

Posters

P1

Quality improvement in point-of-care blood glucose testing with Cobas IT 1000

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Background: The University Hospital Centre Zagreb is the largest hospital in Croatia as well as a unique institution regarding many medical procedures, diagnostic methods and therapeutic procedures. Our hospital has well-developed system of point-of-care testing (POCT). Since POCT is performed outside the central laboratory, it is very important to ensure supervision of each stage of performing the point-of-care testing (quality control, consumption of reagents, critical results, duplicate samples).

Materials and Methods: More than 130 POCT instruments (blood gas analyzers, hematology analyzers, coagulation monitoring system analyzers and glucometers) have been installed so far at the University Hospital Center Zagreb in clinical departments, where the emphasis was to reduce the time span between collecting samples and getting test results (i.e. turnaround time, TAT). 115 glucometers are connected with central laboratory via software Cobas IT 1000 (data management system for hospital POCT). It connects glucometers, collects and stores patient and control test results centrally. Such integration allows thorough analysis of results (reviewing Levey Jennings charts by specific combination of test strip lot and control lot number) and preparation of daily, monthly and annual reports on results obtained from glucometers. Also, it enables adding a new test strip lot to all glucometers in just a few minutes.

Conclusions: POCT group devotes great attention to improving quality of POCT. It is a time-consuming job that requires a lot of patience and understanding. With Cobas IT 1000 we made step forward to better quality of POCT glucometers. At

our hospital, quality means providing safe and appropriate care to our patients. Cobas IT 1000 allows easier and faster control over the use of glucometers and gives us more time for education of non laboratory staff which is one of most important components in quality improvement.

Key words: data management; Cobas IT 1000; point-of-care testing

P2

Results of external quality control of HbA1c in Croatian medical laboratories indicated that POCT system differ from immunoassay methods

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Background: HbA1c as valuable marker in management of diabetes is measured in the most of medical laboratories in Croatia. Aim of this study is to present the results of external quality control assessment (EQA) in Croatian medical laboratories for HbA1c.

Material and methods: Results were obtained from 93 laboratories that participated in cycle of EQA scheme in 2013. The control sample was commercial, lyophilized human blood specimen with normal HbA1c value. The results were entered on-line, as a dual system of reporting: DCCT (%) and IFCC (mmol/mol), and DCCT results were analyzed. Outliers (N=14) were excluded according to Tukey's model and mean (X), standard deviation (SD) and coefficient of variation (CV) were calculated. According to quality specifications established in CROQALM, the allowable deviation from the mean was $\pm 5\%$.

Results: The results were divided in two groups according to the method (immunoassay and colorimetric method). For immunoassay methods (82

participants, six types of instruments), reported results were: $X=5.4\%$, $SD=0.18$, $CV=3.3\%$. For colorimetric method (11 participants, POCT instrument), results were: $X=9.8\%$, $SD=1.94$, $CV=19.8\%$. Immunoassay group indicated acceptable CV ($<5\%$) while results (CV) obtained by colorimetric method (POCT analyzer) were far outside of acceptable limits.

Conclusion: Immunoassay methods for HbA1c determination indicated very good inter-laboratory comparability. High CV in the group of POCT analyzer revealed poor comparability within that method group. Therefore, it can be concluded that only a minority of participants that reported results obtained on POCT analyzers demonstrated low inter-laboratory agreement.

Key words: external quality control; HbA1c; inter-laboratory comparability

P3

Hemolysis effect on coagulation test results

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Background: Due to the high risk of developing thrombosis, the majority of diabetic patients are receiving antithrombotic therapy. During routine laboratory monitoring of these agents a certain number of hemolyzed coagulation samples are usually observed. As hemolysis influence on coagulation test results hasn't been extensively studied, our aim was to investigate the effect of hemolysis on routine coagulation tests: prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen by using serum hemolysis indices (HI) and measuring free plasma hemoglobin concentrations as indicators of the degree of hemolysis.

Materials and methods: We measured PT, aPTT and fibrinogen in 30 hemolyzed and non-hemolyzed plasma samples from the same patients on the coagulation analyzer BCS[®] XP (Sie-

mens Healthcare Diagnostics) on two wavelengths (405 and 570 nm). Free plasma hemoglobin concentrations were determined spectrophotometrically and measurement of HI was performed on biochemistry analyzer Cobas c6000 (Roche). In-vitro hemolysis influence on coagulation test results was studied by adding hemolysate in normal and pathological plasma pool samples (final hemoglobin concentrations: 0-10 g/L). Correlation between free plasma hemoglobin concentrations and coagulation test results in hemolyzed samples was assessed.

Results: Hemolysis affects all routine coagulation tests independently of HI or free plasma hemoglobin concentrations. The major hemolysis influence was observed for aPTT in a normal plasma pool sample, whereas only minor influence was observed for PT in a pathological plasma pool sample. Linear correlation between free plasma hemoglobin concentrations and HI was confirmed.

Conclusions: Although hemolysis affects all three determined coagulation parameters, the influence on each parameter is different, not always correlating with the intensity of hemolysis. Our results indicate that HI is a reliable indicator of the degree of hemolysis. Routine measurement of HI could provide a better estimation of hemolysis influence on coagulation test results and reduce the number of unnecessary and repeated blood testing.

Key words: coagulation tests; hemolysis; hemolysis indices; free hemoglobin plasma concentration

P4

OGTT - National survey

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Background: Slovenia has national guidelines for screening and diabetes detection based on WHO and ADA recommendations (Slovenske smernice

za klinično obravnavo sladkorne bolezni tipa 2 pri odraslih osebah, 2011).

Materials and methods: In May 2013 we sent 120 questionnaires to all registered laboratories in Slovenia at primary, secondary and tertiary level of health care. The questionnaires comprised of 38 questions regarding the type of tests, patients' familiarity with the test procedure, procedure of the OGTT test and methods of glucose determination.

Results: 80 solved questionnaires (67%) were returned. 64 (71%) laboratories confirmed, that they perform OGTT. Most of them perform between 1 and 20 OGTT per day. 60 (97%) laboratories do the 75g-OGTT. 54 (91%) of them also perform 75g-OGTT during pregnancy. The answers in the second part of the questionnaire showed some lack of familiarity with the test procedure. Only 33% of participants had written instructions for the patients. The results about a detailed procedure of OGTT showed that in majority procedures complied with national guidelines. 97% of laboratories determined the first level of glucose from venous blood. 76% of laboratories wouldn't continue the test if the level of glucose was 7 mmol/L for non pregnant patients. For pregnant women the level was 5,1 mmol/L in 92%. We also inquired about the methods of glucose determinations, the results showed that 53% used HK, 30% GOD-PAP and 12% some other amperometric methods. At the end, we tried to find out how many test are performed in our country per month. The conclusion was that we do around 1400 tests, 900 tests are done during pregnancy, which is half of all pregnant women. In UKC Maribor the number of pregnancy 75g-OGTT in 2012 was 405. 48 (11,6%) had glucose levels beyond 5,1 mmol/L and were diagnosed with gestational diabetes.

Conclusion: Slovenian laboratories in majority follow recommendations and national guidelines about OGTT.

Key words: OGTT; gestational diabetes; national guidelines

P5

Urinary protein analysis in diabetic patients

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Background: Diabetic nephropathy is a major late complication in type 2 diabetes mellitus and most frequent cause of end stage renal disease. Proteinuria, especially microalbuminuria is early sign of renal involvement in diabetic patients. The aim of this study was to investigate the relationship of total urinary proteins, microalbumin in urine and the type of proteinuria with diabetes duration.

Material and Methods: This study included 62 patients with diabetes mellitus type 2, average age of patients 45 ± 9 years, duration of disease from 4,6 years to 19,7 years. Microalbumin and total urinary proteins were measured by turbidimetric method in first morning urine sample. Urinary proteins were separated by horizontal thinlayer SDS-PAGE in two urinary samples: first morning urine and urine excreted after routine daily activity.

