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Advanced technologies - New biomarker discovery

Cod: M001

## ASSOCIATION OF NGAL WITH AKI AND MORTALITY IN SEVERE BURN

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Introduction
The reported mortality rates range from 28% to 100% in burn patients who develop acute kidney injury (AKI) and from 50% to 100% among such patients treated with renal replacement therapy. Recently, the serum and urine neutrophil gelatinase-associated lipocalin (NGAL) levels have been introduced as early biomarkers for AKI; the levels are known to increase 24 to 48 hours before the serum creatinine levels increase. In this study, we aimed to estimate the diagnostic utility of the plasma NGAL levels in the early post-burn period as biomarkers for predicting AKI and mortality in patients with major burn injuries.
Methods
From January to September 2016, 26 consecutive patients with a burn wound area comprising ≥20% of the total body surface area (TBSA) were enrolled in this study. Blood samples were obtained for measuring the serum creatinine, and plasma NGAL levels at 24 and 48 hours after admission. Multivariate logistic regression analyses were performed to assess the predictive value of NGAL for AKI and mortality.
Results
In the multivariate logistic regression analysis ,percentage TBSA, hospitalization days, and plasma NGAL levels at 24 hs were independently associated with AKI development (550 mg/dl vs 160 mg/dl) $p<0,001$ ,and mortality (330 ng/ml vs 162 ng/ml) $p<0,001$ .

Conclusions

Massively burned patients who maintained high plasma NGAL levels until 24 hours after admission were at the risk of developing early AKI and early mortality with burn shock. High NGAL levels within 24 hours after admission. were independently associated with AKI development and mortality.The value of NGAL in blood at 48 hours was not conclusive for defined end points.
The current n is even small and at present we extend the experience to establish a ROC curve value of NGAL that reconciles the best sensitivity and specificity for the development of AKI and mortality

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**PREECLAMPSIA : BIOCHEMICAL MARKERS IN EARLY DIAGNOSIS**

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Background: Preeclampisa is multisystemic disease which cause remains unknown, but it is believed that several factors may participate in pathogenesis: angiogenesis disorder, insulin resistance, immuological factors, inflammation and also endothelial disfunction.
Aim of this study was comparison of biochemical markers in early pregnancy between pregnant women with higher risk of developing preeclampsia compared to the healthy women same gestational age, to determine importance of testing these markers in early pregnancy in order of early detection and opportunities of prevention of this disorder.
Materials and methods: Study included total 70 pregnant women divided in two groups: in study group there was 30 women who were in higher risk of developing preeclampsia and control group of 40 healthy women.
All women between 12. and 14. week of gestation after performed bimanuel and ultrasound examination underwant blood sample taking and values of glucose, uric acid, urea, creatinine, fibrinogen and CRP were determined by colorimetric methods.
Results: Values of uric acid in serume were statistically significantly higher in pregnant women of study group than in control group, while the values of glucose, creatinine, fibrinogen and CRP in serum of study group were higher than in control group, and values of urea were similar in both groups.
Conclusion: Results of our research indicate that biochemical markers that we examined can have importance in pathogenesis of preeclampsia, and they can be used in early diagnostic and screening of pregnant women in order of early detection and prevention of this condition.

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Cod: M003

**CLINICAL BENEFITS OF BOIMARKERS: SOLUBLE TYROSINE KINASE (SFLT-1) AND PLACENTAL GROWTH FACTOR (PLGF) IN THE PREECLAMPSIA ASSESSMENT**

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BACKGROUND: Preeclampsia (PE) is a serious complication of pregnancy. The significant importance of this condition is determined by its frequency: 3-5% of the total number of pregnancies and the serious consequences of improper management: PE is the cause of 1 out of every 7 premature births, and more than 4 out of every 10 maternal deaths. An increased blood pressure and a positive result for protein in the urine are the criteria for the diagnosis of PE, but they are weak predictors of the occurrence of this complication. They do not have the sensitivity and specificity required in order to evaluate the severity of the disease. They can not prognosticate how it will proceed. Moreover, in some cases when symptoms are unclear, they may not be enough even for diagnosis. The purpose of this work is through the presentation of several case studies to demonstrate the benefits of implementing new biomarkers for diagnosis and short-term prediction of PE.

METHODS: The analysis of sFlt-1 and PlGF was performed with ECLIA of Cobas 6000 / Roche.

RESULTS: In most of our clinical cases, PE is early onset before 34 weeks' gestation, discrete symptoms - headaches, weight gain or lack of clinical signs. Only in one case there is an increase in blood pressure (RR 150/100). Biomarkers sFlt-1, PlGF, sFlt-1/PlGF in all patients had values explicitly confirming the diagnosis of preeclampsia. Higher levels of sFlt-1/PlGF were associated with adverse outcomes - a preterm delivery or fetal loss.

CONCLUSIONS: sFlt-1, PlGF, sFlt-1/PlGF tests are found difficult to accept by obstetricians in Bulgaria and can not be incorporated in routine practice. However, they have undeniable benefits:
• Confirmed diagnosis of PE with 99.4% specificity and 88.0% sensitivity (literature). They are especially valuable regarding the case of pre-existing diseases - hypertension, kidney or autoimmune diseases and other diseases that make clinical picture unclear.
• They can be used for short-term (within 4 weeks) prediction of the risk of developing PE.
• They can correctly and quickly identify patients in need of hospitalization.
• The high ratio of sFlt-1/PlGF in PE can be associated with an increased risk of an imminent delivery and an adverse outcome.

