

(rs7516341 and rs1186403) deviated from normal distribution. In dominant model (CC + TC/TT) of the first SNP allele C decreases risk of RSA ( $P = 0.034$ , OR = 0.61). The second SNP was significantly different for SP group ( $P = 0.008$ ) where T allele is of limited protective effect in the recessive model of inheritance (Chi-square  $P = 0.082$ , OR = 0.51).

**Conclusion:** It is known that mother's BMI during pregnancy influence maternal and newborn health. *LEP* and *LEPR* are candidate genes for RSA and therefore their influence on mother's BMI during pregnancy and final outcome of pregnancy deserves further investigation.

## P19-08

### The effect of pomegranate seed oil on histological features of testis and sperm quality in male rats

Nikseresht M (1), Mahmoudi R (1), Sharifi A (2), Rahimpour Sisakht F (3), Sane F (4), Jafari M (5)

1) Yasuj University of Medical Sciences, Cellular and Molecular Research Center, Yasuj, Iran, Islamic Republic of

(2) Yasuj University of Medical Sciences, Microbiology, Yasuj, Iran, Islamic Republic of

(3) Jahrom Branch, Islamic Azad University, Biology, Jahrom, Iran, Islamic Republic of

(4) Kazerun Branch, Islamic Azad University, Biology, Kazerun, Iran, Islamic Republic of

(5) Yasuj University of Medical Sciences, Histology, Yasuj, Iran, Islamic Republic of

Corresponding author: nipooya@gmail.com

**Background:** Pomegranate fruit extracts have been commonly marketed as dietary supplements in recent years because its health benefits have been shown in many studies. Many investigators have shown that the pomegranate possesses antioxidant activity and may act as a free radical scavenger.

**Materials and methods:** POMo (pomegranate seed oil) was extracted by petroleum ether. Male Wistar rats were divided into four groups (six per group). One milliliter corn oil, 200, 500 and 1000 mg/kg body weight POMo were given daily for seven weeks by gavage to first- 4th groups, re-

spectively. After this period of time, epididymal sperm were collected and the indexes of sperm quality were determined in all groups. The lipid peroxidation levels and the reduced glutathione contents of sperm were measured. The Diameter of seminiferous tubules ( $\mu\text{m}$ ) and germinal cell layer thickness ( $\mu\text{m}$ ) of testis were determined.

**Results:** A significant increase was found in the percentage of forward progressive sperm motility and a significant decrease was found in the percentage of sperms with slow movement in rats treated with different doses of POMo. The epididymal sperm concentration in rats that eat POMo was higher than control group. The biochemical investigation showed that POMo increased the level of reduced glutathione and decreased the level of lipid peroxidation in the sperm. The Diameter of seminiferous tubules ( $\mu\text{m}$ ) and germinal cell layer thickness ( $\mu\text{m}$ ) were higher in treatment rats.

**Conclusion:** This study shows potential effect of POMo on sperm quality and may use for increasing the fertilizing potency of sperm.

## P20 - Renal replacement

### P20-01

#### Monitoring serial creatinine results in kidney transplant patients using the StatSensor POCT device

Cobbaert C (1), Romijn F (1), van Lint C (2), Schenk P (1), van der Boog P (2)

(1) Leiden University Medical Center, Department of Clinical Chemistry, Leiden, Netherlands

(2) Leiden University Medical Center, Department of Nephrology, Leiden, Netherlands

Corresponding author: c.m.cobbaert@lumc.nl

**Background:** Reference Change Values (RCVs) are helpful to interpret changes in serial diagnostic results. After renal transplantation creatinine is monitored frequently to detect rejection. To judge the usefulness of a device for home monitoring of creatinine, StatSensor POCT device (Nova Biomedical)

and central laboratory creatinine RCVs were determined, using a split sample comparison approach.

**Materials and methods:** Finger pricks were taken from 38 stable post-transplant patients (creatinine 50–450  $\mu\text{mol/L}$ ), and whole blood StatSensor measurements were performed. In addition, venous blood was sampled in lithium-heparin tubes and in serum separation tubes. Heparinised whole blood was used for replicate creatinine measurements on the StatSensor, and serum creatinine was determined using an IDMS-traceable enzymatic method on the Roche Modular P800. RCVs were calculated from:  $2.8 \times (\text{CVa}_2 + \text{CVb}_2)^{1/2}$  ( $P < 0.05$ ,  $\text{CVb} = 5.3\%$ ).