Results: We found strong positive correlation between duration of disease and microalbuminuria ($r= 0,811$; $p< 0,05$) and moderate positive correlation between duration of disease and total urinary proteins ($r= 0,734$; $p<0,05$). According to SDS-PAGE electrophoresis profiles the most common type of proteinuria in patients with shorter duration of diabetes was selective glomerular proteinuria, while in the patients with longer duration of diabetes was detected non-selective glomerular with incomplete tubular proteinuria.

Conclusion: There was positive correlation between microalbuminuria and total urinary proteins with duration of diabetes. Presence of microalbuminuria alerts the physician to prevent further renal damage by timely administration of adequate therapy.

Key words: total urinary proteins; microalbumin; diabetes mellitus; SDS-PAGE.

P6

Oxidized proteins and selenium in patients with type 2 diabetes

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Background: Numerous studies suggest that the diabetic condition is associated with oxidative stress. Measurements of oxidized proteins are used as unspecific indicators of oxidative stress. The primary functions of selenium are defense against oxidative stress through selenium-dependent glutathione peroxidase and other selenoproteins.

The purpose of this study is to evaluate selenium and oxidized protein levels in patients with type 2 diabetes compared to healthy controls.

Materials and methods: Subjects. The study comprehended 54 patients with type 2 diabetes with various complications, including hypertension, nephropathy, neuropathy and retinopathy. Fifty eight age- and sex-matched subjects were included for the control.

Selenium concentrations were determined using Inductively Coupled Plasma-Mass Spectrometry, ICP-MC (Perkin Elmer). Oxidized protein levels were determined using ELISA method with 2,4-dinitrophenylhydrazine (DNP) (Biocell PC Test, Biocell Corporation, New Zealand).

Results: Mean values of selenium in type 2 diabetic patients were 104.57 ± 29.17 $\mu\text{g/L}$. Mean values of selenium in control subjects were 124.09 ± 25.15 $\mu\text{g/L}$ ($P=0,0002$). Mean values of oxidized proteins in type 2 diabetic patients were $0.2585 \pm 0,0511$ nmol/mg. Mean values of oxidized proteins in control subjects were 0.1180 ± 0.0623 nmol/mg ($P=<0,0001$).

Conclusion: From the results it can be seen that selenium concentrations in patients with type 2 diabetes were significantly lower compared to

healthy controls. At the same time concentrations of oxidized proteins were significantly higher in patients with type 2 diabetes compared to healthy controls. Using that data we can assume that lower selenium concentrations subsequently contribute to reduced protection against oxidative stress which results in higher levels of oxidized proteins.

Key words: oxidized proteins; selenium; diabetes mellitus

P7

Hyperglycaemia in children with cancer: two case reports

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Background: Hyperglycaemia induced by stress of disease or steroid therapy may indicate an early defect in glucose regulation. Hyperglycaemia is known to be a common adverse reaction of steroid therapy in 20% to 50% of nondiabetic patients. In cancer patients, corticosteroids are often prescribed as part of treatment or for symptom management.

Aim: We present two cases with hyperglycaemia during chemotherapy: one with corticosteroid-induced hyperglycaemia and the other one with hyperglycaemia induced by stress of chemotherapy.

Patients: Case 1, a 12-year-old girl with acute lymphatic leukaemia; Case 2, a 2-year-old boy with yolk sac testicular tumour. Both children, without a history of diabetes, were treated with chemotherapy at Haematology-Oncology Department, Zagreb Children's Hospital. Corticosteroid therapy was introduced during chemotherapy (2nd cycle) only in Case 1.

Results: Case 1: Before corticosteroid therapy, blood glucose was normal (<5 mmol/L). Hyperglycaemia developed after corticosteroid therapy introduction, increasing glucose concentration to up to 16.4 mmol/L, without acidosis. Hyperglycaemia was present throughout the treatment. Treatment without insulin maintained blood glucose at 11.0 mmol/L or less. Upon completion of corticosteroid therapy and chemotherapy, glucose level returned to the normal range.

Case 2: Before and during chemotherapy without corticosteroids, glucose concentration was within the reference range (<5.0 mmol/L). At the end of the 4th chemotherapy cycle, glucose concentration increased to 23.5 mmol/L (repeated after 12h: 22.8 mmol/L) with ketoacidosis (BE=-17.9). The boy was referred to Endocrinology Department, where type 1 diabetes was verified.

Conclusion: Our results point to the necessity of glucose monitoring in cancer patients undergoing chemotherapy, in particular when corticosteroids are introduced in therapy. Corticosteroid induced-hyperglycaemia is transient in most cases (Case 1). In patients with prediabetes, stress and physical effects of chemotherapy can lead to diabetes (Case 2).

Glucose monitoring and continuous insulin therapy are recommended at glucose levels >7.8 mmol/L. This approach may prevent inflammation, immunosuppression, and symptoms of hyperglycaemia. Also, it may help recognize previously undiagnosed diabetes.

Key words: hyperglycaemia; chemotherapy; corticosteroid therapy; child; diabetes

P8

Urinary patterns analysis of type 2 diabetes mellitus patients by nuclear magnetic resonance spectroscopy method (¹H-NMR)

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Background: Proton nuclear magnetic resonance spectroscopy method (¹H-NMR) was applied to investigate the urinary patterns of type 2 diabetes mellitus (T2DM) patients to identify possible disturbances that may accompany T2DM. We investigate the potential relationship between diabetic retinopathy (DR), diabetic neuropathy (DN), estimated glomerular filtration rate (eGFR), anthropometric indicators (body mass index (BMI), waist circumference (WC), waist to hip ratio (WHR) and waist to stature ratio (WSR)), duration of T2DM and urinary metabolites in T2DM.

Materials and Methods: Serial urine samples of 167 healthy subjects and 185 T2DM patients were investigated by ¹H-NMR. The ¹H-NMR spectra have been recorded on a Bruker Avance DRX 400 MHz spectrometer. The results are evaluated in mmol/mol of creatinine. p<0.05 was taken as significant.

A significant difference between the urinary excretion of valine, 3-hydroxyisovaleric acid, alanine, gamma-aminobutyrate, betaine, citric acid, trimethylamine-N-oxide and glycine at the healthy individuals and T2DM patients was found. The values for 3-hydroxyisovaleric acid and gamma-aminobutyrate increase in T2DM patients with retinopathy vs. without retinopathy. There was no correlation between DN and urinary metabolite picture in T2DM patients.

Results: We found significant correlations between eGFR and dimethylamine ($r=0.194$, $p=0.031$), gamma-aminobutyrate ($r=0.239$, $p=0.049$), acetate ($r=0.29$, $p=0.035$) and pyruvate ($r=0.275$, $p=0.014$) in T2DM patients. Our analysis revealed significant decreased concentrations for citrate, dimethylamine and glycine in T2DM patients with the increase of BMI. WC were positively correlated with gamma-aminobutyrate ($r=0.42$, $p=0.01$) and dimethylamine ($r=0.39$, $p=0.03$) and, no correlation were observed between WHR, WSR and urinary metabolites in T2DM patients. There are higher urinary concentrations for alanine, 3-hydroxyisovaleric acid, citrate and dimethylamine in newly diagnosed type 2 DM patients, while the hippurate increased with the increase of duration of type 2 DM.

Conclusions: $^1\text{H-NMR}$ spectroscopy can be a method to explore urinary metabolite as markers for early detection of associated diseases and complications in diabetes.

Key words: nuclear magnetic resonance spectroscopy method; type 2 diabetes mellitus; urine

P9

Urinary biochemistry of type 1 diabetes mellitus patients using Proton Nuclear Magnetic Resonance Spectroscopy Method ($^1\text{H-NMR}$)

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Background: In recent years, Proton Nuclear Magnetic Resonance Spectroscopy Method ($^1\text{H-NMR}$)

had emerged as a premier research method for the analysis of biological samples.

In the first section of this study we compared the value of the concentrations of different metabolites between normal and type 1 diabetes mellitus (T1DM) patients to obtain basic knowledge of possible differences in the urinary excretion or concentrations of a series of metabolites between patients with type 1 diabetes and nondiabetic individuals and to assess the NMR potential as a diagnostic tool. In the next section of the study T1DM patients were evaluated according to age and duration of T1DM and the NMR profile of metabolites concentrations established.