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Cod: M004

**CONTINUOUS FLOW INDUCES MESENCHYMAL CHARACTER OF OVARIAN ADENOCARCINOMA CELLS IN MICROFLUIDIC 3-DIMENSIONAL CANCER CELL CULTURE**

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Background: Cancer cells have dynamic microenvironment due to the un-controlled angiogenesis and proliferation patterns. Especially alterations in the physicochemical parameters such as increased fluid flow, acid level; can effect progression, immune-escaping and metastasis. Microfluidics techniques supported with three-dimensional (3D) culture techniques of cancer cells unveil the complex biological processes of cancer cells such as cell-to-cell signaling, gene/protein expression, response to external stimuli and growth cycle, their interactions with drugs and hence provide a promising platform to develop novel cancer therapies. Microfluidic 3D culture techniques allow perfectly mimic dynamic tumor microenvironment with precise control of fluids, simultaneous manipulation and analysis of cells. Here, we used microfluidic 3D culture platform, to investigate the effect of continuous fluid flow on metastatic character of cancer cells.
Methods: Microfluidic culture platform was designed and fabricated to perform 3D cell culture with ovary adenocarcinoma cells (EFO-27 and ONCO-DG-1). Specific fluid and gas distribution modeling was performed to validate the microfluidic 3D cancer cell culture platform. To understand the effect of continuous microfluidic flow, viability, proliferation and immunofluorescence stainings for epithelial and mesenchymal markers were performed with the cells cultured in dynamic (under microfluidic flow) and static cell culture conditions.
Results: The proliferation and viability of cancer cells are increasing under microfluidic fluid flow. Immunoluorescence stainings showed that fluid flow induces mesenchymal character of ovarian adenocarcinoma cells with decrease in E-cadherin expression, and increase in N-cadherin and vimentin expressions.
Conclusions: Cancer cells present different characteristics in their dynamic microenvironment. Understanding the effect of continuous fluid flow which is increased in nature tumor microenvironment due to the un-controlled angiogenesis and proliferation patterns, is a key to investigate the mechanisms underlying cancer progression; so this may lead to new diagnostics and therapeutic approaches. (This study was funded by Turkish Scientific and Technical Research Council (TUBITAK-214S334).

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Cod: M005

**REGULATION OF ANGIOGENESIS AND APOPTOSIS SUPPORT THE FLUID FLOW INDUCED EMT IN MICROFLUIDICS BASED COLORECTAL CANCER CULTURE MODEL**

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Background: Gaining metastatic phenotype of cancer cells is highly complex matter, which is caused by the effect of genetic/epigenetic changes and alterations on the physiochemical parameters of dynamic tumor microenvironment. Physical conditions, such as shear forces and aberrant interstitial fluid flow patterns driven by un-controlled tumor bulk; affect progression, immune-escaping, and metastasis. In our previously published studies, it was presented that continuous fluid flow induces epithelial mesenchymal transition (EMT) in cancer cells. Here, we investigate the supportive mechanisms underlying this phenotypic switch.
Methods: To mimic the dynamic tumor microenvironment, colorectal cancer cells (HCT-116 and HT-29) were cultured in microfluidic cell culture platform and they exposed continuous fluid flow. Fluorescence immunocytochemistry staining (E-cadherin, N-cadherin and Vimentin) was performed to characterize epithelial and mesenchymal phenotypes. After presenting the mesechymal transition, miRNA and gene expression analysis were performed by using Affymetrix GeneChip miRNA 4.0 and GeneChip Human Transcriptome 2.0 Arrays, respectively. Target prediction and pathway analysis were further performed to better understand the effect of continuous microfluidic flow at trancriptome and miRNA level.
Results: EMT was showed on continuous fluid flow exposed colorectal cancer cells by the quantification of E-cadherin decrease and N-cadherin and vimentin increase. It was shown that, regulation of angiogenesis and antigen presentation processes, and negative regulation of apoptosis, were observed as statistically significant on the cells which have fluid flow induced EMT.
Conclusions: Continuous fluid flow driven dynamic microenvironment, affects the main processes of cancer cell such as epithelial-mesenchymal transition, angiogenesis, regulation of apoptosis and antigen presentation. Microfluidic technologies can target phenotypic changes driven by physical parameters of tumor microenvironment, can be promising tools to understand complex tumor microenvironment. (This study supported by Dokuz Eylul University- 2012.KB.SAG.003. Calibasi Kocal G was funded by Turkish Scientific and Technical Research Council-TUBITAK BIDEB 2214/A-2013)

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**GALECTIN-3 APPLICATION IN PERIPHERAL ARTERY DISEASE**

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Background:
The specificity and sensitivity of routine laboratory markers for the diagnosis of peripheral artery disease (PAD) are limited. Galectin-3 has an important role in cell-cell adhesion, cell-matrix interactions, macrophage activation and angiogenesis. This study aimed to investigate the PAD diagnostic capacity of the galectin-3 compared to Chemokine (C-C motif) ligand 2 (CCL2), paraoxonase-1 (PON-1), carbonyl content and isoprostanes, whose main characteristic is its duality to estimate the amount of oxidative stress and inflammation.
Methods:
We investigated 86 patients, 18 female and 68 male, between 42 and 89 years, with clinically diagnosed PAD. Their appropriate demographic and clinical characteristics were collected. We used as a control group samples from 72 healthy volunteers, 25 female and 72 male, between 58 and 79 years.
The circulating levels of C-reactive protein (hs-CRP) and beta-2 microglobulin were measured in a Modular P (Roche Diagnostics) automated analyzer. The concentration of CCL2, PON-1, isoprostanes (Cayman® Chemical) and galectin-3 (R&D® system) were analyzed by ELISA. PON1 lactonase activity was done by measuring the ability of hydrolysis of 5-tiobuitl butyrolactone, and PON1 paraoxonase activity, by measuring the hydrolysis of paraoxon. Carbonyl content was determined by a photometric assay (Cayman® Chemical).
Results:
We observed significant increases in hs-CRP, beta-2-microglobulin, CCL2, isoprostanes, carbonyl content and galectin-3 in PAD patients, and significant decrease in all PON1-related variables in ($P<0.001$).
The areas-under the curve of the receiver-operating characteristics curves were:
Marker AUC
PON1 concentration 0,969
CCL2 0,992
PARAOXONASE specific activity 0,791
LACTONONASE specific activity 0,828
ISOPROSTANES 0,999
CARBONYL CONTENT 0,699
B2-MICROGLOBULIN 0,834
hS-PCR 0,805
Galectin-3 0,776

Conclusions:
Galectin-3 does not have as good diagnostic accuracy as Isoprostanes, but it may be considered as an interesting biomarker for PAD because of its near application in clinical laboratories.