**Results:** Mean creatinine levels on the StatSensor were  $161 \pm 86$  and  $154 \pm 81$   $\mu\text{mol/L}$  in finger prick and venous whole blood, respectively. Serum creatinine levels on the Modular were  $172 \pm 82$   $\mu\text{mol/L}$ . Mean overall StatSensor CVa was 10.4% for finger prick and 5.2–6.6% for venous blood. Overall CVa for laboratory serum creatinine was  $< 1.5\%$ . StatSensor RCVs were 35% and 23% for finger prick and venous whole blood, respectively, compared to 15.5% for the laboratory method.

**Conclusions:** Our data illustrate that RCVs are highly affected by overall CVa of POCT devices. Insufficient precision of the StatSensor causes a 2.3-fold increase in RCV as compared to the central laboratory method. A quality mark for POCT devices is recommended to guarantee desirable analytical performance.

## P20-02

### Falsely decreased ionized calcium in a dialysis patient

Lukić I (1), Lapić I (1), Fressl Juroš G (1), Brunetta Gavrančić B (2), Rogić D (1)

(1) Clinical Hospital Center Zagreb, Clinical Institute for Laboratory Diagnostics, Zagreb, Croatia

(2) Clinical Hospital Center Zagreb, Department for Nephrology, Arterial Hypertension and Dialysis, Zagreb, Croatia

Corresponding author: lukic\_iva@hotmail.com

**Background:** Administration of an anticoagulant is required with patients on haemodialysis to pre-

vent thrombosis of the haemodialysis catheter. With patients suffering from heparin induced thrombocytopenia, heparinization is contraindicated and therefore, citrate is used as an alternative.

**Materials and methods:** A 72-year old female patient suffering from kidney failure attending haemodialysis for 25 years has been chosen for this report. Last year the patient was diagnosed with heparin induced thrombocytopenia and therefore catheter-locking in the interdialytic period was performed with citrate as the anticoagulant. Before the haemodialysis and immediately after the removal of the catheter-locking solution, blood sampling for routine laboratory tests was performed. Concentration of ionized calcium was determined potentiometrically using the point-of-care analyzer RapidLab 1265 (Siemens, Germany).

**Results:** The concentration of ionized calcium was measured prior to two haemodialysis treatments and the results were 0.33 mmol/L and 0.37 mmol/L, respectively. The patient, however, did not suffer from any symptoms of hypocalcemia. After the haemodialysis, ionized calcium was determined again, and its values were within the reference range (1.18–1.32 mmol/L).

**Conclusion:** The patient not suffering from any clinical symptoms of hypocalcemia and the concentration of ionized calcium being within the reference range after haemodialysis, indicates that a preanalytical error has occurred. It is highly probable that the removal of the citrate solution from the catheter was not complete, which led to falsely decreased values of ionized calcium due to the capacity of citrate to chelate calcium ions.

## P20-03

### High-sensitive cardiac troponin T predicts survival in patients on chronic hemodialysis

Rajdl D (1), Racek J (1), Eiselt J (2), Trefil L (1), Malánová L (3)

(1) Charles University, Medical Faculty in Pilsen and University Hospital in Pilsen, Institute of Clinical Biochemistry and Hematology, Pilsen, Czech Republic

(2) Charles University, Medical Faculty in Pilsen and University Hospital in Pilsen, 1st Department of Internal Medicine, Pilsen, Czech Republic

(3) B. Braun Avitum, Dialysis center, Pilsen, Pilsen, Czech Republic

Corresponding author: rajdl@fnplzen.cz

**Background and aims:** Cardiac troponins and natriuretic peptide type B (BNP) use is well established in acute myocardial infarction and heart failure diagnosis. Recently, these markers (and especially cardiac troponins measured by high-sensitive assays) are emerging as new prognostic markers in wide variety of diseases. The goal of this study was to compare the prognostic utility of cardiac Troponin I (cTnI, Access 2, Beckman-Coulter), BNP (AxSYM 2, Abbott) and high-sensitive cardiac troponin T (hsTnT, Cobas e411, Roche) in hemodialysed (HD) patients.

**Materials and methods:** We analyzed cTnI, BNP and hsTnT levels in blood samples of 83 chronically HD patients (31 females; median age [interquartile range] = 65 [56-71] years). There were 37 deaths in our study population (median follow-time was 38.3 [17.0-54.0] months). We used Cox proportional hazard model to reveal the possible prognostic role of measured markers.