Materials and Methods: Serial urine samples of 167 control subjects and 132 T1DM patients were investigated by $^1\text{H-NMR}$ method. The patients had a history of T1DM less than 5 years. The NMR spectra were recorded on a Bruker Avance DRX 400 MHz spectrometer. To 0.9 ml urine, 0.1 ml of stock solution of 5 mM sodium 3-(trimethylsilyl)-[2, 2, 3, 3- d_4]-1-propionate in D_2O has been added. The results are evaluated in mmol/mol of creatinine. $p<0.05$ was taken as significant.

Results: A significant difference between the urinary excretion of lactate, citrate, hippurate and gamma-aminobutyrate at the healthy individuals and T1DM patients was found.

The T1DM patients below 35 years old tended to have higher urinary values of lactate, alanine, pyruvate, citrate, choline and hippurate than T1DM patients above 35 years old. The urinary excretion of valine, lactate, citrate, glycine, trimethylamine-N-oxide and gamma-aminobutyrate are higher in patients with duration of T1DM less than 1 year.

Conclusions: Type 1 diabetes mellitus urinary metabolites are interesting in various aspects, such as providing clues for the biochemistry and mechanisms of the disease or potential early diagnostic markers in diabetes renal involvement.

Key words: nuclear magnetic resonance spectroscopy method; type 1 diabetes mellitus; urine

P10**Plasma cholinesterase method comparison**

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Background: Cholinesterases are enzymes capable of hydrolyzing choline esters. There are two types of cholinesterases: Acetylcholinesterase (E.C.3.1.1.7) present in red blood cells and nerve tissues, and Cholinesterase (E.C.3.1.1.8) found in plasma, liver, heart, and other tissues. The biological role of plasma cholinesterase (CHE) has not been clearly established, however, it has been reported that CHE may be involved in lipoprotein metabolism. It is known, that Diabetes mellitus is a low grade systematic inflammation disease. Persistent hyperglycemia causes increased production of free radicals, especially reactive oxygen species, which affect lipoproteins metabolism. For this reason the uses of plasma CHE measurements in diabetes are nowadays increasing.

Materials and methods: We evaluated two methods for plasma CHE determination with different detection principles: dry chemistry test with reflectance measurement (Vitros Chemistry Cholinesterase), and liquid chemistry test with absorbance measurement (Simens Advia Chemistry Cholinesterase). Both methods are based on the catalytic activity of CHE to hydrolyse butyrylthiocholine to thiocholine, which afterwards reacts with a dyeing compound. The rate of change in the measured signal is proportional to CHE activity in the sample.

We collected 58 heparin plasma samples with routinely ordered CHE determination. The data was statistically evaluated with least-square analysis method.

Results: Both methods showed a very good agreement ($r=0,996$), although dry chemistry method results are significantly lower than liquid chemistry results ($p<0,0001$). The difference can be

overcome by taking into account the correlation equation (intercept=8,753 and slope=0,721) and different method linearity limits.

Conclusions: We have shown that both methods meet appropriate expectations for plasma cholinesterase determination in diabetes patients.

Key words: analysis comparison; cholinesterase; lipoprotein metabolism; diabetes

P11**Is there a role for HbA1c in diagnosing gestational diabetes in high-risk pregnancies?**KRHAČ M^{1*}, RADIŠIĆ BILJAK V¹, RADELJAK A¹, BOŽIČEVIĆ S¹, PRAŠEK M²,
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Background: The use of HbA1c had been recently proposed for the diagnosis of diabetes mellitus, but its diagnostic use for gestational diabetes (GDM) had not been recommended. Thus, a cumbersome and poorly reproducible 75 g oral glucose tolerance test (oGTT) remains the only recommended diagnostic tool for GDM.

The aim of this study was to assess the diagnostic accuracy of HbA1c in pregnant women with high risk for developing GDM.

Materials and Methods: 785 pregnant women, referred to our clinic for the diagnosis of GDM underwent a standard 75 g oGTT procedure with venous plasma glucose measured with an automated hexokinase procedure (Beckman Coulter AU680, USA) at fasting, 1h and 2h after glucose load (FPG, 1h-PG and 2h-PG, respectively). Mean gestational age was $28 \pm 5,2$ weeks. The classification of glycaemic status was carried out according to the IADPSG/WHO criteria. HbA1c was sampled at fasting and measured with an automated im-

munoturbidimetric procedure (TinaQuant-Integra 400Plus, Roche Diagnostics, USA).

Results: Women with GDM (N=367) were older (P=0,006), and had higher both pre-gestational BMI and gestational body-weight (P<0,001) than healthy women (N=418). HbA1c was significantly higher in the GDM-group (P<0,001), as were FPG, 1h-PG and 2h-PG, respectively (P<0,001). ROC-curve analysis for HbA1c revealed an AUC of 0,647 (P<0,001), and a cutoff at 5,1%/32 mmol/mol (69,5% sensitivity, 50% specificity). Stepwise linear regression analysis identified FPG, 1h-PG and gestational age as the most significant determinants of HbA1c level (P<0,001), followed by the body weight (P=0,0303) and age (P=0,0327), whereas 2hPG and pre-gestational BMI had no influence on HbA1c level.

Conclusions: Despite significantly higher HbA1c in GDM, there is a substantial overlap of HbA1c values between the healthy and GDM population, which might compromise its diagnostic use in a high-risk population. However, our results suggest that assessment of HbA1c-utility for screening of GDM in general population merits further investigation.

Key words: gestational diabetes; oral glucose tolerance test; HbA1c

P12

Antioxidative Effect of Naringenin on the Activity of Superoxide Dismutase and Glutathione Peroxidase in HepG2 Cells under Hyperglycaemic Conditions

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Background: Oxidative stress is believed to play a pivotal role in the etiology and pathogenesis of diabetes mellitus and its late complications. Hyper-

glycaemia-induced oxidative stress in diabetes is a consequence of both increased production of free radicals and reduced capacity of antioxidative defence. The citrus-derived flavonoid, naringenin, possesses antioxidative, free radical scavenging and metal chelating properties. The aim of this work was to investigate the antioxidative effect of naringenin on the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in HepG2 cells under hyperglycaemic conditions.

Materials and methods: Activities of SOD and GPx were measured in lysates of HepG2 cells (ATCC HB8065, Manassas, USA). HepG2 cells tested were divided in six groups: control group, diabetes group (30 mM glucose) and four diabetes-naringenin groups (30 mM glucose + naringenin in one of the following concentrations: 1000, 100, 10 and 1 µg/mL). Activities of both enzymes were measured spectrophotometrically and expressed by protein concentrations.

Results: The results were reported as specific activities of SOD and GPx. All groups tested were compared to diabetes group. Student t-test was used to compare tested groups. P-value <0.05 was adopted as statistically significant. Diabetes-naringenin group (30 mM glucose + 1 µg/mL naringenin) showed significantly increased activities of SOD (P<0.001) and GPx (P=0.007). On the other hand, in diabetes-naringenin group (30mM glucose + 10 µg/mL naringenin) only SOD showed increased activity (P=0.002). The other two diabetes-naringenin groups did not show significantly higher activities of SOD and GPx.

Conclusion: The results obtained from this work indicate that naringenin exhibits its antioxidative effect on the activity of SOD and GPx in HepG2 cells under hyperglycaemic conditions when applied in lower concentrations, 1 µg/mL and 10 µg/mL.

Key words: naringenin; oxidative stress; hyperglycaemia; antioxidative enzymes

P13

Performance of equations for estimating glomerular filtration rate in diabetic patients

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Background: Acetoacetate, glucose and hydroxybutyrate have been reported as interference in the Jaffé method for creatinine determination. They can be found in urine of diabetics. They could be responsible for overestimation of GFR by creatinine clearance (CrCl), or be a source for variability in CrCl when compared with eGFR.