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Advanced technologies - New biomarker discovery

Cod: M007

## THE ROLE OF BIOMARKERS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE(COPD)

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Abstract
Background: COPD is a non-specific inflammation, which involves the airways, lung parenchyma and pulmonary vessels that causes a progressive lost of lung function.
Aim: To measure and assess the diagnostic value of some biomarkers of inflammation in COPD patients.
Methods: 118 COPD patients (113 males mean age 69 and 5 females mean age 70). 68 current smokers, 34 exsmokers , nonsmokers 17. We divided them in two groups. First COPD group (stageI/II) with mean FEV1 74.4%(62-96) predicted) and second COPD group(stageIII/IV) mean FEV1 36.5%(14-70) predicted Respiratory failure was present in 86 cases.Blood tests including total CBC, biochemistry panel and biomarkers of inflammation Fibrinogen ,CRP and Il6 were performed .The clinical and radiologic data were included as well.
Results: We found significant increased mean values of CRP,IL6,Fibrinogen and Leukocytes : 59mg/L, 30 ng/ml,580mg/dl and 11350/uL respectively. Correlation between leukocytosis&neutrofilia and COPD stages was r=0.303 p=0.002; LR 13.4,p=0.004. CRP levels of 30-40mg/L had a SE 61.1-87.6 % for COPD patients stage III/IV,p<0.05. CRP levels >25-100mg/L had the highest SE 71.4 ,AUC (60.82.4) , p=0.0001 for COPD cases with respiratory failure. IL6 had a SE 78.1% AUC(64.7-91.4) ,p 0.015 with mean IL6 values of 16.8ng/ml and 31.5ng/ml in stg I/II and III/IV COPD cases respectively. In stg III/IV COPD cases fibrinogen SE was 76.4 ,AUC(66.3-88.5),p=0.022. FEV1 negatively correlated with neutrophils (r=-0.244,p=0.049), fibrinogen(r=-0.344,p0.014) and CRP(r=-0.279,p0.016).FEV1/FVC negatively correlated with neutrophils(r=-0.352,p=0.004), fibrinogen (r=-0.423.p=0.002) and with CRP(r=-0.247,p=0.034).
Conclusions: Taking into consideration of major inflammation biomarkers with clinical , respiratory, laboratory and imagery studies play a crucial role in evaluation of the progress of lung inflammation and lung function in COPD

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Cod: M008

**LUNG SURFACTANT PROTEIN A(SPA) AND D (SPD) IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE(COPD)**

R. Hasa [1], D. Mema [2], J. Gjerazi [5], A. Teferici [4], E. Nushi [4], E. Tashi [4], D. Todri [4], H. Hafizi [4], P. Kapisyzi [4], A. Bulo [3], J. Bushati [4]

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Abstract
Background: COPD is coused by progressive inflammatory obstruction of lung airways. Aim is to evaluate SPA and SPD,other biomarkers and correlations between them in COPD patients.
Methods: SPA, SPD, IL6 ,CRP were assessed in 118 subjects with COPD,and 38 controls group. (113 males,mean age 69 and 5 females,mean age 70). 68 current smokers, 34 exsmokers ,17 nonsmokers .
Results: Control grup had mean levels of IL6 5.78+/-2.1ng/ml,CRP 5.12+/-2.2mg/L, SPA 26.97+/-16.28 ng/ml and SPD 90+/-36.8ng/ml. COPD patients had higher mean levels of IL6 31+/48ng/ml ,CRP60+/76mg/L, SPA 47+/-35ng/ml and SPD 176+/-98.6ng/ml all p=0.000 . FEV1 negatively correlated with neutrophils (r=-0.244,p=0.049), fibrinogen(r=-0.344,p0.014) and CRP(r=-0.279,p0.016). ROC curves showed that these biomarkers had high sensitivity to differentiate stage IV COPD patient and those with respiratory failure. SPA has SE 81.7AUC (74.6-88.7); SPD SE 72 AUC(63.6-80.3), CRP SE 86.4 AUC(80-92.40), IL6 SE 82.4 AUC (75.5-89.3) all, p=0.000 . SPA had higher SE than SPD for respiratory failure patients {SE 68.9 AUC (55-82.8) p<0.005 versus SE 54 AUC(39-69) p=0.6}. ROC curve showed SPA was significantly higher in both ex/current smokers than nonsmokers ( 44.9+/-25 and 46+-40 versus 31+/-21 ng/ml, p<0.005). SPD was significantly higher in exsmokers than in nonsmokers (183.5+ /-100 versus130+/-76.4ng/ml, p<0.05). FEV1/FVC negatively correlated with neutrophils(r=-0.352,p=0.004), fibrinogen (r=-0.423.p=0.002) ,and CRP(r=-0.247, p=0.034) SPA had significant positive correlations with IL6,CRP,Fibrinogen,WBC, pCO2 and pack years cigarettes (r=0.247; r=0.309; r=0.401; r=0.429; r=0.321 and r=0.217 respectively all p<0.05) SPD positively correlates with pCO2 and COPD stages with r=0.242,p=0.023 and r=0.382,p=0.000 respectively, but had no correlation with inflammation markers such CRP,IL6,fibrinogen and WBC.
Conclusion: It seems that except for SPD, all other biomarkers of this study including SPA associated with clinic data can give more useful aid and information about the progression and severity of COPD .

