P<0.001$) and significantly higher PLR value ($P<0.006$) were found in patients with more advanced tumor stage, with lack of significant differences in NLR. When analysing values of the studied factors in respect to the local extent of tumor (T1-2 vs.T3-4), significantly higher CEA, NLR and PLR values ($P<0.03$; $P<0.001$; $P<0.003$, respectively) were determined in the group of patients with T3-4 stage. In the group with involved lymphatic nodes (N1-3), the concentration of CEA and PLR value were significantly higher ($P<0.002$; $P<0.02$; respectively), in comparison with those without nodal involvement (N0). Univariate analysis showed, that shorter disease-free survival was associated not only with III and IV tumor stage ($P<0.004$), T3-4 local extent of the tumor ($P<0.0006$), lymph node metastases ($P<0.002$), but also with CEA levels exceeding 4.9 ng/mL ($P<0.0001$), NLR value greater than 2.8 ($P<0.005$) and PLR value greater than 175 ($P<0.003$). Three relevant independent predictors of poor DFS were identified in multivariate analysis: stage of disease (HR=2.03), CEA (HR=2.93) and PLR (HR=2.28).
Conclusion
Apart from stage of disease, also an elevated preoperative concentration of serum CEA and PLR value were an independent predictors of poor disease-free survival in patients with colorectal cancer.

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Biomarkers in cancer

Cod: M268

MOLECULAR CHARACTERIZATION OF IN-VIVO ISOLATED EPCAM-POSITIVE CIRCULATING TUMOR CELLS IN BREAST CANCER

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background: In the early stages of cancer, the chance to detect rare CTCs is increasing by increasing the sample volume. The aim of our study was to evaluate the diagnostic sensitivity of a novel clinical device for the in-vivo isolation of EpCAM-positive CTCs (CellCollectorTM, GILUPI, GmBH), by using highly sensitive RT-qPCR molecular assays.
Methods: 47 breast cancer patients without overt metastases before the beginning of adjuvant chemotherapy (M0), 15 early breast cancer patients after the completion of therapy, 26 breast cancer patients with overt metastases before starting of therapy (M1) and 14/26 of them before the second cycle of therapy (M2), as well as 20 healthy donors participated in the study. After in-vivo isolation, total RNA was extracted from captured cells, lysed in Trizol, followed by cDNA synthesis. RT-qPCR was used for the molecular characterization of captured cells, for: CK-19, HER-2, TWIST1, VEGF, ER, PR, EGFR, CD44, CD24, and ALDH1, while B2M was used as a reference gene. Peripheral blood was also collected for CTC analysis by the FDA cleared CellSearchTM system. In addition, immunofluorescence staining of cytospins was performed and screened for CTCs using the ARIOL system, using ER, HER2, CK (8, 18, 19) and CD45 for CTC identification.
Results: At least one gene was expressed in 22(46.8%) of M0, 7(46.7%) of M0 after the completion of therapy, 16(61.5%) of M1 and 5(35.7%) of M2 patient groups, but in none of healthy donors 0/20(0%). CellSearchTM gave positive results in 9(19.6%) and 6(42.6%) of M0 before and after the completion of therapy, 10(38.5%) of M1 and 1(7.1%) of M2. Immunofluorescence (Ariol system) was positive for ER, HER2, CK (8, 18, 19) in 8/37(21.6%) M0, 3/15 (20%) after the completion of therapy, in 6/16(37.5%) M1 and in 2/14(14.3%) M2 groups.
Conclusions: In-vivo isolation of CTC is minimally invasive, and in combination with high specific and sensitive RT-qPCR assays for CTC detection and molecular characterization seems promising. These results should be validated in large patient cohorts, and in respect to the clinical outcome.

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Biomarkers in cancer

Cod: M269

THE RELATIONSHIP BETWEEN OBESITY, AND IL-6, VEGF, MMP-9 AND TIMP-1 IN BREAST CANCER PATIENTS

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Background: Obesity is associated with an increased risk of breast cancer developing, especially in postmenopausal women. It is associated not only with an increased risk of oestrogen receptor (ER)-positive breast cancer in postmenopausal women, but also is associated with a worse clinical outcome regardless of menopausal status. Of particular interest in obesity is its association with chronic inflammation. The aim of the performed study was the analysis of relationships between IL-6 and MMP-9, TIMP-1 and VEGF in groups of breast cancer patients selected in respect to BMI values.
Material and methods: The study was performed in 217 breast cancer patients qualified for surgery, and in the reference group of 47 healthy women. The VEGF, MMP-9 and TIMP-1 determinations were performed by ELISA method using reagents from eBioscience and miniBos analyzer. IL-6 concentration was determined by ECLIA method with the use reagent kits and Cobas e411 analyzer (Roche Diagnostics).
Results: In breast cancer patients, compared to the reference group, were found significantly higher concentration of MMP-9, TIMP-1, VEGF, IL-6 and also significantly higher BMI values. In the group of patients there were significant relationships between the BMI vs IL-6 and VEGF as well as the correlations between IL-6 vs. MMP-9 and TIMP-1 and MMP-9 vs. TIMP-1. However, in the group of breast cancer patients aged over 55 years compared to the younger found only significantly higher BMI values and the concentration of IL-6, with no significant differences in other parameters. Moreover, comparison of IL-6, VEGF, MMP-9 and TIMP-1 between the groups separated due to the BMI (<30>) revealed only a significantly higher levels of IL-6 in patients with BMI greater than 30 at the lack significant differences in other paameters.
Conclusions:
1. In patients with breast cancer, obesity (BMI greater than 30) is associated with increased concentration of IL-6.
2. The significant correlations between IL-6 and MMP-9 and TIMP-1 seem to confirm the important role of inflammation in the progression of breast cancer.
3. The correlation between MMP-9 and VEGF indicates the participation of this metalloproteinase in the activation of angiogenesis in breast cancer patients.