Materials and methods: Study enrolled 235 males (ages 18 to 83 years, mean 56.00 ± 14.34) and 265 females (ages 19 to 86 years, mean 58.38 ± 13.93). There were 267 diabetics and 233 non-diabetics. We tested performance of Cockcroft-Gault, MDRD and CKD-EPI equations. Serum creatinine was analyzed by enzymatic method (with creatininase, creatinase, sarcosine oxidase and phenol-aminophenazone peroxidase), and urine creatinine by Jaffé method on Beckman Coulter AU680. Calibration material was Beckman Coulter system calibrator (traceable to NIST SRM 967) and Beckman Coulter urine calibrator (traceable to ID-MS reference method). Statistical analysis was done by Kolmogorov-Smirnov test, bivariate correlation and ROC (MedCalc).

Results: In diabetics, as well as non-diabetics, MDRD showed the highest correlation with CrCl. In the group of diabetics CKD-EPI equation showed the highest AUC at CrCl cut off 90 ml/min, which would make this formula the best predictor of CrCl when it comes to discriminating normal and slightly decreased GFR. In identifying diabetics with moderately decreased GFR (cut off of 60 ml/min) MDRD had higher AUC than CKD-EPI. Observed differences between AUC were not significant (only in non-diabetics CKD-EPI was significantly better at predicting CrCl than CG). Results were considered significant at $p < 0.05$.

Conclusions: All equations had better correlation with CrCl in non-diabetics than in diabetics. Possibly the reason is hyperfiltration of the glomeruli in the initial stage of diabetes that affects CrCl (increasing urine creatinine and volume), but not the eGFR or ketone interfering with Jaffé method. However, this didn't have an effect on the eGFR performance in diabetics in this study. All eGFR had great performance and no one was statistically better than the others.

Key words: Diabetes mellitus; creatinine clearance; estimated glomerular filtration rate

P14

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Background: The most common cause in the incidence of end stage renal disease (ESRD) is diabetes mellitus. The main cause of mortality in diabetes positive dialysis patients is cardiovascular disease (CVD). It's important to identify and prevent cardiovascular risk factors in dialysis patients. The serum omentin-1 level was found to be associated with cardio- metabolic disorders such as insulin resistance, diabetes, dyslipidemia and CVD. Omentin-1 may play an important role in the pathogenesis of atherosclerosis and it may be predictive parameter of co morbidities associated with obesity and glucose metabolism.

The aim of this study was to examine possible clinical significance of serum omentin-1 level in haemodialysis (HD) patients.

Methods: A total of 60 prevalent HD patients at Clinical Hospital Center Rijeka were included in the cross-sectional study. Patients were divided into two groups according to presence of diabetes. Venous blood sample was withdrawn after an overnight fasting before midweek HD session. Serum omentin-1 level was assessed by enzyme-linked immunosorbent assay.

Results: Diabetes negative group was comprised 26 subjects: 16 males and 10 females of average ages 67 (range 33-88) and diabetes positive group was comprised 34 subjects: 25 males and 9 females of average ages 70 (range 31-87). Omentin-1 levels of diabetic HD patients were found to be lower than of non-diabetic HD patients ($8,4\pm 3,8$ $\mu\text{g/l}$ vs. $12,7\pm 2,9$ $\mu\text{g/l}$, respectively; $P < 0,001$).

Conclusions: Serum omentin-1 levels were significantly lower in diabetic HD patients. We believe that decreased omentin-1 levels could play an important role in progression of atherosclerosis through its action on the vascular endothelial inflammatory state in this patients group. Further investigation in prospective clinical study with greater number of patients is needed.

Key words: dialysis; diabetes; omentin-1 protein

P15

How well are the patients in Croatia informed about the OGTT procedure?

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Background: Pre-analytical errors still constitute the largest source of errors in laboratory work. The accuracy and reliability of any diagnostic procedure is strongly dependent on the proper patient preparation and patient's knowledge about the certain procedure. We hypothesized that most of

the patients are not informed well enough about the proper OGTT procedure for blood sampling. The aims of this study were to investigate: i) how well patients are informed about the OGTT procedure for laboratory blood testing; ii) the most commonly used way to inform the patients and iii) whether the particular ways of informing differ anyhow.

Materials and methods: The anonymous questionnaire was conducted across the country in 23 Croatian primary and secondary healthcare centres. All 329 patients were instructed by laboratory staff on how to answer the questionnaire, but not about the OGTT procedure itself. The participants filled the questionnaire before the first blood draw. Data analysis was performed by using non-parametric tests were appropriate, with the P value less than 0.05, as the level of significance.

Results: The results showed that: (i) a greater proportion of participants (90%) had enough information about adequate preparation for the OGTT, but only a minority of the patients (42%) were completely familiar with the entire OGTT procedure, (ii) their major source of instructions was their gynaecologist (66%), and (iv) the ways of informing patients made a difference in the patients' awareness ($P = 0.030$).

Conclusion: In general patients are not familiar enough with the whole OGTT procedure. In order to improve the pre-analytical phase of the laboratory work it is very important that the laboratory provides the doctors with clear and understandable written instructions for the preparation of the patients as well as that all changes and updates are available to all users of laboratory services in an adequate timeframe.

Key words: pre-analytical errors; survey; patient education

P16**Hb1Ac comparison and evaluation in patients with cardiovascular diseases**

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Background: Type II Diabetes Mellitus(T2DM) is the most frequent endocrin disease. Elevated HbA1c level is reported as a risk factor in cardiovascular diseases whereas cardiovascular diseases cause death and disability among diabetic patients.

The aim of the study is to compare the immunoturbidimetry and high performance liquid chromatography(HPLC) methods and determine the HbA1c status among patients who were admitted to our hospital and policlinics to select the best method for determining HbA1c in order to meet the needs of the patients.

Materials-Methods: HbA1c levels from 20.503 patients (12288 females and 8215 males) were collected from the Laboratory Information system(LIS) retrospectively. Patients were admitted to our Cardiology and Cardiovascular Surgery Hospital for cardiovascular reasons and to our Internal Medicine policlinics for non-cardiovascular reasons between 2011 and 2012, whereas HbA1c levels were analyzed with high performance liquid chromatography (HPLC). Also venous blood samples obtained in EDTA containing tubes from 237 in-and-out patients of our hospital (155 females and 82 males) were analyzed in Premier Hb 9210(Trinity Biotech –USA) and Cobas c501(Roche –Germany) using HPLC and immunoturbidimetry respectively. This two methods were compared.

Results: Patients with HbA1c level $\leq 6\%$ were 47,5 %, ≤ 7 were 74,5 %, ≤ 8 were 85,2% and ≥ 8 were 14,8 % of all patients. After the comparison of the two methods there was found a strong correlation from all patients ($n= 237$)($r^2=0.90$) and from patients with HbA1c level $\geq 6.0\%$ ($n=125$) ($r^2=0.88$).

Conclusion: The importance of HbA1c in patients with cardiovascular diseases and the HbA1c levels among our patients indicate that HPLC represents a better choice to determine HbA1c in our hospital. But immunoturbidimetry can be the choice of the method in laboratories with low number of patients. Each laboratory should consider which method is the best.

Keywords: HbA1c; HPLC; immunoturbidimetry; cardiovascular disease; comparison

P17**Evaluation of the analytical quality of B-ANALYST as a POCT analyzer for HbA1c determination**

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Background: Type 2 diabetes mellitus (DM2) represents a major public health issue. The incidence and prevalence of DM2 continue to grow worldwide. The risk of DM2 is associated with morbidity and mortality, which increase healthcare costs. Glycated hemoglobin (HbA1c) is a good marker for glycemic control. Point-of-care testing technology (POCT) for HbA1c determination allows a better glycemic monitoring of diabetic patients and avoids complications of poorly controlled diabetes.

Materials and Methods: we randomly selected samples received in our routine laboratory for HbA1c determination. Samples were collected by venipuncture into EDTA tubes and analyzed in duplicate. We use the HA-8180 (Menarini Diagnostics®) as a reference method which employs cationic exchange High Performance Liquid Chromatography (HPLC). The B-Analyst (Menarini Diagnostics®) is based on the principle of latex agglutination immuno-turbidimetric method. For the com-

parison study we selected 120 samples divided in 4 ranges. For inter-assay variability, we repeated the same sample during 12 consecutive days. For intra-assay study we repeated each sample ten consecutive times during the same day.