**Results:** Our results indicate that the best prediction of overall survival can be obtained from hsTnT (relative risk (RR) of overall mortality with its 95 % confidence intervals (CI): 1.022 [1.03 to 1.042],  $P = 0.03$ , followed by BNP (RR [CI]: 1.0001 [to 1.00004 to 1.001],  $P = 0.05$ ). cTnI was not significantly associated with survival of HD patients in this setting.

**Conclusion:** High-sensitive troponin T and BNP can help in risk stratification of hemodialysed patients.

## P20-04

### Interpretation of cardiac markers in end stage renal disease patients in hemodialysis (HD)

Aakre K (1), Røraas T (2), Petersen P (2), Svarstad E (3), Sandberg S (1)

(1) Haukeland University Hospital, Laboratory of Clinical Biochemistry, Bergen, Norway

(2) University of Bergen, The Norwegian Quality Improvement of Laboratory Services in Primary Care (NOKLUS), Department of Public Health and Primary Health Care, Bergen, Norway

(3) University of Bergen, Renal Research Group, Institute of Medicine, Bergen, Norway

Corresponding author: kristin.moberg.aakre@helse-bergen.no

**Background:** The aim was to calculate the within-subject variation (CVi) and reference change value (RCV) for TnT and pro-BNP in HD patients.

**Materials and methods:** 19 patients (treated with HD for median 16.5 month) and 20 healthy volunteers were included. Samples were collected once a week (immediately before dialysis and during morning hours, respectively) for ten weeks. Results below the detection limit of the high sensitive TnT and NT-proBNP assay (Modular Roche Diagnostics; 3 ng/L and 1 pmol/L) were excluded. CVi and RCVs were calculated after ln-transformation of the data.

**Results:** Half of the healthy controls were female and median age was 61 years. Median age of patients was 71 years, four patients were female. Eight patients had nephrosclerosis and six glomerulonephritis as main cause for renal disease. Four patients had clinical events during the ten week observation period and were excluded from calculations. Mean TnT and pro-BNP concentrations ranged from 18 to 189 ng/L and 22 to 6189 pmol/L, respectively in the HD patients. Most control subjects had TnT values below 3 ng/L (CVi could not be calculated) whilst mean NT-proBNP concentrations ranged from 2-63 pmol/L. In HD patients TnT CVi was 8.2%; TnT RCVs were -18% and +22%; NT-proBNP CVi was 26% and RCVs were -51% and +105%. In healthy controls NT-proBNP CVi was 55% and RCVs were -77% and +336%.

**Conclusion:** In cardiac stable HD patients TnT show less variation compared to NT-proBNP. The NT-proBNP variation in HD patients is lower as compared to healthy controls.

## P20-05

### The role of NGAL as predictor of delayed graft function after kidney transplantation

Glisic B (1), Lalic N (1), Naumovic R (2), Durutovic O (3), Majkic-Singh N (1)

(1) Clinical Center of Serbia, Center of Medical Biochemistry, Belgrade, Serbia

(2) Clinical Center of Serbia, Clinic for Nephrology, Belgrade, Serbia

(3) Clinical Center of Serbia, Clinic for Urology, Belgrade, Serbia

Corresponding author: biljaglisic@yahoo.com

**Background:** Delayed graft function (DGF) is very common complication after kidney transplantation which can increase the risk of early or late graft loss. Commonly, it is monitored by measuring the serum creatinin levels, unreliable parameter for assessing acute changes in graft function. Recently, NGAL has emerged as a novel biomarker for predicting the onset of DGF. We performed a pilot study to assess the role of NGAL, measured 3 hours after the transplantation, as a potential predictor of DGF.

**Materials and methods:** Our study included 20 kidney transplanted patients, divided into two groups: IGF (immediate graft function, 6 patients) and DGF (14 patients). NGAL was measured using the ARCHITECT® Urine NGAL assay (Abbott Diagnostics, Illinois, USA) in urine samples collected exactly 3 hours after the transplantation.

**Results:** The mean uNGAL values in IGF and DGF groups were  $1215.3 \pm 766.7$  ng/mL and  $1630.0 \pm 1023.9$  ng/mL, respectively. We found no statistically significant differences between the groups. After expressing the results as uNGAL to urinary creatinin ratio (NGAL/Cr), the differences were statistically significant ( $P < 0.01$ ) with levels of  $143.6 \pm$

$79.4$  ng/mg for IGF and  $905.6 \pm 656.7$  ng/mg for DGF.

**Conclusions:** Our results have shown that NGAL measured in urine samples collected three hours after the transplantation can be used as a predictor of DGF, if expressed as NGAL-to-creatinine ratio.