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Biomarkers in cancer

Cod: M270

THE UTILITY OF IL-6, suPAR, NEUTROPHILS AND LYMPHOCYTES IN THE QUALIFICATION OF PATIENTS WITH INVASIVE BLADDER CANCER WITH NON-RADICAL TURBT FOR THE CONSERVATIVE TREATMENT

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Introduction: The treatment of patients with invasive bladder cancer is a multistage process, requiring consideration of a number clinical factors, including surgical radicality of transurethral resection of the bladder tumor (TURBT). In contrast to patients with totally resected bladder tumor, the majority of which can be routinely qualified for conservative definitive therapy, the lack of radical TURBT may hinder treatment decision making. The aim of this study was to evaluate the potential usefulness of the serum IL-6 and suPAR, as well as the number of neutrophils and lymphocytes in the selection of non-radical TURBT patients for further therapy.
Material and methods: The determinations of IL-6, suPAR, neutrophils and lymphocytes were performed in the group of 115 invasive bladder cancer patients with non-radical TURB and in the reference group of 30 healthy subjects.
Results: In the group of invasive bladder cancer patients after non-radical TURBT compared to the reference group were found: significantly higher level of IL-6, suPAR and the number of neutrophils, the absence of significant differences in the number of lymphocytes. Moreover, between the group of patients qualified to definitive conservative treatment (based on clinical evaluation) and patients unsuitable for this form of therapy significant differences in the concentration of IL-6 and suPAR and the number of neutrophils has been found. Analysis curves for IL-6, suPAR, neutrophils and lymphocytes plotted for group non-eligible to conservative treatment against group eligible to this form of treatment showed that the sensitivity and specificity in the differential diagnosis of these two groups are respectively 55.4% and 84.l7% (IL-6), 73.9% and 71.2% (suPAR), 60.0% and 76.3% (neutrophils), and 63.8% and 77.8% (lymphocytes).
Conclusion: The determination of IL-6 and suPAR seems to be useful in the correct qualification of bladder cancer patients with non-radical TURBT for further therapy.

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Biomarkers in cancer

Cod: M271

TUMOR-ASSOCIATED GLYCANS FROM SERUM GLYCOPROTEINS FOR THE DIAGNOSIS OF EARLY-STAGE SEROUS EPITHELIAL OVARIAN CANCER

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Background: Epithelial ovarian cancer (EOC) is the most frequent cause of death from all gynecological malignancies because of its late diagnosis. As N-glycosylation is modified in the course of ovarian cancer, it is a promising source of tumor biomarkers. In this work, we investigated the glycome of total serum of primary serous ovarian cancer patients, patients suffering from benign ovarian tumors and healthy controls. We also investigated for the first time the N-glycome profiles of ascetic fluid from primary serous EOC patients and compare them with the serum N-glycome of the same patients as well as healthy controls.

Methods: Serum N-glycans were released from total serum proteins, permethylated and measured by MALDI-TOF-MS. The areas of the glycan structures that were significantly up- or downregulated were combined as a score named GLYCOV. The diagnostic performance of the GLYCOV value was compared with CA125 using Receiver Operating characteristics curves. Sensitivity and specificity were calculated using binary logistic regression.

Results: GLYCOV was able to diagnose early-stage as well as late-stage serous EOC better than CA125 and even allowed the discrimination between malignant and benign ovarian tumors. Ascites showed qualitatively as well as quantitatively different N-glycosylation pattern compared to healthy serum. Overall, increased antennarity, branching, sialylation and LewisX motives were observed in ascites samples. Indeed, different intensities of N-glycans were detected especially for the highly branched N-glycans. In addition, a correlation was established between ascites volume and degree of sialylation.

Conclusion: We reported for the first time the N-glycome of ascetic fluid and showed that the glycome modulations, previously detected in EOC serum were also present in ascites. Both serum and ascetic fluid from EOC patients exhibited typical features of inflammatory conditions, when compared with healthy serum.

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Biomarkers in cancer

Cod: M272

DETECTION OF BREAST CANCER ASSOCIATED GLYCANS ON MUC1/CA15-3 WITH LECTINS COATED ON EUROPIUM NANOPARTICLES

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BACKGROUND: The cancer antigen 15-3 assay (CA15-3) has been widely used for the detection of breast cancer (BCa) recurrence; however, its sensitivity and specificity are inadequate for early detection of the disease. CA15-3 is known to be differentially glycosylated in BCa, potentially offering a way to construct CA15-3 assays with improved cancer specificity. The goal of our study was glycoprofilling of BCa associated CA15-3 for sensitive and specific assay development.

METHODS: CA15-3 from a breast cancer cell line was captured by anti-CA15-3 antibody immobilized on microtitration wells. A panel of lectins, coated onto fluorescent Europium-chelate-doped nanoparticles (Eu^{+3}-NPs), was used for the detection of immobilized CA15-3 by measuring the time-resolved fluorescence of Eu after the separation of unbound particles. Clinical evaluation was done on serum samples from metastatic BCa patients (n=44) and healthy women (n=31).