Results: regression analysis of the data for the method comparison between HA-8180 and B-Analyst showed a slope of 1,0097 and an intercept of 0,1287. The Pearson's correlation coefficient was 0,9894 ($p < 0,0001$). Bias study of HbA1c measurements for B-Analyst showed a mean difference respect to HA-8180 of 0,205 with a 95% confidence interval. The concordance correlation coefficient to asses accuracy was 0,9904 (0,9866-0,9932). The CV for the inter-assay study was 1,4%. For the intra-assay study we analyzed 3 samples with different HbA1c%, whose CV were 1,03% (4,7% HbA1c), 0,46% (6,4% HbA1c) and 0,78% (8,1% HbA1c).

Conclusions: the evaluated B-Analyst showed good linear correlation with the reference method. It also showed good accuracy both in the inter-assay and in the intra-assay. The B-Analyst carried out with quality specifications required for monitoring of diabetic patients.

Key words: POCT; diabetes; HbA1c

P18

Application of the guidelines for the request of microalbuminuria in diabetic patients

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Background: Microalbuminuria is defined as excretion of 30–300 mg of albumin/24 h. American Diabetes Association and National Kidney Foundation guidelines, but also Serbian clinical guidelines for diabetes, emphasise significance of microalbuminuria determination in diabetic patients because of early diagnosis of incipient diabetic nephropathy. It is recommended that annual testing for mi-

croalbuminuria in patients without clinical proteinuria should begin 5 years after diagnosis of type 1 diabetes and at the time of diagnosis of type 2 diabetes. The diagnosis of microalbuminuria requires the demonstration of increased albumin excretion on 2 of 3 tests performed within 6 months.

Aim of this paper is to show frequency of microalbuminuria testing among population of diabetic patients in Railway Healthcare Institute, Belgrade.

Materials and methods: Data were extracted from laboratory information system about number of patients with diagnosis of diabetes mellitus and laboratory tests which were ordered for them in 2013.

Results: In 2013. 2269 patients with diabetes mellitus made 3915 visits in our laboratory. 61051 laboratory analyses were done for them. But among all those tests only 111 were microalbuminuria testing, which means that only 4.89% of diabetic patients in our institution had their annually microalbuminuria testing done.

Conclusions: Microalbuminuria is under requested test, potentially affecting longer-term health outcomes. Causes for this could be that doctors don't apply national and international recommendations in their everyday practice, but also the fact that health insurance in Serbia doesn't accept costs of this test at the primary health care level, so patients have to pay for it. Corrective measures could be: education of primary health care doctors (we have organized two lectures about microalbuminuria clinical significance in 2013), education of diabetic patients and putting microalbuminuria on the list of tests which are covered by health insurance.

Key words: microalbuminuria; diabetes; guidelines

P19**Plasma glucose concentration, HbA1c together in assessing stability of diabetes**Nake A^{1*}, Hatibi S²¹Medical University of Tirana, Faculty of Tech Medical Sciences; Tirana² Regional Health Authority, Tirana

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Introduction: Diagnosis and monitoring of diabetic patients performs with two basic examinations, measurement of plasma glucose concentrations and HbA1c values as a condition for stability of average plasma glucose values and delaying of clinical complications by endocrinologist.

Aim: Argumentation of necessity of evaluation of plasma glucose concentrations and HbA1c values together for stability of plasma glucose concentration.

Material and methods: We've studied plasma glucose values (mg/dL) and HbA1c (%) determined by reference standard methods in 37 patients with diabetes, with average age 67.2 ± 10.1 . We've calculated and compared average values of plasma glucose concentrations between two groups of patients those with HbA1c = 4-8 (first group) and those with HbA1c > 8 (second group). We've calculated Pearson correlation coefficient between plasma glucose and HbA1c, respectively.

Results: In our diabetic patients: HbA1c = 8.79 ± 1.74 ; plasma glucose 191 ± 61.5 ; Pearson correlation coefficient between plasma glucose concentrations and HbA1c levels, resulted : $r = 0.52$; Calculation and comparison of average values of plasma glucose concentrations between two groups of patients resulted respectively: 132 ± 54 and 231 ± 70.3 . It was observed statistically significant difference between these values ($p < 0.0001$).

Conclusion: HbA1c monitoring is an indispensable examination to evaluate the degree of instability of diabetes together with plasma glucose concentration. The value of Pearson correlation coefficient tells us the necessity of performing these

two examinations, especially for monitoring purposes.

Key words: blood glucose; glycated hemoglobin HbA1c; diabetes

P20**Dried Blood Spot: Could potassium solve hematocrit issue?**

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Background: Dried blood spots (DBS) on filter paper is one of the easiest and noninvasive way to collect samples from different population, with several advantages over using conventional sampling. It brings lot of diagnostics possibilities in different diseases including diagnostics and monitoring diabetes. However, it is still associated with some issues. Hematocrit (Hct) is the most widely discussed challenge, as deviation of Hct values may significantly impact DBS-based quantitation and different diffuse on filter paper which results in sample concentration differences per punch area. It is particularly important in newborn samples where Hct values tend to change hourly. To solve this issue we measured potassium (K⁺) concentration, endogenous intracellular electrolyte that also varies with Hct.

Materials and Methods: We used 85 DBS samples from newborns, children and adults. To evaluate K⁺ extraction from DBS several procedures were tested. After extraction, K⁺ was measured by indirect potentiometric using the ISE module on Cobas 6000 Clinical Chemistry Analyzer. Hct was measured on Sysmex XE-5000.

Results: 0.25% Tritona X-100 with freezing and defrosting showed as the most effective way to release as much as K⁺ from 3-mm DBS punches in the least possible volume. Potassium concentration was from 0.36 to 1.89 mmol/L, with hematocrit values from 0.158 to 0.697 L/L. We noticed a

significant dispersion of results when potassium concentration was greater than 1.3 mmol/L or hematocrit was greater than 0.6 L/L.

Conclusion: We developed a procedure to predict the approximate Hct of DBS, based upon K⁺ measurement. Comparing measured and calculated hematocrit showed good correlation between their values. From measured potassium concentration calculated hematocrit values can be used for correction of the measured DBS analyst values. For K⁺ values above 1.3 mmol/L and hematocrit above 0.6 L/L (neonatal population) a special curve relationship with concentrations of potassium has to be made.

Key words: dried blood spot; hematocrit; potassium concentration; newborns

P21

Association of serum nesfatin-1/NUCB2 with metabolic risk factors in non-obese, normoglycemic subjects

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Background: Nesfatin-1 is a polypeptide encoded in the N-terminal region of Nucleobindin2 (NUCB2), expressed in the hypothalamus, pancreatic islets, gastric endocrine cells and adipocytes. Recent studies indicate its role in regulation of satiety and stimulation of insulin secretion. We assessed the relationship between serum nesfatin-1/NUCB2 and selected metabolic risk factors in normoglycemic individuals.

Materials and Methods: Study included 80 normoglycemic, non-obese (BMI <30 kg/m²) subjects aged 25-40 years (32 women, 48 men). Basic anthropometric parameters (weight, BMI, WHR) and blood pressure measurements were performed. Laboratory tests: fasting plasma glucose, glycated

hemoglobin (HbA1c), lipid profile, insulin, CRP, apolipoproteins AI and B were measured on automatic analyzers (Abbott Architect ci8200, Roche Cobas e411). Adiponectin and nesfatin-1/NUCB2 were assayed by commercially available ELISA kits (BioVendor R&D, Phoenix Pharmaceuticals Inc.).

Results: Nesfatin-1/NUCB2 levels ranged 0.53-14.38 ng/mL and were significantly higher in women compared to men (1.28 vs. 0.82 ng/mL; p=0.02). In men nesfatin-1/NUCB2 correlated negatively with glucose (R= -0.51; p=0.009), insulin (R= -0.33; p=0.038) and HOMA-IR (R= -0.42; p=0.027), while inverse relationship was observed in women. Multivariable regression analysis with glucose, insulin and HOMA-IR in females and with glucose, HOMA-IR and adiponectin in males explained 87% and 32% of nesfatin-1/NUCB2 variability.