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Advanced technologies - New biomarker discovery

Cod: M009

**RECOMBINANT APOA-I/HDL ANTIBODY TWO-SITE IMMUNOASSAYS IN CORONARY ARTERY DISEASE DIAGNOSIS**

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Coronary artery diseases (CAD) belong to one of the main causes of mortality. Recent studies have questioned the use of high density lipoprotein cholesterol (HDL-C) as a sole risk marker for reduced protective activity of HDL particles (HDL-P). These apolipoprotein A-I (apoA-I) containing particles are highly heterogeneous, differing in size, density, lipid and protein composition. In diagnosis of CAD analysis of certain subclasses of HDL-P might improve risk estimation. According to some studies small HDL are associated with severity of atherosclerotic disease but also opposite findings have been made. Our aim is to develop assays with possible value in CAD risk estimation.

We developed three apoA-I/HDL recognizing two-site immunoassays. In these assays apoA-I/HDL is recognized with biotinylated scFv-alkaline phosphatase and detected with scFv-phage and europium-labeled anti-phage Mab combined with time resolved fluorescence (TRF) based detection. Immunoassays were here evaluated with 36 samples from Corogene study; 12 patients who had died of acute coronary syndrome (ACS), 12 patients with stable CAD and 12 individuals without CAD. Samples were sex and age matched. ApoA-I, HDL-C, total cholesterol (TC), triglyceride (TG), phospholipid transfer protein activity (PLTP) and cholesteryl ester transfer protein activity (CETP) had been measured earlier and low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula.

Spearman's correlation was moderate between assays 109-122 and 22-454 and TC, LDL-C, apoA-I, HDL-C, PLTP and CETP ($p<0.005$). Assay 110-525 had moderate correlation with TC, LDL-C, apoA-I and HDL-C ($p<0.05$) and possibly mild correlation with CETP ($p=0.06$). Differences between the study groups were analysed by Kruskal-Wallis test. ApoA-I and 110-525 had significant difference between the patient groups with fatal ACS, stable CAD and less severe conditions ($p<0.05$). With assays 109-122 and 22-454 study groups had close to significant difference ($p=0.06$ and 0.08). The new apoA-I/HDL assays had mild, moderate and high correlation with each other ($p<0.05$).

Although the sample number is small we assume that by looking at combined differences in subclasses of HDL-P it may be possible to improve CAD risk assessment. Our next aim is to improve the assays by reducing assay steps and to evaluate them with larger set of clinical samples.

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Cod: M010

**PUTATIVE NEW INFLAMMATORY AND METABOLOMIC MARKERS AS TOOLS TO IDENTIFY THE ENDOMETRIOSIS PROGRESSION**

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BACKGROUND
Endometriosis is defined by the presence of resembling endometrial tissue outside the uterus. At present, the gold standard invasive method for its diagnosis is a visual inspection by laparoscopy and biopsy of the tissue. New technologies belonging to "omics" science are focusing on this disease. Metabolomics profiling has emerged as a powerful tool for the identification of total metabolites present in different biological systems since only small sample volumes are required, and the test is nondestructive.
In this work we applied metabolomics profiling coupled to the evaluation of two known inflammatory markers, PTX3 and calprotectin, to search new markers able to improve the endometriosis diagnosis and to identify its progression.

METHODS
PTX3 and calprotectin levels were measured on follicular fluids in three groups of patients: control group, patients with stage I-II and patients with stage III-IV endometriosis (age range:25-38 years) by ELISA test, (Eurospital,Italy). Extractions of the polar and lipophilic fractions on all the follicular fluids were performed using methanol:H20:chloroform mixture (2:1:2). 1H-NMR spectra at 300K were acquired on 600-MHz Avdance Bruker spectrometer and integrated in buckets. OPLS-DA was used to compare the proton signals obtained for controls and endometriosis patients.

RESULTS
1H NMR spectra were obtained from aqueous and lipophilic extracts of all the collected follicular fluids. OPLS-DA analysis evidenced that the controls and the patients with stage I-II and stage III-IV endometriosis grouped in separate clusters. In details, we identified that some molecule metabolites as well as lactate and some lipids showed different levels in the patients compared to control group and also between patients with stage I-II and stage III-IV endometriosis. Since endometriosis is an inflammatory disease, we evaluated PTX3 and calprotectin levels and evidenced that PTX3 increased in endometriosis with stage III-IV whereas calprotectin levels did not differ significantly across the three groups.

CONCLUSIONS
The data obtained on follicular fluids from group control and patients with stage I-II and stage III-IV endometriosis have evidenced the correlation between metabolite and PTX3 levels and there was not relationship among PTX3 and calprotectin concentration.

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Cod: M011

## COMPARISON OF PERIOSTIN LEVELS IN PATIENTS WITH BENIGN PROSTATE HYPERPLASIA AND PROSTATE CANCER

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**Objective**: Prostate cancer is the one of the most frequent type of cancers in males. Benign Prostate Hyperplasia is the mostly seen complaint of lower urinary tract. PSA is widely used in prostate cancer screening. Trans rectal ultrasound (TRUS) guided biopsy is the gold standard method for differentiating Benign Prostate Hyperplasia (BPH) and Prostate cancer. Biopsy is performed in patients by evaluating risks with Serum Prostate specific antigen (PSA) value and urological examination findings. According to the results of the biopsies, only 20-40% of patients were diagnosed as prostate cancer. In this study, evaluation of the availability of serum periostin levels to make diagnose of patients with prostate cancer because of decreasing the frequency of unnecessary biopsies is aimed.
**Material and Methods**: Patients are categorized into control, benign prostate hyperplasia and prostat cancer groups in terms of the biopsy findings. Number of patients of groups that mentioned above were 54,56 and 51; respectively. Serum total PSA, serum free PSA and serum Periostin levels were measured in all groups. Moreover, % free PSA, PSA density, prostate volume and gleason scores in patients with prostate cancer were also evaluated.
**Results**: Serum periostin levels of BPH group were significantly higher than those in prostate cancer group (p=0,028)
**Conclusion:** It is thought that, this elevation is due to TGF-B, which has the role of prostate enlargement, causes fibroblast and organizes periostin productions. It is concluded that, serum periostin levels should be evaluated in larger populations via standardized-commercial kits.