RESULTS: We found that wheat germ agglutinin (WGA) and macrophage galactose-type lectin (MGL) -Eu^{3+} NPs recognize BCa associated CA15-3 ($CA15\text{-}3^{Lectin}$). Serum $CA15\text{-}3^{Lectin}$ measurement better discriminated patients with metastatic BCa from healthy women as controls compared to a conventional CA15-3 immunoassay. The clinical sensitivities of the assays were 59.1, 70.5 and 77.4 % for conventional CA15-3, $CA15\text{-}3^{MGL}$ and $CA15\ 3^{WGA}$, respectively.

CONCLUSIONS: Our results suggest that the new $CA15\text{-}3^{Lectin}$ concept could substantially increase the clinical sensitivity without affecting specificity compared to the conventional CA15-3 immunoassays. Therefore, the $CA15\text{-}3^{Lectin}$ assay should be an excellent alternative to the conventional CA15-3 tumor marker for tracking the recurrence and metastasis of breast cancer.

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Biomarkers in cancer

Cod: M273

PERFORMANCE EVALUATION OF THE ATELLICA IM* PSA, COMPLEXED PSA, AND FREE PSA ASSAYS†

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Background: The ADVIA Centaur® PSA†, Complex PSA (cPSA)†, and free PSA (fPSA)† assays are intended to quantitatively measure prostate-specific antigen (PSA) in human serum using the ADVIA Centaur Immunoassay Systems. The primary objective of this study is to demonstrate the analytical performance of similar PSA, cPSA, and free PSA assays for use on the Atellica™ Immunoassay (IM) Analyzer,** an automated and high-throughput immunoassay analyzer under development by Siemens Healthineers.
Methods: The Atellica IM PSA, cPSA, and free PSA assays use the same reagents and calibrators as the ADVIA Centaur PSA, cPSA, and free PSA assays. The Atellica IM PSA, cPSA and fPSA assays are two-site sandwich immunoassays that employ direct chemiluminometric technology, which uses constant amounts of two antibodies. Precision of the Atellica IM PSA, cPSA, and fPSA assays was evaluated according to CLSI protocol EP05-A3, and a method comparison of the Atellica IM and ADVIA Centaur PSA, cPSA, and fPSA assays was performed according to CLSI protocol EP12-A2.
Results: The observed repeatability for the Atellica IM assays ranged from 1.4 to 2.2% CV for the PSA assay, 1.2 to 1.9 % CV for the cPSA assay, and 1.5 to 2.3% CV for the fPSA assay. The within-lab precision ranged from 2.8 to 5.1% CV for the PSA assay, 2.5 to 3.5%CV for the cPSA assay, and 3.0 to 4.2% CV for the fPSA assay. The method comparison between the Atellica IM and ADVIA Centaur assays was $y = 0.93x + 0.59$ for PSA, $y = 0.92x + 0.8$ for cPSA, and $y = 0.95x - 0.08$ for free PSA with native patient samples.
Conclusions: The Atellica IM PSA, cPSA, and free PSA assays have demonstrated analytical performance capable of measuring prostate-specific antigen with accuracy and precision for use as an aid in the diagnosis of prostate cancer.
*Not CE Marked. Not available for sale. Any features listed are part of the design goals. Future availability cannot be guaranteed.†Assays under development.

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Biomarkers in cancer

Cod: M274

THE VARIABILITY OF ELECTROPHORETIC DIMER/MONOMER PATTERN OF URINE FREE IMMUNOGLOBULINE LIGHT CHAINS IN THE COURSE OF MONOCLONAL GAMMAPATHY.

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BACKGROUND
Immunoglobulin free light chains (FLCs) are laboratory hallmark of monoclonal gammapaties (MG). FLCs are secreted by monoclonal plasma cells in monomeric and/or dimeric form. The median duration of survival for patients with MG varies in a wide range. It is difficult to predict whether patient will become stable or progress to malignant condition within short period of time. The study on FLCs' dimer/monomer (D/M) patterns has revealed the significance of dimerization process of FLC. But the question whether D/M pattern of FLC may determine the severity of the disease still remains open. We suggest that D/M FLCs' pattern is characteristic of individual plasmatic cells clone and may be relevant regarding prediction of disease course and response to therapy.
METHODS
54 patients diagnosed with MG was included in this study. We evaluated the fluctuation of dimer/monomer (D/M) pattern of urine FLCs in individual patients in the course of disease. We retrospectively examined SDS- electrophoresis – based D/M patterns of FLCs. For FLCs kappa (κ) type we examined 130 FLCs' patterns (in 38 patients; 2-8 patterns for individual patients), for FLCs lambda (λ) type 53 FLCs' patterns (in 16 patients; 2-8 patterns for individual patients). We measured the intensity of the bands with digital image analysis. Because for FLC lambda the main form is dimeric we focused on variability of dimeric form. Conversely for FLC kappa we calculated variability of monomeric form.
We used following formula:
[(Individual value of dimeric λ or monomeric κ form – mean value of dimeric λ or monomeric κ form of respective patient)/ mean value of dimeric λ or monomeric κ form of respective patient] *100%
RESULTS
The percentage of dimeric FLC λ form and monomeric FLC κ form in individual patients showed minior variation. The upper and lower quadrille was respectively -0,2% and 2,3% for monomeric FLC κ form and -3,4% and 3,6% for dimeric FLC λ form. We compared the values of consecutive measurements in respective patients with Wilcoxon signed-rank test. There were no significant differences between consecutive values neither for dimeric FLC λ form ($p = 0,6$) nor for monomeric FLC κ form ($p = 0,6$).
CONCLUSIONS
The results suggest that the FLCs' D/M pattern is characteristic of individual patients and it doesn't change in the course of disease. There is need for more detailed study based on relationship between FLCs' D/M pattern clinical manifestations of diseases.