## P20-06

### Nutritional status assessment in patients undergoing hemodialysis

Crnokrak S, Mujagić R, Honović L

General Hospital Pula, Clinical laboratory, Pula, Croatia

Corresponding author: slavica\_crnokrak@net.hr

**Introduction:** Approximately 40 to 70 percent of patients with end-stage renal disease are malnourished. The assessment of nutritional status should be a routine care of dialysis patients to permit early recognition and the institution of appropriate therapy. Most of the standard methods of assessing nutritional status can be applied to patients with renal failure; however, some of these parameters are altered by uremia. There is no single measurement that can be used to determine the presence of malnutrition.

**Materials and methods:** Seventy male hemodialysis patients were included in this study. Nutritional parameters (body mass index, cholesterol, triglyceride, transferrin, albumin, pseudocholinesterase, uric acid, blood lymphocyte count) were measured by standard biochemical tests. Results were evaluated by a Forward stepwise multiple linear regression method.

**Results:** There were revealed an standard equation; body mass index ( $\text{kg}/\text{m}^2$ ) =  $1.795 \times$  cholesterol ( $\text{mmol}/\text{L}$ ) +  $2.657 \times$  transferrin ( $\text{g}/\text{L}$ ); that shows a significant association of body mass index, especially, with cholesterol and transferrin (multiple  $R = 0.675$ ;  $P < 0.001$ ). However, body mass index were not correlated ( $P > 0.05$ ) with zinc, albumin and pseudocholinesterase in serum and blood lym-

phocyte count. Thereafter, there were revealed an improved equation; body mass index ( $\text{kg/m}^2$ ) =  $0.016 \times \text{uric acid } (\mu\text{mol/L}) + 0.756 \times \text{triglyceride } (\text{mmol/L}) + 0.955 \times \text{cholesterol } (\text{mmol/L}) + 1.875 \times \text{transferrin } (\text{g/L})$ ; that shows a higher correlation of body mass index (multiple  $R = 0.727$ ;  $P < 0.001$ ) with uric acid, triglyceride, cholesterol and transferrin.

**Conclusion:** We were suggested equation that may confidentially predict the nutritional status in patients undergoing hemodialysis.

### P20-07

#### Visfatin is not associated with inflammatory markers in patients on hemodialysis

Čelap I (1), Šimundić AM (1), Nikolac N (1), Miler M (1), Altabas K (2), Šefer S (2)

(1) University Hospital Center Sestre milosrdnice, Clinical Institute of Chemistry, Zagreb, Croatia

(2) University Hospital Center Sestre milosrdnice, Center for Hemodialysis, Department of Nephrology, Zagreb, Croatia

Corresponding author: ivana.celap@gmail.com

**Background:** Patients with chronic renal disease often suffer from diverse cardiovascular complications. Endothelial dysfunction has been involved in development of different forms of the cardiovascular diseases, including chronic renal disease. In recent years, ubiquitous adipokine called visfatin has been considered as a novel marker of endothelial dysfunction and inflammation. Thus, the aim of our study was to investigate the association of serum visfatin concentrations and well-established markers of cardiovascular risk in the patients on hemodialysis.

**Materials and methods:** Serum and plasma samples from 66 patients (40 males and 26 females) treated by hemodialysis were analysed for visfatin, fibrinogen, CRP and PAI-1 levels. Visfatin was determined by ELISA method while CRP, fibrinogen and PAI-1 were obtained by standard laboratory methods.

**Results and conclusion:** Visfatin did not correlate with the studied markers of inflammation (CRP ( $r =$

$-0.05$ ,  $P = 0.152$ ); PAI-1 ( $r = -0.08$ ,  $P = 0.558$ ) and fibrinogen ( $r = 0.04$ ,  $P = 0.772$ )). Statistically significant correlation between visfatin level and fibrinogen ( $r = 0.51$ ;  $P = 0.008$ ) and the time on dialysis ( $r = 0.70$ ;  $P < 0.001$ ) was observed in female patients. In conclusion, visfatin is not associated with CRP, fibrinogen and PAI-1 in patients on hemodialysis.