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Advanced technologies - New biomarker discovery

Cod: M012

**SERUM ISCHEMIA-MODIFIED ALBUMIN LEVELS REFLECT LONG-TERM HYPOXIA IN RESPIRATORY DISEASE; A PILOT STUDY.**

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Background:
Hypoxia is usually evaluated as decreased partial pressure of oxygen in arterial blood (PaO2), or per-cutaneous oxygen saturation (SaO2). So far, there is no blood markers reflecting degree of hypoxia in long-term. On the other hand, ischemia-modified albumin (IMA) is produced irreversibly by reactive oxygen species during ischemic states. When pH drops, eight amino acids from N terminal of albumin reduce their ability to bind transition metals. Applying this phenomenon, we hypothesized as relationships of blood glucose and glyco-albumin, IMA may contribute as a long-term hypoxia marker reflecting hypoxemia in recent weeks. The aim of this study is to clarify if serum IMA levels are increased in patients with respiratory insufficiency.

Materials and Methods:
Nineteen respiratory disease patients, 14 males, 5 females, Ages 57.7±15.0 (mean ±SD) were enrolled. They were 11 COPD, 3 pneumonia, and 4 other diseases. As normal control, sera from 24 healthy adult volunteers (12 males), ages 48.8±17.5 were collected. Subjects with ischemic heart, cerebrovascular diseases were excluded. IMA was assayed by the albumin-cobalt-binding test (Clinical Chemistry 49:581,2003) and expressed as absorbance arbitrary units (AU) using Versa Max microplate reader.

Results:
Average concentration of IMA in respiratory disease patients was 0.48AU, which was significantly higher than that of healthy adults (0.36AU, p=0.000138). IMA had mild regression with SaO2 (r=0.337), and PaO2 (r=0.343). Concentration of IMA decreased after successful treatment of hypoxia such as oxygen inhalation and anti-microbial administration. For example, a 42-yo female patient with pneumonia had IMA 0.71AU, SaO2 92.0%. After treatment, she recovered with lower IMA and higher SaO2 levels (0.46AU, 97.0%). A 43-yo male with C. pneumoniae infection, IMA decreased from 0.46 to 0.38AU, SaO2 increased from 95.0 to 97.0% with treatment.

Discussion and Conclusion:
We previously reported increase of IMA in neonates' sera with fetal distress (Gugliucci A. Clin Chim Acta. 362:155,2005). Serum IMA concentration is increased in hypoxia patients. After successful treatment, decrease of IMA was observed. Although sample number is limited, our data suggest that IMA could be an indicator of long-term hypoxia. More study is needed to confirm the result.

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Cod: M013

## A NOVEL NANOPARTICLE-BASED SENSITIVE IMMUNOASSAY FOR THE DETECTION OF URINARY EXTRACELLULAR VESICLES

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**Background:** Almost, all cells secrete extracellular vesicles (EVs) whose number, size and their composition are altered in disease condition. This makes the EVs attractive biomarkers for early diagnosis of various malignancies. However, the process for isolation of such EVs from bio-fluids is tedious and often, time-consuming. Thus, we report a simple, robust, and highly sensitive method for the detection of EVs from minimally processed native urine (uEVs).

**Methods:** The developed time-resolved fluorometry (TRF) based immunoassay uses biotinylated antibodies against the members of tetraspanin family (CD9, CD81, and CD63) for capturing uEVs from minimally processed healthy urine samples. The captured uEVs are detected using 97 nm europium doped nanoparticles, polysterene bead packed with ~30,000 $Eu^{3+}$ chelates, conjugated either with antibodies against CD9, CD81, CD63, or mannose recognizing human lectins, like Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) or Mannose-Binding Lectin (MBL).

**Results:** In this study, we have shown that the uEVs can be captured and detected with all possible nine combinations of antibodies. We, also, showed that DC-SIGN and MBL lectins, immobilized on Eu3+-NP, could detect uEVs through surface glycoproteins enriched in mannose, thus providing signal amplification through avidity effect.

**Conclusion:** The developed $Eu^{3+}$-NP approach combined with tetraspanin-antibodies can be used for the detection of EVs from minimally processed urine samples. This approach can also be applied to detect EVs from other biofluids and with the right combination of antibodies against tumor-associated surface antigens the method could be explored for EV-based cancer diagnosis. On the other hand, the use of lectin-coated NP-tracers provides possibilities for the detection of cancer-related altered glycosylation patterns reflected on the surface of EVs.

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Cod: M014

**NON-INVASIVE VIABILITY ASSESSMENT OF IN VITRO FERTILIZED HUMAN EMBRYOS USING THE ALPHA-1 CHAIN OF HAPTOGLOBIN AS A QUANTITATIVE BIOMARKER.**

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**Background**
Infertility nowadays is a growing health issue in the developed world meaning that every year more and more couples visit an assisted reproduction (ART) centre. However, the success rate of the process does not exceed 30-35%. An effort is made worldwide to find new additional indicators of embryo viability complementary to the routinely used morphological evaluation.

**Methods**
Following appropriate ethical approval, spent embryo culture medium samples (n=160) were measured using liquid chromatography coupled mass spectrometry (Bruker Daltonics, ESI-TOF) in a series of retrospective, blind experiments. A 15 µl of the sample was directly injected into the instrument.