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Biomarkers in cancer

Cod: M275

EVALUATION OF TWO STRATEGIES FOR THE INTERPRETATION OF TUMOR MARKERS IN ASCITIC FLUID

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Background: Ascitic fluids present a diagnostic challenge. Approximately more than 15% are associated with cancer and approximately 40% require invasive procedures to perform diagnosis. Determination of tumor markers (TM) may help to identify patients who are or not candidates for invasive tests. Two strategies are used to obtain high specificity in the differential diagnosis of malignant ascitic fluids: a) high cut-off points, and b) fluid/serum (F/S) ratio and low cut-off points. The aim of this study is to compare these two strategies and to establish whether the identification of possible false positives using Adenosine deaminase (ADA), C reactive protein (CRP) and % of polymorphonuclear cells improves diagnostic accuracy.
Methods: We studied 116 ascitic fluids, 56 of them malignant. ADA, CRP and %PN were determined in ascitic fluid, and Carcinoembryonic antigen (CEA), Cancer antigen 72-4 (CA72-4), Cancer antigen 19-9 (CA19-9) and Cancer antigen 15-3 (CA15-3) in ascitic fluid and serum.
Results: Establishing a cut-off value for each TM for a specificity of 100%, a joint sensitivity of 72.2% was obtained. With the F/S strategy and low cut-off points, sensitivity was 74.5% and specificity 95%, Subclassifying cases with negative ADA, CRP and %PN, both strategies achieved a specificity of 100%; sensitivity was 67.5% for the single determination and 81% for the F/S ratio. In fluids with positive ADA, CRP or polymorphonuclear cells, the specificity for the single determination was 100% while the sensibility was 85.7%, and for the F/S ratio the specificity was 66,7% and sensibility 57.1%.
Conclusions: The best interpretation of TM in the differential diagnosis of malignant ascites is obtained using the F/S ratio and low cut-off points in the group with negative ADA, CRP and %PN and single determination with high cut-off in group with ADA, CRP or %PN positive.

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Biomarkers in cancer

Cod: M276

THE CLINICAL UTILITY OF mir-125B AND mir-221/222 FOR BLADDER CANCER PROGNOSIS AND PATIENTS SURVIVAL OUTCOME FOLLOWING TREATMENT

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Background: Bladder cancer (BlCa) remains the fourth most common type of malignancy in the male populations of the developed countries. MicroRNAs (miRNAs or miRs) are small (21-25 nt) non-coding RNAs able to regulate gene expression and thus to control cellular homeostasis, including cell growth, proliferation, migration and apoptosis. Recent studies have highlighted the implication of miR-125b and miR-221/222 in human malignancies, including urothelial carcinoma, however, their clinical significance for disease outcome is unknown. In the present study, we have analysed the expression of miR-125b, miR-221 and miR-222 in bladder tumors and adjacent normal urothelium in order to evaluate their clinical significance as novel biomarkers and possible therapeutic targets for bladder cancer.
Methods: Bladder tumor and adjacent normal urothelium tissue specimens were obtained from 165 surgically treated patients. Total RNA was extracted following pulverization and was polyadenylated at the 3'-end by E. coli Poly(A) polymerase. Thereafter, poly(A) RNA was reversed transcribed to cDNA using a poly(T) primer. SYBR-Green based qPCRassays were developed, validated and applied for the quantification of miR125b, miR-221 and miR-222 levels using the comparative CT method $2^{-\Delta\Delta CT}$. Extensive statistical analysis was finally performed for the evaluation of miRNAs clinical significance for BlCa patients.
Results: The levels of miR-125b, miR-221/222 were significantly downregulated in bladder tumors compared to their normal counterparts. However, increased expression of miR-125b, miR-221/222 observed in muscle-invasive (T2-T4) compared to superficial (Ta, T1). Focusing on non muscle-invasive tumors (Ta, T1), loss of miR-125b and miR-221/222 was correlated high grade TaT1 tumors and higher EORTC-risk group patients. Kaplan-Meier survival curves and Cox regression analysis revealed the significant and independent clinical value of miR-125b and miR-221/222 for the prediction of non muscle-invasive bladder cancer (NMIBC) patients' risk for disease relapse and progression to muscle invasion stages, as well as for the estimation of muscle-invasive (MIBC) patients overall survival expectancy.
Conclusions: Our data clearly demonstrate the clinical utility of miR-125b, miR-221 and miR-222 to serve as novel biomarkers for bladder cancer prognosis and patients' survival outcome following surgically treatment.

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Biomarkers in cancer

Cod: M277

CORRELATION BETWEEN TUMOUR MARKER CA 125 WITH CEA AND CEA 15-3 FOR OVARIAN CANCER DIAGNOSTIC

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Background: Tumour marker CEA 125 can be used to detect and monitor ovarian cancer. Also, tumour markers CEA and CEA 15-3 can be used as additional parameters. Very often is a trade-off between sensitivity and specificity. Ovarian cancer has the highest death rate among gynaecological cancers. Most ovarian cancers are not detected until a late stage and survival rates than are desperately low.
Methods: The study included 20 patients with ovarian cancer. The concentration of tumour markers were measured by IMMULITE 2000 Analyzer.
Results: Obtained results have shown significant differences between concentration of tumour markers before and after surgery. The tumour marker CEA 125 is elevated in about 87% of patients with ovarian cancer but only in about 43% of early stage disease. The concentration of CEA 125 before surgery was 125U/ml but after surgery was 110U/ml. The specificity of tumour markers CEA and CEA 15-3 was not significant for diagnostic of ovarian cancer.
Conclusions: Tumour markers are valuable diagnostic parameters to support clinical decision making. Specificity of CEA 125 can be very significant and useful for diagnostic and therapy in patients with ovarian cancer. No significant correlation between CEA 125 with CEA and CEA 15-3 was found in this study.