Conclusions: Association of serum nesfatin-1/NUCB2 with metabolic risk factors differs essentially by gender, however this issue requires further investigation in large, population-based study.

Key words: nesfatin-1/NUCB2; adipocytokines; metabolic risk; insulin resistance; type 2 diabetes

P22

Importance of the analysis of the fructosamine 3-kinase gene promoter region: experience in an Italian cohort of diabetic patients

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Background: Nonenzymatic glycation is one of the most important factors in the pathogenesis of diabetic complications. Fructosamine 3-kinase (FN3K) appears to be responsible for the removal

of fructosamine from proteins, suggesting a protective role in nonenzymatic glycation. Recently, genetic variants in the FN3K gene have been found in diabetic patients.

The aim of the present study is the conclusion of the molecular investigation of the FN3K gene in an Italian cohort of diabetic patients and healthy subjects, by analyzing its promoter region. We have evaluated the presence of two polymorphisms, the c.-385A/G (rs3859206) and the c.-232A/T (rs2256339), known to be associated with FN3K enzymatic activity in erythrocytes.

Materials and methods: Seventy diabetic subjects, 35 T1DM and 35 T2DM and 33 healthy blood donors with no history of diabetes were evaluated using PCR and direct sequencing of the FN3K gene promoter region. The allelic frequencies for each polymorphism were calculated and the Hardy-Weinberg equilibrium was estimated using the χ^2 -test.

Results: Among the diabetic patients for the polymorphism c.-385A/G we detected 40 patients GA, 15 AA and 15 GG; for c.-233A/T, we observed 46 patients AT, 11 TT and 13 AA. Regarding the control subjects, we observed for the c.-385A/G 18 individuals GA, 11 AA and 4 GG, and for the c.-233A/T 16 individuals AT, 8 TT and 9 AA. Moreover, our screening identified two new variants (c.-421C/T, c.-429delATCGGAG). Furthermore, combining these results with our previous investigation, we observed that T2DM patients with GG genotype for the polymorphism c.-385A/G and CC for c.900C/G presented a lower concentration of HbA1c.

Conclusions: The analysis of FN3K promoter region allowed us to complete the screening of the gene in our cases, reinforcing the idea that some variants could act together and influence the FN3K enzymatic activity as reflected on the levels of HbA1c.

Keywords: Diabetes; Fructosamine 3-kinase; FN3K; HbA1c.

P23

Evaluation of the performance of an immunoturbidimetric HbA1c reagent applied to the Siemens ADVIA 2400 automatic analyzer

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Introduction: Glycated Hemoglobin (HbA1c) is recognized as the gold standard for long-term glycaemic control. The use of HbA1c to screen and diagnose diabetes mellitus has increased, leading to the suggestion of a HbA1c level of 48 mmol/mol (6.5%) as a diagnostic cut-off. Our aim was to evaluate a new immunochemical reagent based on latex particles (Axis Shield), using Siemens ADVIA 2400.

Material and Methods: Intra-assay and total imprecision, interferences studies (bilirubin ~850 μ mol/L, triglycerides ~16.9 mmol/L, total protein ~140 g/L, sodium cyanate ~50 mg/dL, ascorbic acid ~50 mg/dL, urea ~24.99 mmol/L, glucose ~105.46 mmol/L, rheumatoid factor ~700 U/mL), method comparison vs Sebia Capillary Electrophoresis, lot to lot reproducibility, linearity and carry over were conducted on ADVIA 2400 according to CLSI protocols. Additionally, 40 samples with target values by NGSP were measured by the two methods.

Results: CVs % obtained by intra-assay imprecision, on 3 human specimens at different concentrations (<48 between 48-64, and >64 mmol/mol) in 20 replicates, were <4%. CVs% by total imprecision, performed in 20 days with 4 calibrations on 5 materials [control low, control high and 3 human samples], resulted <4%. Interferences were stud-

ied on two human samples without obtaining significant biases (<10%). Methods comparison, performed on 120 samples ranging 23–137 mmol/mol, obtaining $r=0.9809$ as regression coefficient and a mean bias at decisional level (48 mmol/mol) <2.0%. The results obtained with the 40 NGSP samples has allowed the certification of the new reagent.

Conclusions: The availability of fully automated method for the determination of HbA1c will not be only desirable but will become an important clinical need. The ADVIA 2400 is able to perform the analysis in 10 minutes. Furthermore, this method showed good performance in our evaluation, robustness with respect to endogenous interference, and a good correlation when compared with routinely used CE especially against NGSP materials.

Keywords: HbA1c; Glycated Hemoglobin; NGSP

P24

Validation of five point-of-care glucometers

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Background: Self-monitoring of blood glucose is a convenient way in the management of patients with type 2 diabetes mellitus (T2DM). Although numerous glucometers are available in the market, only rare can achieve requested performance characteristics given by International Federation in Clinical Chemistry and Laboratory Medicine (IFCC) and, especially, American Diabetes Association (ADA). ADA sets analytical error at <5% while IFCC allowable error is <20%. The aim of the study was to investigate analytical performance characteristics of five glucometers versus reference laboratory method.

Materials and methods: Glucometers of five different manufacturers [Conture (Bayer Vital GmbH,

Leverkusen, Germany), Xceed (Abbott Diabetes Care Inc., Alameda, CA, USA), AccuCheck Active (Roche Diagnostics, Mannheim, Germany), Bionime GM550 (Bionime GmbH, Switzerland) and X-meter (Glucocard, Arkray Factory Inc, Shiga, Japan)] were validated. Laboratory glucose was measured with the IFCC reference method with the hexokinase on AU 2700 (Beckman Coulter, Brea, CA, USA). Venous blood was drawn from 30 T2DM patients and healthy volunteers in sodium fluoride/potassium oxalate vacutainers (Greiner Bio-One, Kramsmünster, Austria). Immediately after venous blood sampling, capillary blood glucose was determined on all glucometers for each patient.

Results: All glucometers have shown satisfactory imprecision (Conture 5%; AccuCheck 4.2%; Xceed 5.1%; X-meter 5.2%, Bionime 3.3%). Relative mean biases between glucometer and reference method were: Conture (4.02%), Bionime GM550 (14.31%), AccuCheck Active (3.16%), Xceed (6.08%), X-meter (12.46%). Passing-Bablok regression analysis have shown systematic shift for AccuCheck Active ($y=0,2906 [-0.6162 - (-0.1500)]+1,0156 (1,0000 - 1,0541)$) x and proportional difference for Bionime GM550 ($y=0,06113 (-0,3079 - 0,5261) + 0,8473 (0.7826 - 0.8947))x$. Error grid analysis showed 100 % results in A zone for all glucometers except Bionime with only 4 % results in A zone.

Conclusion: Results of the study showed that glucometers Conture and Xceed satisfy analytical performance according to IFCC recommendations while only Conture fulfill ADA criteria.

Keywords: glucose; point-of-care; glucometer; validation

P25**Estimation of the detection of gestational diabetes mellitus - do we need to resign from 1h50g load?**

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Background: The aim of the study was to estimate and compare detection of Gestational Diabetes Mellitus (GDM) using criteria from Polish Diabetes Association (PDA) recommendation 2013 and 2014, and verify sensitivity and specificity of 1h50g load.

Materials and methods: Tests were performed on group of 23440 pregnant women between 2008-2013 at Diagnostyka Company, Cracow, Poland. The cutoff values for the OGTT and 1h50g load were estimated according to criteria from PDA.

Results: OGTT was made on 16155 women (one step model). GDM were diagnosed in 1511 (9.4%) using 2013 criteria and in 1523 (9.4%) using 2014 criteria respectively. 1h50g test were performed in 7285 women. Glucose intolerance was observed in 1388 women, GDM had been confirmed with OGTT (two step model) in 404(2013yr) and in 312 patients (2014yr). Detection of GDM were 5.8% (2013yr) and 4.5% (2014yr). For evaluated sensitivity and specificity of 1h50g load according to new and old cutoffs, 2719 women were chosen, and divided into two groups: first with glucose intolerance and diabetes (1405 women) and second with negative test results (1314 women). In first group GDM was diagnosed in 421 (2013yr) and 329 (2014yr). In second group detection was 102 (3.8%) and 40 (1.5%) patients. Sensitivity and specificity were compared using both diagnostic criteria: 2013 recommendation-sensitivity: 80.5%, specificity: 55.1%, negative predictive value (NPV): 92.2%, positive predictive value (PPV): 29.9%, negative likelihood ratio (LR): 0.35, positive LR 1.8 and odds ratio (OR): 5.084; 2014 recommendation-sensitivity: 89.2%, specificity: 54.2%, NPV: 97.0%, PPV: 23.4%, negative LR: 0.2; positive LR: 1.9; and OR 9.7.