**Results**
A protein marker was found which significantly ($p<0.001$) differed in quantity between the samples of embryos which did, as opposed to those which did not implant. This protein was identified as the alpha-1 chain of the human haptoglobin molecule. A significant correlation ($p<0.001$) was also found when comparing the clinical outcome (clinical pregnancy) and the outcome predicted by the biochemical measurements.

**Discussion**
The haptoglobin fragment quantitation serves as an additional tool along the process of morphological viability assessment. The blind, retrospective results offer a positive predictive value of more than 50%. The negative predictive value of the analysis was 100%. The results provide a contraselection tool, i.e. screening the embryos with good morphological aspects, but no implantation potential.

**Acknowledgements**
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Cod: M015

## COMPARISON OF IFN-Γ AND IL- 2 RESPONSES TO MYCOBACTERIAL ANTIGENS AS MARKERS FOR DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION.

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**BACKGROUND:** One-third of the world's population is estimated to have Latent tuberculosis infection, LTBI, that they may develop active TB in the near or remote future. Since the distinction between LTB1 and active TB is not clear, we aimed to produce the recombinant mycobacterial antigens, L-Alanine Dehydrogenase (AlaDH) and ESAT-6/CFP-10 fusion proteins, in order to achieve markers for diagnosis of LTBI.
**METHODS:** The study population (n = 99) from the TB center in Shiraz/Iran, were divided into three groups: newly diagnosed active TB cases (n = 33), their household contacts (n = 33) and control group (n = 33). AlaDH and ESAT-6/CFP-10 fusion proteins were produced through PCR and cloning methods. Using Enzyme-Linked Immunospot Assay (ELISPOT), responses of these antigens to interferon-γ (IFN-γ) and interleukin-2 (IL-2) were determined. Differences between the groups were assessed with the Kruskal-Wallis and Mann-Whitney tests for nonparametric data analysis. The p-values of 0.05 or less were regarded as significant. Statistical analysis was performed by the software program SPSS version 16.
**RESULTS:** IFN-γ ELISPOT assays responses to both ESAT-6/CFP10 ($p = 0.81$) and AlaDH ($p = 0.18$) revealed that there were no significant differences between individuals with LTBI and active TB infection. The same results were determined for IL-2 ELISPOT responses to ESAT-6/CFP10 between the two groups. While significantly higher IL-2 ELISPOT responses to AlaDH were observed in LTBI vs. active TB infection. Using ROC analyses, a cut off value of 275 SFC showed a sensitivity of 75.8% and specificity of 78.8% for distinguishing latent vs. active TB by IL-2 responses to AlaDH.
**CONCLUSIONS:** The current study suggests that it may discriminate LTBI from active TB infection by IL-2 T-cell responses to AlaDH.

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Cod: M016

## HEME OXYGENASE-1 IN PLASMA IN PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA

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BACKGROUND Community-acquired pneumonia (CAP) is one of the most common infectious diseases all over the world. CAP is an important cause of mortality and morbidity worldwide. Induction of cytoprotective heme oxygenase-1 (HO-1) during acute lung processes is a crucial defense mechanism (Raval C.M., Lee P.J., 2010) Because of the properties of inducible HO-1, we hypothesized that one of the mechanism of CAP progression would be connected with decreasing of HO-1 activity. We aimed to study whether HO-1 is changed in plasma of CAP patients with varying degrees of severity.
METHODS. HO-1 was measured in plasma of 6 patients with community-acquired pneumonia moderate severity (1-st group) and in plasma of 8 patients with severe community-acquired pneumonia (2-nd group). All patients underwent a detailed clinical examination. As control group 10 healthy subjects were enrolled. The study was approved by the local Ethical Committee at the Medical University of Karaganda. Informed consent was obtained from the subjects before they were recruited into the study. The concentration of HO-1 in plasma was measured with immunoassays using ELISA kit.
RESULTS Plasma HO-1 concentrations were higher in 1-st group patients (median 40.4, range 35.2-47.7), compared with controls (median 30.5, range 25.64-45.99). Plasma HO-1 concentrations were lower in 2-nd group patients (median 7.95, range 5-32.9), compared with 1-st group patients (median 40.4, range 35.2-47.7), ($p < 0.01$) and control ones.
CONCLUSIONS. Our results have demonstrated the alteration of HO-1 in plasma of CAP patients depending on severity of the disease. Early we have demonstrated that CAP development was accompanied by changes in the concentration of oxidative modified proteins in blood of patients (L. E. Muravlyova et al., 2015). The increasing of plasma HO-1 concentrations in response to oxidative stress regarded as a protective mechanism against oxidative tissue injury. The causes of HO-1 suppression in patients with severe CAP are unclear and need further investigations. In any case, taken together, our results suggest that plasma HO-1 levels might serve as biomarker of CAP progression

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Cod: M017

**CELL-FREE DNA: THE SEARCH OF PROGNOSTIC BIOMARKERS IN PROSTATE CANCER**

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Background: Several studies have shown the potential role of cfDNA levels in the prognostic assessment of different solid malignancies. However, the quantification of pure cfDNA is a prerequisite for a reliable genotype analysis focused on the detection of cancer-specific DNA mutations signatures and/or epigenetic modifications. In this study, the quality and quantity of cfDNA were assessed by two different quantification procedures, furthermore cancer-specific DNA mutations as prognostic biomarkers in prostate cancer patients were tested.

Methods: A total of 25 prostate cancer patients and 30 aged matched healthy controls were enrolled into the study. Blood samples were collected at the diagnosis of prostate cancer, and at 6 and 12 months following the radical prostatectomy operation. cfDNA was extracted from plasma through Qiagen kit and Promega automatic extractor. Qubit 2.0 was utilized for measurements of total amount cfDNA before qPCR quantification performed targeting of the single copy gene APP. Methylated GSTP1 and RASSF1A tumour specific cfDNA markers were determined.