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Biomarkers in cancer

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PIK3CA MUTATIONAL STATUS IN CIRCULATING TUMOR CELLS (CTCS) AND CORRESPONDING CIRCULATING TUMOR DNA IN BREAST CANCER PATIENTS

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AIMS: Molecular characterization of Circulating Tumor Cells (CTC) and analysis of circulating tumor DNA (ctDNA) in cancer patients holds promise as an extremely powerful and reliable non-invasive clinical tool for the individual molecular profiling of each patient in real time. In this study, we analyzed PIK3CA hotspot mutations (1633 G>A, 3140 A>G) in CTC and corresponding ctDNA of early and metastatic breast cancer patients. We also examined whether there is a correlation between the presence of PIK3CA mutations in CTC and ctDNA.

METHODS: We used our highly sensitive methodology for the detection of PIK3CA hotspot mutations in exons 9 (1633 G>A) and 20 (3140 A>G), based on a combination of allele-specific PCR, asymmetric rapid PCR and high resolution melting analysis (Markou et al, CCR 2014). We analyzed PIK3CA hotspot mutations in the EpCAM-positive CTC fraction and the corresponding plasma samples of: i) a group of 21 patients with operable breast cancer, ii) a group of 46 breast cancer patients with verified metastasis and iii) 80 healthy female volunteers. All ctDNA samples were examined for their DNA quality; to verify DNA quality, primers specific for the wild type in exactly the same PIK3CA gene region for exon 9 were used to assess for hotspots mutations. The mutation status of PIK3CA gene in ctDNA samples was detected by the developed methodology exactly as previously described.

RESULTS: The assay is highly sensitive as it can detect 0.05% of mutated dsDNA in the presence of 99.95% wtDNA for both exons (9 and 20) and highly specific (0/30 healthy donors). PIK3CA hotspot mutations were identified in ctDNA in 21/46 (45.6%) of metastasis-verified breast cancer patients and 6/21 (28.6%) of operable breast cancer patients. In metastasis-verified breast cancer patients, the concordance between EpCAM- positive CTCs and ctDNA for 1633 G>A mutation was 74%, whereas the corresponding concordance for the 3140 A>G mutation was 82.6%. In operable breast cancer patients, the concordance between EpCAM-positive CTC and ctDNA for the 1633 G>A mutation was 71.4%, whereas the corresponding concordance for the 3140 A>G mutation was 100%.

CONCLUSIONS: Detection of PIK3CA hotspot mutations in EpCAM-positive CTC and corresponding ctDNA has shown a correlation both in metastasis-verified and operable breast cancer patients. We will further evaluate our findings in a large cohort of patients to evaluate response to molecular targeted therapies in breast cancer.

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Biomarkers in cancer

Cod: M279

MOLECULAR-GENETIC APPROACH TO DETECT MALIGNANT PRECURSORS IN CERVICAL INTRAEPITHELIAL NEOPLASIAS (CIN).

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Background
Human papillomaviruses with high oncogenic risk (HR-HPV) are associated with nearly 100% of exocervical cancers. Malignant prekursors are expressing E6/E7 viral oncogenes (genome instability initiation) and cellular genes associated with cell proliferation. They may be "hidden“ in the neoplasias of lower stages, so may escape and the patients are at high risk for malignancy development.

Methods
Using molecular-genetic approaches (DNA and RNA extraction, RT-PCR, quantitative PCR) we analyzed the exocervical scrubs from 26 patients with various degrees of CIN before and after conization.
For malignant precursors detection we used: 1) HR-HPV genotyping, 2) E6/E7 viral oncogene expression detection and 3) relative quantification of TOP2A and Ki67 cellular gene expression ($\Delta\Delta$Ct). We used IPO8 gene as reference gene and exocervical scrab without atypia as endogeneous controle.

Results
We found, that there is only 50% correlation between oncocytology prior conisation and histology results after conisation. In our study, all samples L SIL prior conisation were underdiagnosed because after conisation CIN2 and CIN3 were confirmed. In these samples we detected E6/E7 oncogene expression and TOP2A and Ki67 ower-expression prior conisation. In the group of H SIL patients (atypical cells were spread to 2/3 of tissue), nearly 20% of patients were underdiagnosed and nearly 25% of patients were owerdiagnosed. In the underdiagnosed samples we detected E6/E7 oncogene expression and TOP2A ower-expression. Ki67 over-expression was not in all cases associated with TOP2A over-expression.
In the group of H SIL patients (atypical cells were spread to 3/3 of tissue), more than 50% of samples were overdiagnosed. In samples of all groupes, which were diagnosed as CIN1 after conisation, we detect neither the E6/E7 expression, nor TOP2A and Ki67 over-expression prior conisation.

Conclusions
Using molecular-genetic approaches we detected in underdiagnosed neoplasias E6/E7 viral oncogene expression (initiation of cell cycle deregulation) and over-expression of cellular genes TOP2A and Ki67 (accelerated cell prolifaration). Both genetic markers – viral E6/E7 oncogenes and cellular genes TOP2A and Ki67 - could be used as good markers for "hidden“ malignant precursors detection.

