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 Domić 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

within run imprecision was 3.01% and beetwen-run imprecision was 2.82%. Linearity was confirmed with calibration curve in 6 points in concentration range from 12.5 to 150 µg/mL. Passing-Ba-block regression analysis of valproic acid comparison on two analyzers showed statistically significant, but clinically insignificant deviation in slope of regression equation (b = 0.9410; 95%CI = 0.9042-0.9766). The Cusum linearity test proved that there was a linear relationship between two methods.

Conclusion: Analytical validation of valproic acid by PETINIA method on Abbott Architect c8000 system fulfilled all previously established criteria and could be implemented in a routine laboratory work.

P21-02

The possible anti-cholinesterase properties of biologically synthesized platinum nanoparticles

Arowolo A, Whiteley C

Rhodes University, P.O. Box 94, Grahamstown, South Africa., Biochemistry, Microbiology and Biotechnology, Grahamstown, South Africa

Corresponding author: afolakesennuga@yahoo.com

Biomineralization, using protein cavity (cage) as a limiting growth field for the synthesis of uniformed sized and shaped nanoparticles (nps), is fast becoming a common approach in biological synthesis of nps. This is due to the fact that this method of production is by far simple, environmentally friendly and cost effective with great potential application in drug-delivery. However, little is known on the possible effect of these synthesized nps on the natural biological function of this protein cages. Also, the toxic effect of these nps on various biomedical target is unknown. In this work, spherical platinum nanoparticles (Pt-nps) of relatively uniform sized (3-5 nm) were biologically synthesized within the cavity of horse spleen apoferritin (HSA). Nanoparticles were characterized using UV, Transmission electron microscopy (TEM) and Electron dispersion analysis of X-rays (EDAX). The effect of synthesized Pt-nps on the ferroxidase activity of HSA was investigated. Finally, their potential anti-cholinesterase or neurotoxic properties were also studied. Synthesized Pt-nps significantly increased the ferroxidase activity of HSA by up to 9-fold. Results showed further, no significant inhibition on AChE activity compared to about 80% inhibition earlier reported with chemically synthesized nanoparticles. Pt-nps are well known to be efficient catalyst and may explain the rapid increase in the ferroxidase activity of HSA observed. We also believe the protein shell of HSA shielded the Pt-nps from eliciting any significant effect on the activity of AChE.

P21-03

Recreational drugs in clinical toxicology: new challenges in the everyday routine

Lakatos A (1), Lajtai A (1), Porpaczy Z (2), Mayer M (2), Kovacs G (1)

(1) University of Pécs, Med. School, Dept. of Laboratory Medicine, Pécs, Hungary
(2) University of Pécs, Med. School, Dept. of Forensic Medicine, Pécs, Hungary

Corresponding author: agnes.lakatos@aok.pte.hu

In clinical laboratory toxicology we use biological samples (mostly urine) of the poisoned patient to confirm the consumed dangerous substances. The anamnesis in this field is usually inefficient. The patients are often unconscious or don't want to tell or don't even know what was the substance they used for suicide or abuse. The spectra of most often used poisonous agents are changing in the time. It is also true for the medical drugs but in the field of the recreational drugs weekly appearing and explosively spreading new substances result everyday challenge for the clinical toxicologists. Not to be illegal for a while helps diffusing the usage of them. The lack of experience of the application of emerging designer drugs often draw the user to run into serious status and need emergency medical treatment. Neither the widespread immunological rapid tests, nor the medical experi-

ence can help the diagnosis of them. We use HPLC-DAD (Shimadzu TOX.I.S) system for toxicological screening and identifying the poisonous basic drugs. Its library can be expanding by the user. To validate the „reference materials" we use MALDI TOF method. We have managed to analyze mephedrone, flephedrone, 4-MEC, MDPV, Benzo-fury (5APB), 4FA, pentedrone and methoxetamine in the last 2 years in increasing number (about 300) of clinical cases. The HPLC method is convenient for the detection and identification of a broad spectrum of drugs it can be either well known or new.

P21-04

Cannabimimetics – A new challenge in routine clinical toxicology

Grobosch T, Binscheck T

Labor Berlin - Charité Vivantes GmbH, Laboratoriumsmedizin & Toxikologie, Berlin, Germany

Corresponding author: thomas.grobosch@laborberlin.com

Background: A 24-yr-old woman with a history of anorexia nervosa/bulimarexia was found unresponsive, breathless and asystolic in bed. After 30 min reanimation attempts of the emergency doctor the patient was defibrillated and transported to hospital. On admission, the EGC showed a prolonged QT interval and developed a significant metabolic acidosis with severe hypokalaemia.

Materials and methods: Systematic toxicological analysis: CEDIA, HPLC-DAD, HS-GC. Qualitative Screening (XLC-QQTOF). Quantitative analysis: Cannabimimetics, fentanyl, midazolam (LC-MS/MS), amiodarone (LC-MS).

Results:

Urine: Drug screening by CEDIA was positive for benzodiazepines. Additional analytics, including diuretics, laxantia, etc. resulted all negative. XLC-QQTOF analysis identified midazolam, fentanyl, and the N-(5-hydroxypentyl) metabolite of JWH-018 (110 µg/L). The quantitative LC-MS/MS analysis for cannabimimetics identified another metabolite of JWH-018, N-pentanoic acid (43 µg/L).

Plasma: STA revealed midazolam (640 µg/L). XLC-QQTOF analysis identified midazolam, amiodarone and fentanyl. Additional quantitative analysis of the plasma sample identified JWH-018 (58 µg/L), the N-pentanoic acid metabolite (40 µg/L). Three days after reanimation, the neuron specific enolase in serum was significantly increased (80 µg/L) - indicating hypoxic brain damage.