Results: Preliminary data showed that patients with high cfDNA concentration at baseline had worse disease free time and overall survival.

Conclusion: The automated cfDNA extraction associated to the quantification by Qubit 2.0 seems to be the best approach to quantify the patient's cancer-specific DNA mutations by qPCR assay. The combination of multiple mutational/methylation cancer biomarkers is suitable to determine the total amount of cfDNA in prostate cancer patients. cfDNA detection can be used as a prognostic and predictive tool for stratification, clinical management and follow-up of prostate cancer patients.

Advanced technologies - New biomarker discovery

Cod: M018

**EVALUATION OF A HEPATITIS B CORE RELATED ANTIGEN TEST (LUMIPULSE® G HBCRAG) IN PATIENTS WITH "PRECORE MUTANT STRAINS".**

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BACKGROUND
Quantification of hepatitis B core-related antigen (HBcrAg) has been proposed as a new serum marker for monitoring HBV infected patients. The assay quantifies the proteins HBeAg, Core and p22cr transcribed from preC/C gene (3.5kb mRNA; pregenomic RNA in viral cycle). HBcrAg seems to be a potential surrogate marker for HBV replication but also for HBV transcription even in the case of low or undetectable HBV activity such as that observed in inactive carriers and successful antiviral therapy. Some HBV genome variants affect the components of HBcrAg, the preC mutation G1896A results in a stop codon which abrogate HBeAg and p22cr. Besides the presence of BCP mutations A1762T and G1764A are associated to a 50% and 70% decrease. This fact could disqualify the application of HBcrAg marker in the presence of such frequent variants.
The aim of this study is to analyse if the presence of any of these mutations leads to interference in the results of HBcrAg measurements. We evaluated the correlation between HBcrAg and the viral load depending on the presence of mutations.
METHODS
Fifty serum samples from chronic hepatitis B patients (HBV-DNA 5.8 Log UI/mL ± 1.7) were evaluated. The HBV-DNA was quantified by PCR RealTime assay (Cobas6800 Roche). BCP and preC mutations were detected by PCR and reverse hybridization (INNO-LiPA HBV PreCore, Fujirebio Europe) and the HBcrAg quantification was measured using an automated CLEIA-method (Lumipulse G HBcrAg, Fujirebio). The samples were classified in wildtypes (WT: absence of mutations), mutants (MT: presence of mutations), heterozygous (HT: simultaneous presence of WT and MT signals).
RESULTS
The global Pearson correlation coefficient between the viral load and HBcrAg is 0.69 ($p<0.01$) and in the groups is:
Group pre-C: WT 0.76($p<0.01$), HT 0.62($p=0.03$) and MT 0.5($p=0.12$).
Group BCP: WT 0.68($p<0.01$), HT 0.84($p<0.01$) and MT 0.63($p=0.01$).
It shows a strong positive correlation statistically significant in all the groups except preC mutants that are near significant.
CONCLUSION
Measurement of HBcrAg levels seems to be useful in HBV patients, even when infected by variants which affect HBeAg expression. The most probable reason is that the HBcrAg assay detects not only HBeAg but also HBcAg and p22cr. In the preC mutants group that has no HBeAg the HBcrAg levels seem clearly lower ($p<0.01$).
Nevertheless, more samples must be tested to confirm this finding and allow further analysis of the significance in preC mutant variant.

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Cod: M019

## PRELIMINARY CLINICAL RESULTS OF A METABOLISM-BASED METHOD TO DETECT CIRCULATING TUMOR CELLS

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### Background
The number of circulating tumor cells (CTC) in blood is correlated with the progress of metastatic cancer, and is emerging as a minimally-invasive diagnostic tool for therapy monitoring and recurrence detection.
In a previous work (Del Ben, Turetta et al., Angew. Chem. Int. Ed. Engl. 2016) we demonstrated a label-free technique for CTC detection based on their altered metabolism, by measuring the secretion of H+ or lactate production of individual, viable tumor cells compartmentalized in microfluidically prepared micro-droplets. We present here additional clinical data and current challenges.

### Methods
Clinical samples (2mL of whole blood collected in EDTA-tube) were lysed and CD45-depleted using Milteniy LD columns and beads. Optionally, a membrane staining is performed to further characterize “acid-producing” population. Droplets are formed by water-in-oil emulsification in a microfluidic flow-focusing junction. Cell suspension is divided into pL droplets by a phase of perfluorinated oil - 2% surfactant. Cells are suspended in Joklik’s EMEM, together with a ratiometric pH-sensitive dye (SNARF-5F). Droplets are incubated at 37 $^{o}$C for 30 min and reinjected for fluorescent pH measurement. A triggered camera collects pictures of selected drops.

### Results
According to selected cut-off (pH=6.4) we detected significantly more events in metastatic breast (median 41/mL, range [5-3125], n=6) and lung cancer (49/mL, [28-87], n=4) vs healthy donors (3/mL, [1-9], n=7), $p<0.005$.
In breast cancer, we demonstrated the presence of both EpCAM(-) and EpCAM(+) acid-producing cells, while CD45 showed dim or no expression. We sorted out selected events by dielectrophoresis with an efficacy close to 100% and a false positive rate (droplets going randomly into the sorting channel) of 0.4%, $n>50.000$. To characterize isolated cells, the non-trivial task of efficiently harvesting rare cells from the collected emulsion must still be solved.

### Conclusion
We presented additional evidence supporting the validity of our metabolism-based method to detect CTC. We observed a significant difference between patients and healthy donors, with the number of events/mL comparable to existing literature on CTC. To further validate the technology, we set up a comparison study against Veridex CellSearch®, and we aim at characterizing isolated cells in order to prove their neoplastic phenotype and describe their molecular and genetic composition.