### P20-08

#### Effect of hemodialysis on the expression of TLR-4 on monocytes

Dobrošević B (1), Bilandžija M (2), Barbić J (3), Šerić V (1)

(1) Clinical Hospital Centre Osijek, Department of Clinical Chemistry, Osijek, Croatia

(2) Clinical Hospital Centre Osijek, Department of hemodialysis, Osijek, Croatia

(3) University of Osijek, Medical School, Osijek, Croatia

Corresponding author: dobrosevic.blazenka@kbo.hr

**Background:** Infections are frequent complications in patients undergoing hemodialysis. End-stage renal disease is simultaneously associated with inflammation. It was recently shown that family of Toll-like receptors (TLR) play a critical role in innate immunity. It has been reported that expression of TLR-4 was lower in patients on dialysis. The purpose of this study was to determine the effect of hemodialysis on the expression of TLR-4 on CD14+ monocytes.

**Materials and methods:** In 33 hemodialysis patients, we measured expression of TLR-4 on CD14+ monocytes at the beginning (0 min.) and following (180 min.) hemodialysis. The hemodialysis procedure was performed by using polysulphone dialysers. Expression of TLR-4 on CD14+ monocytes was determined by staining with PE labeling anti-TLR-4 monoclonal antibody (eBioscience) and analyzed by flow cytometry (FACSCalibur, BD).

**Results:** The percentage of TLR-4 on CD14+ monocytes was significantly lower 180 min. after hemodialysis ( $21.7 \pm 6.5$ ) compared with beginning ( $24.8 \pm 6.4$ ;  $P < 0.01$ ).

**Conclusions:** The expression of TLR-4 on monocytes becomes down-regulated in uremic patients. The hemodialysis procedure may suppress the expression of TLR-4.

## P20-09

### Markers of the obesity and inflammation in patients with metabolic syndrome and on dialysis

Miler M (1), Segulja D (2), Nikolac N (1), Celap I (1), Kackov S (3), Simundic AM (1)

(1) Sestre milosrdnice University Hospital Center, University Department of Chemistry, Zagreb, Croatia

(2) University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia

(3) Polyclinic Bonifarm, Medical biochemistry laboratory, Zagreb, Croatia

Corresponding author: marijana.miler@gmail.com

**Background:** Dialysis is an invasive treatment in chronic kidney failure associated with increased activation of inflammatory system. The aim of this study was to examine the difference between concentrations of classic inflammatory marker (C-reactive protein, CRP) and new markers from adipokine family (leptin and resistin) in patients on dialysis and with metabolic syndrome.

**Materials and methods:** Total of 140 patients were included in the study, 55 of them with metabolic syndrome (according to NCETP ATP III criteria), 66 on hemodialysis and 18 on peritoneal dialysis. For all patients body mass index (BMI) was provided. CRP concentration was determined with turbidimetric method on Beckman Coulter AU2700 analyzer (Beckman Coulter, Brea, USA). Concentrations of leptin and resistin were determined with fluorescent bead immunoassay (Bender MedSystems GmbH, Vienna, Austria) on the flow cytometer (Beckman Coulter, Brea, USA). Differences between 3 groups were tested with Kruskal-Wallis test.

**Results:** According to the results, CRP concentration was higher in group of dialyzed patients than

in patients with metabolic syndrome ( $P < 0.001$ ) whereas leptin and resistin concentrations did not differ between groups ( $P = 0.115$  and  $P = 0.569$ , respectively). BMI was lower in group of patients on hemodialysis regarding to patients on peritoneal dialysis and with metabolic syndrome ( $P < 0.001$ ).

**Conclusions:** CRP is increased in dialyzed patients and that probably indicates inflammatory response in dialysis. Since leptin and resistin concentrations are not increased in dialyzed patient, we hypothesize that those markers share some other regulatory mechanisms.

## P21 - Toxicology and TDM

### P21-01

#### Analytical validation of valproic acid on the Abbott Architect c8000 clinical chemistry system

Čepić K, Dujmov I, Mladina B, Šupe D, Stanišić L

Split University hospital, Department of Clinical Chemistry, Split, Croatia

Corresponding author: katarina.cepic@kbsplit.hr

**Background:** Valproic acid is a broad-spectrum anticonvulsant drug. High concentration has been associated with hepatic toxicity and acute toxic encephalopathy. The aim of this study was to evaluate analytical performance of Abbott Architect c8000 analyzer for therapeutic drug monitoring (TDM) of valproic acid.

**Materials and methods:** Analytical validation of valproic acid determination by particle enhanced turbidimetric inhibition immunoassay (PETINIA) on Abbott Architect c8000 system included: inaccuracy (bias), within-run imprecision, between-run imprecision and method comparison with analytical system Architect i1000, CMIA method, for 66 human samples.

**Results:** Inaccuracy (bias) result was -0.24% to 2.4%. The highest coefficient of variation (CV) for