Conclusions: In two step model using old cutoffs 3.8% of patients were undiagnosed, detection of GDM was lower than using one step model. The numbers of undiagnosed patients using two step model and new criteria decreased, sensitivity of 1h50g load elevated, but detection was still lower than using one-step model

Key words: gestational diabetes; pregnancy; recommendation

P26**User verification for HbA1c on AU 480 Beckman Coulter**Sablek J^{1*}, Todorčić Z², Sablek Lj¹¹ Medical biochemistry laboratory Ljerka Sablek, Požega, Croatia² Medical biochemistry laboratory, Labplus Polyclinics, Split, Croatia

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Background. Glycated hemoglobin (HbA1c) is formed by non-enzymatically binding of glucose to the free amino group of the N-terminal end of the β -chain of hemoglobin A. HbA1c is used to measure average glucose blood levels over the lifespan of the erythrocytes (120 days), and is a longlasting indicator of blood glucose levels, which makes it good indicator of diabetic disease control.

With this study, we wanted to evaluate new method in laboratory by defining precision and trueness for the determination of HbA1c at the AU 480 (Beckman Coulter, USA), by immunoturbidimetric method.

Materials and methods: We determined the concentrations of total hemoglobin and HbA1c. HbA1c is measured in a latex agglutination inhibition test. If the sample does not contain HbA1c, there will be a microparticle agglutination marked with antibodies and agglutinators. The presence of HbA1c in the sample results in reduced levels of agglutination. The increase in absorption is inversely proportional to the concentration of HbA1c in the sample.

Results: We determined concentrations of HbA1c, during five days, in triplicate, in commercially available control samples for two concentration levels declared by the manufacturer. From the obtained values of concentrations of HbA1c, we calculated repeatability, intermediate precision, intra-laboratory precision and systematic error (bias).

To evaluate precision, we compared our variation coefficient of intra-laboratory precision (for level 1 it's 1.3 %, for level 2 it's 1.5 %) with the desirable specification for imprecision from Westgard biological variation database (0.9 %).

To evaluate trueness, we compared our bias (at level 1 is 0.54 %, at level 2 is 4.06 %) with desirable specification for inaccuracy from Westgard biological variation database (1.5 %).

Conclusion: Based on the obtained values, we concluded that our method for HbA1c determination is not ready to use in routine yet, we have to do corrections in all preanalytical and analytical steps.

Key words: glycosylated hemoglobin; automation; verification

P27

Low-grade inflammatory state in pregnancy complicated by diabetes

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Background: A low-grade systemic inflammation is concomitant in diabetes. The aim of the study was to investigate the relation between low-grade systemic inflammation expressed as C-reactive protein in gestational diabetes.

Material and methods: The CRP concentrations in pregnant women with normal glucose tolerance (NGT) and those with gestational diabetes mellitus (GDM) were measured at the same time as the oral glucose tolerance test (OGTT). Tests were performed on group of 603 pregnant women in

2013 at Diagnostyka Company, Cracow, Poland, 64 patients were diagnosed as gestational diabetes.

Results: CRP level were significantly higher in GDM patients compared to NGT: GDM median 3.9 (quartile 1.4-4.8) mg/l; NGT median 2.3 (quartile 1.1-3.3) mg/L; $p=0.019$. GDM patients were significantly older: median 33 (quartile 29-38) yr; median 31 (quartile 28-35) yr $p=0.003$. 584 women with CRP between 0 and 5 mg/L were chosen and divided into two groups: first with CRP level 0-3 mg/L (400 women: 362 NGT and 38 GDM patients), and second with CRP 3-5 mg/L (184 women: 172 NGT and 12 GDM). CRP were significantly higher in GDM patients in second group: GDM median 4.3 (quartile 4.1-4.7) mg/L; NGT median 3.8 (quartile 3.3-4.3) mg/L; $p=0.014$; whereas, no change was found in CRP in first group: GDM median 1.5 (quartile 1.0-2.0) mg/L; NGT median 1.5 (quartile 0.8-2.1) mg/L; $p=0.875$. Using ROC-analysis the cutoff point for CRP were estimated as 4.9 mg/L for second group of CRP, and 5.8 mg/L for whole population. There were no significant correlation between CRP level and fasting plasma glucose nor glucose level after 2 hour 75 gram load.

Conclusions: CRP as a marker of low-grade inflammation state was associated with gestational diabetes mellitus in statistically significant group number two.

Key words: gestational diabetes; C-reactive protein; pregnancy

P28

Correlation between two analytical methods of HbA1c measuring in aligned and misaligned diabetic patients

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Backgrounds: Glycosylated hemoglobin (HbA1c) is representative of the mean blood glucose level over three months. The aim of the study was to

compared the HbA1c value using both HPLC and turbidymetric (T) assay in diabetic patients with aligned diabetes (AD) and with misaligned diabetes (MD).

Materials and Method: HbA1c (T), HbA1c (HPLC), glucose level and hemoglobin (Hb) were performed on group of 53 men (21AD, 22MD) and 79 women (46AD, 26MD), between 2011-2013 in Diagnostyka Company, Cracow, Poland.

Results: Significant differences were observed between both HbA1c (T), HbA1c HPLC and glucose leveling women: median [quartile] for: HbA1c (T) 5.2 (4.9-5.4)[%]; 5.8 (5.4-6.2)[%] $p < 0.001$; HbA1c (HPLC): 33.1 (29.9-35.3) [mmol/mol]; 39.7 (35.3-44.0) [mmol/mol], $p < 0.001$; glucose 13.3 (12.7-14.4) [mmol/l]; 13.4 (12.7-14.2) $p < 0.001$ (AD, MD respectively). In men: HbA1c (T) 5.3 (5.2-5.6) [%]; 5.8 (5.4-6.5) [%] $p = 0.004$; HbA1c HPLC: 34.2 (33.1-37.5) [mmol/mol], 39.11 (36.4-47.3) [mmol/mol] $p < 0.001$; glucose: 4.9 (4.5-5.2) [mmol/l]; 6.0 (5.6-7.2) [mmol/l] $p < 0.001$ (AD, MD respectively) Significant correlation were found between HbA1c (T) and HbA1c (HPLC) in men: $R = 0.7112$, $p = 0.003$; $R = 0.7108$, $p = 0.003$ (AD, MD respectively) and in women: $R = 0.6452$, $p = 0.003$; $R = 0.6331$; $p = 0.011$ (AD, MD respectively). No significant correlation were found between both HbA1c (T) nor HbA1c (HPLC) and glucose level in AD or MD group however, in combined group of women and men, the significant correlation were found: HbA1c (T) $r = 0.4786$, $p = 0.001$; HbA1c HPLC $r = 0.4338$, $p = 0.003$; HbA1c (T) $r = 0.6115$, $p < 0.001$; HbA1c HPLC $r = 0.8731$, $p < 0.001$ (women, men respectively).

Conclusions: Significant correlation were found between both analytical methods of HbA1c measuring in aligned and misaligned diabetes (women and men). No significant correlation were found between both HbA1c (T) nor HbA1c (HPLC) and glucose level in AD and MD patients (women and men). Although the significant correlation were found in combined group of women and men (AD+MD)

Key words: HbA1c; HPLC; diabetes mellitus

P29

Testing paracetamol interference with glucose analysis in serum

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Background: Paracetamol (acetaminophen) is widely used over-the-counter pain reliever and fever reducer. Studies have shown that paracetamol interferes with some home blood-glucose-measurement systems. The aim of this study was to investigate if the paracetamol interferes with glucose analysis in serum.