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Biomarkers in cancer

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ESTIMATION OF OXIDATIVE STRESS STATUS AND PON1 ACTIVITY IN PATIENTS WITH COLORECTAL CANCER

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Background: Colorectal cancer is one of the leading causes of cancer-related morbidity and mortality. Growing interest in cancer prevention imposes the need for exploration of novel risk factors and disease progression contributors. It is generally observed that cancer cells exhibit an increased reactive oxygen species (ROS) production rate and an altered redox environment compared to normal cells. Nevertheless, the ROS's contradictory roles in tumorigenesis are debatable. Redox regulation and redox signaling play a key role in tumorigenesis. Antioxidants may play a dual role through inhibition of tumorigenesis by preventing oxidative injuries to DNA, but also through promoting tumor cell survival by an antiapoptotic effect. In the present study, we assessed the oxidative stress parameters in colorectal cancer-patients in order to get a more comprehensive insight into its diagnostic and prognostic potential. Particulary, we were interested in exploring high-density lipoprotein's (HDL) protective properties through antioxidative effect of paraoxonase (PON1) and sulfhydryl (SH) groups.
Methods: The study included 105 patients with colorectal cancer (CRC) and 109 healthy controls. Oxidative stress status parameters such as PON1 activity, SH groups, total antioxidative status (TAS), prooxidative-antioxidative balance (PAB) and ischaemia-modified albumin (IMA) were determined along with the traditional lipid status parameters.
Results: CRC patients had lower HDL-cholesterol ($p<0.001$) than controls as well as decreased PON1 activity ($p<0.01$). Plasma SH groups concentration was also significantly lower in patients compared to the controls ($p<0.01$), whereas patients had higher PAB values ($p<0.001$). The observed increase in TAS values and decreased IMA concentrations in controls compared to the patients did not reach statistical significance. In patients, PON1 activity correlated positively with HDL-cholesterol ($\rho=0.317$, $P=0.006$), as well as SH groups with IMA levels ($\rho=0.320$, $P=0.023$).
Conclusion: Lower HDL-cholesterol levels, alongside with the decreased PON1 activity and SH groups concentration in patients with colorectal cancer are indicators of the disturbed HDL functionality resulting in reduced oxidative protection. Other oxidative stress parameters were also in concordance with this finding.

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Biomarkers in cancer

Cod: M281

DETERMINATION OF PLASMA METHOXYTYRAMINE AS A METASTATIC PHEOCHROMOCYTOMA BIOMARKER

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Objective
Determination of metanephrines (catecholamine metabolites) from blood plasma plays an important role in the diagnosis of pheochromocytoma (PHEO) and paraganglioma (PGL) – chromaffin cell tumors. We studied whether it is possible to utilize plasma 3-methoxytyramine, the O-methylated metabolite of dopamine, as a biomarker for malignant PHEO and/or PGL. In agreement with recent scientific articles, increased methoxytyramine can be used for distinguishing between patients with and without metastases.

Design and method
We tested patients with and without diagnosis of PHEO and/or PGL. The diagnosis of several selected patients was PGL of the head and neck accompanied by metastases.
All selected patients were fasting overnight and on a special diet before blood taking. Heparin was used as an anticoagulant. The blood corpuscles were separated by centrifugation. 3-methoxytyramine from plasma matrix was extracted by solid phase extraction (SPE) and subsequently determined by high performance liquid chromatography (HPLC) with electrochemical detection (ED).

Results
In all patients with PGL of the head and neck accompanied by metastases, we found that the concentrations of plasma 3-methoxytyramine was at least ten times higher than in patients without metastases.
We observed no significant differences in methoxytyramine concentrations in patients with PHEO and/or PGL without metastases as well as in patients without tumor.

Conclusions
According to our results, plasma 3-methoxytyramine can be used successfuly as a biomarker for distinquishing between patients with metastatic malignant PGL and/or PHEO and patients with malignant PGL and/or PHEO without metastases. Furthermore, the methoxytyramine levels in patients with metastatic tumor and in patients without tumor did not distinct significantly.

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Biomarkers in cancer

Cod: M282

ADVANCED MESOTHELIOMA INDEX (AMI), MMP-9, TIMP-1 AND VEGF IN SMALL CELL LUNG CANCER.

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In the assessment of cancer patients prognosis, additionally to the classical prognostic factors, the indicators describing intensification of inflammation and nutritional status of patients have been frequently used. These indicators are related to the host organism response for developing tumor. One of such indicators is the AMI (advanced mesothelioma index) which is calculated on the basis of BMI values, albumin concentrations, and the ratio of platelets to lymphocytes.
The aim of the studies undertaken in patients with SCLC was the analysis of MMP-9, TIMP-1 and VEGF in relation to the value of AMI in terms of their impact on the patients prognosis.
Material and methods:
Blood cell counts, and concentrations of MMP-9, TIMP-1, VEGF, and albumin were performed before therapy in a group of 146 patients with small cell lung cancer in different advancement of the disease and in the reference group of 63 healthy person. AMI was calculated for each of investigated person.
Results:
In SCLC patients, compared to the reference group, was found significantly higher concentration of MMP-9, TIMP-1, VEGF and significantly lower AMI value. In the group of patients were found inverse correlations between values of AMI and MMP-9, TIMP-1 and VEGF. Moreover, positive correlations were also observed between VEGF vs.MMP-9 and TIMP as well as MMP-9 vs. TIMP-1. Between groups selected according to the stage of disease, there were significant differences for the concentrations of TIMP-1 and VEGF. Univariate analysis showed a significant relationships between 5-year survival of patients and stage of disease, gender, concentrations of MMP-9, TIMP-1, VEGF and AMI values. The multivariate analysis revealed that apart from stage of disease, only AMI lower than 5.0 was an independent unfavourable prognostic factor in SCLC patients. However, between the two groups separated due to the AMI, there were no significant differences in relation to the advancement. In patients with AMI values lower than 5.0 as compared to patients with higher values of this index significantly higher levels of MMP-9, TIMP-1 and VEGF were observed.
Conclusion
Significantly higher levels of MMP-9, TIMP-1 and VEGF in patients with AMI < 5 confirm the value of this index as an independent, unfavorable prognostic factor.