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Cod: M020

**ROLE OF HYDROPEROXIDES AS MARKERS OF OXYDATIVE STRESS IN ESSENTIAL HYPERTENSION**

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Background: Oxidative stress contributes to blood vessel damage and also plays a role in the pathogenesis of essential hypertension (EH). It leads to an impaired regulation of blood flow, increased platelet aggregation as well as stimulation of leukocyte adhesion. The aim of the study was to determine plasma concentration of hydroperoxides in patients with EH, compared with the control group, and to consider the possibility of introducing FOX1(ferrous ion oxidation) method in routine laboratory measurement of plasma hydroperoxides, as a marker of oxidative stress.
Materials and methods: The study was performed on 45 patients with EH, aged 50.7±5.6 years. Exclusion criteria were smoking, alcoholism, history of coronary heart disease, myocardial infarction, cardiac, renal, hepatic insufficiency, impaired glucose tolerance, diabetes mellitus. Control group was consisted of 20 voluntary blood donors from Department of transfusiology, aged 52.6±5.7 years. Plasma levels of hydroperoxides were measured by using spectrophotometric FOX 1 method (Pharmacia Biotech Ultrospec 2000 Spectrophotometer, England). Difference between two groups was tested with Mann-Whitny test. The level of significance was set at 0,001.
Results: The mean level of plasma hydroperoxides in the study group was 3.49±1.9 μmol/L, which was 3.5 fold greater ($p<0.001$) compared to control group (1.17±0.11 μmol/L).
Conclusions: Increased plasma concentrations of hydroperoxides in the study group indicate the presence of oxidative stress in blood vessels in patients with EH. FOX 1 method can be used in routine laboratory monitoring of patients with oxidative stress-mediated diseases, such as EH.

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Cod: M021

## DETECTION OF URINARY PROTEOME IN PATIENTS WITH GLOMERULONEPHRITIS USING ADVANCED PROTEOMICS TECHNIQUES

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BACKGROUND
A multitude of proteins and peptides can be identified in normal human urine, which is an ideal source of biomarkers that provides a non-invasive approach to diagnosis and prognosis. Mass spectrometry-based approaches to urinary protein and peptide profiling can reveal changes in excretion rates of specific proteins/peptides that can have predictive value in the clinical arena. The main aim of this work was detection of differences in urine proteins in patients with glomerulonephritis and healthy controls and to identify abnormal proteins.
METHODS
Twenty patients with IgA nephropathy (IgAN), twenty patients with Systemic lupus erythematosus (SLE) and appropriate healthy controls were selected. Pooled urine samples were processed by Filter Aided Sample Preparation (FASP) method. Obtained peptide mixtures were labeled using 127C and 128C labels from TMT10 Plex labelling kit, separated by nano-LC with C18 column and analyzed on an Orbitrap Fusion MS (TMT-FASP-Orbitrap Fusion). Data were analyzed and quantified with the Proteome Discoverer 1.4. Spectrum of urinary proteins was monitored also by two-dimensional electrophoresis.
RESULTS
We found out that 602 proteins and 590 proteins were detected in patients with IgAN and SLE using method TMT-FASP-Orbitrap Fusion respectively. Namely, serotransferrin and alpha-1-antitrypsin/ prostaglandin D synthase and alpha-1-acid glycoprotein were significantly differentially expressed (with p value $<0.05$) in the urine of patients with IgAN/ SLE in comparison with healthy controls.
CONCLUSIONS
The results showed that the TMT-FASP-Orbitrap Fusion method indicate a differences in the urine proteome characteristic for each diagnosis.
Work is supported by RVO-VFN64165, AZV 15-31662A

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Cod: M022

**A NOVEL PARTICLE ENHANCED TURBIDIMETRIC IMMUNOASSAY FOR PLASMA CALPROTECTIN**

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BACKGROUND
ELISA is the most commonly used method for measuring calprotectin in plasma, but it is expensive and is usually associated with long test turnaround times. A particle enhanced turbidimetric immunoassay (PETIA) is developed by Gentian Diagnostic As which is believed to be cheaper with reduced reagent cost and faster with random test access by running samples continuously.

The immunoparticles developed for the Gentian plasma calprotectin prototype immunoassay were coated with polyclonal avian antibodies. The polyclonal antibodies are raised to detect MRP8/MRP14 complexes. The advantages of avian antibodies are that they do not react with rheumatoid factors, human anti-mouse IgG antibodies (HAMA) or the human complement system.

The purpose of the present study is to demonstrate a high performance of the Gentian plasma calprotectin prototype immunoassay for the detection of human calprotectin in plasma samples, namely EDTA plasma and lithium heparin plasma.

METHODS
Plasma calprotectin concentrations were measured using a newly developed turbidimetric prototype assay run on a clinical chemistry analyzer (Mindray BS400). A within run precision study, limit of quantitation (LOQ), linearity study, security zone and interference study was performed.

The calibration range was chosen from 0 mg/L to 20, 0 mg/L; Control low was 1, 0 mg/L and control high was 10, 0 mg/L.

RESULTS
Gentian plasma calprotectin prototype immunoassay demonstrated a limit of quantitation (LOQ) around 0,20 mg/L and a security zone up to 60 mg/L on Mindray BS400; The within run precision study showed that the samples ranged from 0,5 mg/L to 17,0 mg/L have a total CV below 5 %; The linear range was shown from 0, 3 mg/L to 20,0 mg/L with a recovery criteria between 90% to 110%; In the interference study, a concentration of 10,0 g/L hemoglobin, 600 mg/L bilirubin and 10,0 g/L intrilipid were tested on Mindray BS400, no significant interferences were discovered.

CONCLUSIONS
Gentian plasma calprotectin immunoassay can be used as a sufficient tool for professional users to measure the calprotectin concentrations in plasma samples, which could be used as a potential inflammation marker for the patients.