Materials and Methods: Testing was performed in accordance with IFCC Drug Effects in Clinical Chemistry. Part 2. Guidelines for Evaluation of Analytical interferences, 1984. Two paracetamol concentrations have been tested to cover the concentration spectrum (200 µg/mL and 1000 µg/mL). Two serum pools (normal and high glucose concentration) have been used as samples. Determination of glucose (Siemens, UK) was done on Dimension Xpand Plus analyser (Siemens, UK). All measurements were performed in duplicate. Bias exceeding 10% is considered „interference“.

Results: There were no difference between mean glucose concentration in test samples (5.0 mmol/L) and control samples (5.0 mmol/L) after adding 200 µg/mL and 1000 µg/mL of paracetamol in serum pool with normal glucose concentration. After adding 200 µg/mL and 1000 µg/mL of paracetamol in serum pool with high glucose concentration there were no difference present between mean glucose concentration in test samples (11.1 mmol/L) and control samples (11.1 mmol/L).

Conclusion: Paracetamol has no significant effect (bias less than 10%) on glucose analysis when present in serum at concentration of 200 µg/mL or 1000 µg/mL.

Key words: drug interference; glucose; paracetamol

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Determination of potential urine biomarkers of kidney disease by LC-MS / MS

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Background: Proteinuria may be caused by either increased glomerular basement membrane permeability, or by impairment of secretion-reabsorption tubular processes. It is often used as a surrogate marker in monitoring and predicting outcome in patients with chronic kidney disease but it is non-specific. We established a proteomics approach for identification of nephropathy-related biomarkers in urine, including Diabetic nephropathy (DN), which is a well-known complication of long-standing diabetes. The main aim of this work was to assess differences in urine proteins in patients with nephropathies and healthy controls and to identify abnormal proteins as potential biomarkers of the renal disease.

Materials and Methods: In our pilot project, we have analyzed protein composition of urine in 20 patients with IgA nephropathy and an appropriate healthy control group. The proteins were separated by the isoelectric focusing method (IEF) in the first step. The second dimensional separation and protein characterization was performed using iTRAQ quantitative analysis followed by mass spectrometry LC-MS/MS.

Results: We have identified 46 urinary proteins significantly up- or down regulated in the urine of IgA nephropathy patients. We have found changes in proteins of complement system, Regulation of actin cytoskeleton or retinol biosynthesis. Whilst these preliminary data require further studies that will correlate the results with clinical, laboratory and

histopathological data, some of the proteins, namely Cathepsin, Retinol-binding protein, Protein AMBP were also represented in urine of patients with diabetes mellitus in previous studies made by others.

Conclusion: We have optimized proteomic methodology that is able to identify potential urine biomarkers of kidney disease. Study of changes of urinary proteins could help to establish the diagnosis, assess the disease activity and/or predict the prognosis of different renal diseases, including different glomerulonephritis or DN.

Key words: kidney disease; LC-MS/MS; urine proteins

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Demand management of hemoglobin A1c testing according to the American Diabetes Association's Standards of Care 2014

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Background: Recently, American Diabetes Association (ADA) has considered the A1c test to diagnose diabetes ($\geq 6.5\%$). Furthermore, ADA has defined a category of increased risk of diabetes (pre-diabetes) whose values of HbA1c are between 5.7%-6.4%. The low difference of the HbA1c levels between the categories defined requires a strictly control of testing interval. ADA recommends measurement of HbA1c at least two times a year whether a patient's glycemic targets have been reached and maintained. Unstable patients may require four analyses a year.

The aim is the evaluation of time interval of the HbA1c during the last year in Aljarafe's area.

Materials and Methods: This study of management of the HbA1c test is based on the consensus among primary care and Aljarafe's Hospital to im-

prove diabetes outcomes (Plan Integral Diabetes de la Junta de Andalucía). Intervals testing conditions were: 180 days for stable patients and 90 days for unstable and non-maintained subjects. The A1c test was performed using a method that is NGSP certified and standardized to the DCCT assay.

Results: The total of HbA1c tests solicited was 23017, with 1174 (5.10%) were rejected for being out of the time intervals considered. The number of tests was: 18157 (72.2%) with 1 test/year, 5638 (22.4%) with 2 tests/year, 1020 (4.1%) with 3 tests/year and with 327 (1.3%) 4 tests/year. The average of HbA1c tests for each group was: 6.28%, 6.96%, 7.62% and 7.9%, respectively. The prevalence of diabetes in the sample studied was 5.1%.

Conclusions: The consensus approved in our Hospital about the time interval of the HbA1c has contributed to improve the diabetes management with reasonable criteria for demanding.

The strategies used for demanding of A1c suppose the reduction of the number of duplications. Unnecessary tests do not make a positive contribution in the appropriate diabetes diagnose.

Key words: Diabetes; HbA1c test; demand management; primary care

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Activities of superoxide dismutase and glutathione peroxidase in plasma of patients with Balkan endemic nephropathy

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Background: Balkan endemic nephropathy (BEN) is a familial chronic tubulointerstitial disease with insidious onset and slow progression to terminal renal failure. The aim of this study was to investigate the influence of oxidative stress on BEN by

measuring the activities of antioxidative enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX).

Material and methods: Activities of plasma GPX and SOD were determined spectrophotometrically in 30 BEN patients and 31 controls with nephrolithiasis.

Results: The activity of GPX was significantly lower in patients with BEN compared to the control group (368.1 ± 133.8 , 425.7 ± 82.5 , respectively, $p < 0.05$). When the BEN patients were divided according to eGFR, in patients with $eGFR > 90 \text{ ml/min/1.73m}^2$ GPX activity was significantly higher compared to those with $eGFR < 60 \text{ ml/min/1.73m}^2$. Moreover, significant negative correlation was observed between GPX activity and creatinine, urea and proteinuria ($r = -0.530$, $p < 0.05$; $r = -0.704$, $p < 0.001$; $r = -0.475$, $p < 0.05$; respectively). However, SOD activity was about 10% higher in plasma of BEN patients in comparison with controls, but without statistical significance (48.7 ± 9.6 , 44.2 ± 10.8 , respectively, $p > 0.05$).

Conclusions: The reduced GPX activity in patients with BEN might be a consequence of the atrophy of the kidneys present in these patients. As a result of the reduced enzyme antioxidant capacity, it may be assumed that the increased production of free radicals may be an important factor in the progression of BEN.

Key words: Balkan endemic nephropathy; oxidative stress; superoxide dismutase; glutathione peroxidase.

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Pharmacovigilance - case report

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Background: a brief introduction. National Blood Transfusion Institute is producing medicines which are obtained from human plasma. Medi-

cines which we are producing human is albumin, specific immunoglobulin - tetanus and rabies immunoglobulin. Our Directive for medicines and medical devices (Sl.Glasnik 84/2004) define that is necessary to use pharmacovigilance for all medicines.

The term pharmacovigilance comprises all scientific and data gathering activities relating to the detection, assessment, and understanding of adverse events. This includes the use of pharmacoepidemiological studies. Pharmacovigilance activities are undertaken with the goal of identifying

adverse events and understanding, to the extent possible, their nature, frequency, and potential risk factors.

Materials and methods: The little girl, after dogs bit her received usual anti rabies prophylaxis in the health center The little girl, after dogs bit her received usual anti rabies prophylaxis in the health center She react with severe reaction. After we received sample from patient to analyze it. We decided to analyze in her sample subclass of IgA. We reanalyzed sample of drug (Human rabies immunoglobulin) from the same batch.

Results:

Name of sample	IgA g/L	IgM g/L	IgG g/L	Subclases IgA1	Subclases IgA2
Normal values	0.9-4.5	0.6-2.5	8-19	0.61-3.04	0.10-1.13
Patient M. J.	2.42	0.65	9.0	2.15	0.18
Humani rabies imunoglobulin	2.26	0.78	130	1.90	0.21

Conclusion:

After comparison of these results with normal values we concluded that, this reaction is not connected with IgA immunodeficiency, or with Hu-

man rabies immunoglobulin. Any reaction could possible to manifest for all population with or without diabetes.

Key words: pharmacovigilance; rabies; diabetes