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Biomarkers in cancer

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DIAGNOSTIC POWER OF VEGF, TIMP-1 AND CA 15-3 IN EARLY BREAST CANCER STAGES BASED ON ROC ANALYSIS

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Background. Breast cancer (BC) is one of the most common malignancies in the world. It should be emphasized that there is an increase in the incidence of this type of cancer among young and middle-aged women. It is associated with the fact that the available tumor markers show low sensitivity, and thus, the cancer is undetected in early stages. Considering the increasing number of cases, early diagnosis is vital, especially in the initial stages of the tumor. The substantial progress in this field has been made by screening methods. However, in the case of small lesions, they are not effective. That is why the search for new parameters that could be helpful in early cancer detection is needed. The aim of this study was to evaluate the diagnostic usefulness of VEGF, TIMP-1 and CA 15-3 in early stages of breast cancer.
Methods. The study group consisted of 80 women with diagnosed BC (I or II stage). The control group consisted of 30 healthy women and 30 women with benign breast tumor. The tested parameters were determined in plasma by enzyme-linked immunosorbent assay (ELISA) and the comparative marker (CA 15-3) by chemiluminescence (CMIA). Diagnostic utility has been determined based on parameters such as diagnostic sensitivity (SE), specificity (SP), and area under the ROC curve (AUC).
Results. The highest SE in I stage of BC was found for TIMP-1 (85%), in II stage all parameters showed the same SE (75%). The highest SP in I and II stage of BC was found for VEGF (85%). The AUC represents the overall accuracy of a test, with a value approaching 1.0 indicating a perfect SE and SP. Our results showed that in I stage of BC, only VEGF has shown AUC which was statistically significant (AUC=0.691; p=0.002). In II stage of BC, also VEGF has shown the better results (AUC=0.716; p<0.001) when compared to TIMP-1 (AUC=0.640; p=0.003). CA 15-3 results were non significant in both stages.
Conclusions. These results indicate a much higher diagnostic usefulness of the tested parameters than CA 15-3. VEGF occurred to be the best candidate for cancer diagnostics (better than commonly used tumor marker) in early stages of BC as well as in the differentiation between BC and benign tumor.

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Biomarkers in cancer

Cod: M284

K-RAS MUTATIONS DETECTION IN CIRCULATING EXOSOMES OF PATIENTS WITH PANCREATIC DUCTAL ADENOCARCINOMA: A STUDY ON ANALYTICAL FEASIBILITY.

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Background: early diagnosis of pancreatic ductal adenocarcinoma (PDAC) is a key element to improve patients prognosis. k-ras gene mutations establish early in carcinogenesis and are present in almost 90% of PDACs. Exosomes shed in the extracellular compartment also by cancer cells are a non invasive and enriched source of circulating tumour DNA (exoDNA). Aim of this project is to verify whether the recognition of k-ras gene mutations in exoDNA is analytically feasible.
Methods: We verified pre-analytical processing (sample type, temperature, duration of storage, centrifugation protocol and exosome isolation procedures) on exoDNA. Blood from 5 donors was collected in serum and EDTA tubes and kept at room temperature (RT) or refrigerated (COLD). Samples were centrifuged one or two times after 30 minutes (30min) and 3 hours (3h). Aliquots (-80°C) were used for exosome isolation by two commercial kits: Total Exosome Isolation Kit (Life Technologies) (A) and ExoQuick precipitation solution Kit (System Biosciences) (B). DNA was extracted and quantified (fluorimetric assay).
exoDNA was obtained from sera of 11 PDAC patients (T2-3, N0, M0). Gly12Asp and Gly12Val k-ras mutations were analyzed by CAST PCR (Life Technologies). Results were compared with those from matched neoplastic and normal adjacent tissues.
Results: Mean efficiency of exoDNA isolation (ng DNA/mL of sample) was significantly higher in serum (15,94±11,51ng/mL) than in plasma (5,13±2,27ng/mL) ($p<0,001$) being independent on the number of centrifugations. Among sera mean efficiency of exoDNA isolation was significantly higher in (RT, 3h) samples (kit A=27,75±9,03ng/mL and kit B=30,61±7,72ng/mL) than in (COLD, 30min) samples (kit A=13,10±8,07ng/mL and kit B=14,22±7,75ng/mL) ($p<0,05$). k-ras mutations were detected in 10/11 tumour samples (7/11 Gly12Val and 3/11 Gly12Asp). No mutation was detected in normal adjacent tissues. Mean concentrations of exoDNA extracted from PDAC patients' sera was (57,74±51,20 pg/μL) and no k-ras mutation was detected.
Conclusions: the overall low concentration of exoDNA extracted from peripheral blood hampers K-ras mutation detection due to a low probability of sampling tumour exoDNA in the analytical phase.

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Biomarkers in cancer

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PD-L1 EXPRESSING CIRCULATING TUMOR CELLS (CTCS) IN PATIENTS WITH BREAST CANCER

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Background: Programmed cell Death receptor Ligand 1 (PD-L1) is a very promising biomarker for the selection of patients for cancer immunotherapy. We recently developed a highly sensitive, specific and robust RT-qPCR assay for PD-L1 mRNA. The aim of the present study was to study the expression of PD-L1 in CTCs from breast cancer patients with verified metastasis.

Methods: We quantified the expression of PD-L1 mRNA transcripts in EpCAM-positive CTCs, by using our recently developed RT-qPCR assay, based on the following procedure: i) immunomagnetic enrichment of EpCAM-positive CTCs from 20mL of peripheral blood, ii) total RNA isolation iii) cDNA synthesis and iv) RT-qPCR for PD-L1. PD-L1 expression in respect to the expression of B2M as a reference gene, was normalized using the $2^{-\Delta\Delta}$Ct approach. Peripheral blood samples (20mL) were obtained from 22 breast cancer patients with verified metastasis and 14 healthy donors.

Results: According to our results, 11/22 (50%) of metastasis-verified breast cancer patients were found to be positive for PD-L1 overexpression in CTCs. These are preliminary results and these percentages may change, since the number of samples that we are analyzing is continuously increasing. Our results are in concordance with a recent study by Mazel et al (Mol Oncol 2015), that by using the CellSearch(®) system found PD-L1((+)) CTCs in 11/16(68.8%) patients with metastatic breast cancer.

Conclusion: This is the first time that a quantitative RT-qPCR molecular assay is used for the evaluation of PD-L1expression levels in EpCAM-positive CTCs in metastatic breast cancer patients. The assay is closed-tube, quantitative, highly specific and sensitive, and high-throughput. We are currently evaluating the assay in a large number of clinical samples.

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Biomarkers in cancer

Cod: M286

THE PLASMA LEVELS AND DIAGNOSTIC UTILITY OF MACROPHAGE–COLONY STIMULATING FACTOR (M-CSF) AND METALLOPROTEINASE-9 (MMP-9) IN ENDOMETRIAL CANCER PATIENTS

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Background. M–CSF and MMP-9 may play a role in the pathogenesis of cancer disease, especially in cell growth, proliferation and angiogenesis. Additionally MMP-9 plays an important role in degradation of the extracellular matrix which provides to the metastasis. We investigated the plasma levels of M-CSF and MMP-9 in comparison to tumor marker (CA125) in patients with endometrial cancer (adenocarcinoma endometrioides) and in relation to the control groups: patients with benign tumor (myoma uteri) and healthy subjects.
Methods. Plasma levels of M-CSF and MMP-9 were determined using immunoenzyme assay (ELISA), CA125 - by chemiluminescent microparticle immunoassay (CMIA).
Results. Plasma levels of M-CSF (620,00 pg/ml), MMP-9 (299,50 ng/ml) and tumor marker (CA125 – 119,44 U/ml) were significantly higher in ovarian cancer patients as compared to the healthy control (280,50 pg/ml; 157,00 ng/ml; 19,94 U/ml) ($p<0,01$ in all cases) or benign cancer patients (418,44 pg/ml; 179,00 ng/ml; 24,44 U/ml) ($p<0,01$ in all cases). The plasma levels of M-CSF, MMP-9 and tumor markers were also significantly different in the advanced tumor stages (III-IV) (804,22 pg/ml; 348,40 ng/ml; 209,15 U/ml) than those found in the early stages (I-II) (402,48 pg/ml, $p=0.0430$; 278,2 ng/ml, $p=0,05$; 72,45 U/ml, $p<0,001$). The M-CSF and CA 125 diagnostic specificities received high values (94%; 94%; 92%). The diagnostic sensitivity, the positive and the negative predictive values of M-CSF (71%; 94%; 60%) were higher than for MMP-9 (48%; 90%; 47%), and CA 125 (68%; 92%; 59%). The higher area under the ROC curve (AUC) was observed for M-CSF (0,8798) than for MMP-9 (0,8432) and CA 125 (0,8508). The combined use of M-CSF or MMP-9 with tumor marker resulted in the increase of the sensitivity range and AUC (95%; 0,9286 or 91%; 0,9004). The highest values were obtained by analyzing all tested parameters (97%; 0,9504) as a new diagnostic panel.
Conclusions. These results suggest a potential usefulness of M-CSF and MMP-9 in the diagnostics of endometrial cancer, especially in combine use with CA 125 as a new diagnostic panel of tumor markers.

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Biomarkers in cancer

Cod: M287

THE SPECIFIC RECEPTOR FOR INTERLEUKIN-8 (IL-8) IN ADENOCARCINOMA OF ESOPHAGUS

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Background. Esophageal cancer (EC) is an aggressive malignant tumor of the gastrointestinal tract. It has been estimated that adenocarcinoma of esophagus (AC) became the most common type of this malignancy. In our investigation we aim to measure the serum concentration of specific receptor for interleukin-8, known as CXC chemokine type-2 receptor (CXCR-2), to assess the potential usefulness of this protein in the diagnosis of AC patients. Moreover, the serum CXCR-2 level was compared to the concentrations of classical tumor marker for AC (CEA – carcinoembryonic antigen) and marker of inflammation (CRP – C-reactive protein).
Methods. The study included 20 patients with AC and 30 healthy controls. The serum levels of CXCR-2, CEA and CRP were assessed by immunoenzyme assays.
Results. The concentration of specific receptor for IL-8 (CXCR-2) was higher in sera of AC patients when compared to healthy controls. Similar results were obtained for serum levels of CEA and CRP. The percentage of increased levels of CXCR-2 was found to be higher than CEA as well as CRP and increased to 75% for combined use with CRP.
Conclusions. Our data indicate the potential role of specific receptor for IL-8 in the diagnosis of patients with adenocarcinoma of esophagus.
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