Conclusions: Clinicians, and the users need to be aware of the severe clinical effects following consumption of synthetic cannabinoid preparations marketed as partly legal cannabis alternatives. The cannabinomimetics cannot be detected by commonly used immunoassays for THC, therefore mass spectrometry is essential for identification. It is not clear whether the disturbance of myocardial repolarisation in this case was specifically induced by JWH-018 or might be facilitated by other specific circumstances in this case.

P21-05

Extreme case of designer drug abuse

Lajtai A (1), Lakatos A (1), Benkő A (2), Mayer M (2), Porpáczy Z (2), Kovács L. G (2)

(1) University of Pécs, Medical School, Department of Laboratory Medicine, Pécs, Hungary
(2) University of Pécs, Medical School, Department of Forensic Medicine, Pécs, Hungary

Corresponding author: agnes.lakatos@aok.pte.hu

The identification of new designer drugs means the largest problem in the clinical and forensic toxicology examinations; reference materials are not available, their metabolisms are unknown, their versatility is unlimited. The medical condition of a drug user is frequently critical, requiring intensive care, not to mention the possibility of long-term adverse reactions. Since January 2012 nine designer drugs became illegal in Hungary (e.g. MDPV, 4- Fluroamphetamin, some of the

JWH compounds). Illicit marketing and abuse of these compounds seem to be reduced, in contrast to those drugs which are still legal but we haven't met them before in our diagnostics practice (e.g. tryptamine derivates, 3-FA, pentedrone). In the near past, we analyzed serum and urine samples of a 27 year old chronic male drug user (GC, Abbott Axsym, HPLC-DAD: Shimadzu TOX.I.S). In the urine sample we detected several kinds of drugs in large quantities. The main component was pentedrone. However, the interpretation of the results was not simple. The complication was caused by some unknown peaks that appeared on the chromatogram. These peaks are likely to be the products of the metabolic reduction of pentedrone. Spectra of these metabolites are very similar to those of ephedrine, but their retention times are different. In conclusion, consumers of various designer drugs might be viewed as the participants of a pharmaceutical study, who voluntarily accept to take part in a dangerous and uncontrolled experiment and help us to discover new drugs,metabolic routes and adverse effects.

P21-06

Identification of acidic compounds in acute intoxications by on-line SPE-HPLC-DAD

Mut L (1), Grobosch T (1), Binscheck T (1), Frenzel W (2)

(1) Labor Berlin - Charité Vivantes GmbH, Laboratory Medicine and Toxicology, Berlin, Germany
(2) Berlin Institute of Technology, Department of Environmental Technology, Berlin, Germany

Corresponding author: SwetlaMut@t-online.de

Background: Most therapeutic drugs relevant in clinical toxicology are characterized by basic and neutral properties, but a number of acidic medications (NSAID's, barbiturates) can cause life-threatening poisonings. Therefore, an on-line SPE-HPLC-DAD screening method was developed to identify acidic compounds in poisonings. An in-house library was generated to support this method.

Materials and methods: To reduce matrix effects and to deproteinate the plasma, precipitants (acetone, methanol), ratios (1/2, 1/3, 1/4, 2/3, 3/4 plasma/precipitant), buffers (pH: 6, 2.3, 9), injection volumes (0.25 mL, 0.50 mL, 1.0 mL) were tested for maximum performance. Four SPE cartridges were examined in terms of extraction efficiency of acidic compounds. Six HPLC-columns were studied for optimum peak intensity and symmetry. Gradients for an on-line extraction and chromatographic separation of acidic compounds were created. Data of analyzed substances and poisonings were used to establish an in-house library. The method was applied to acute intoxications.

Results: Optimal results for sample preparation were precipitation of plasma with acetone (1/2), dilution of supernatant in buffer (pH = 6) and injection volume: 1.0 mL. Only the SPE-column StrataX-A (pH = 6), allowed the extraction of acids. The most appropriate column for analytical separation and peak symmetry was a 150 x 4.6 mm, 3 µm C6-Phenyl column. The analysis time was 44 min., including on-line extraction (12 min). An in-house library included > 150 entries of acidic compounds and metabolites. Such substances as salicylic acid, or ibuprofen were identified in intoxications.

Conclusion: The described method proved to be efficient and sensitive for screening of acidic therapeutic drugs in human plasma in case of acute poisonings.

P21-07

Automated online solid phase extraction UPLC/MS/MS for the analysis of mycophenolic acid

Eastwood M (1), Chusney G (2), Calton L (1)

(1) Waters Corporation, Clinical, Manchester, United Kingdom
(2) Hammersmith Hospital, Imperial College Renal & Transplant Centre, London, United Kingdom

Corresponding author: martin_eastwood@waters.com

Background: The recent consensus report evaluating therapeutic drug monitoring of mycophenolic acid (MPA) highlighted the need for accurate drug dosing strategies to minimize the incidence of drug-related toxicity while maintaining efficacy. Here we evaluate the potential of a new online solid phase extraction (SPE) system coupled to UltraPerformance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) for the automated sample preparation and analysis of MPA in human plasma.

Materials and methods: Commercially available kits were used for calibrators and QC material. Method comparison was carried out using anonymized patient samples quantified using a validated LC/MS/MS assay. All samples were pre-treated with zinc sulphate and methanol. Automated online extraction was carried out with a Waters® ACQUITY UPLC coupled to a Waters MassTrak Online SPE Analyzer* and analysed using a Waters ACQUITY® TQD mass spectrometer.

Results: Following CLSI-EP6-A, the assay was shown to be linear from 0.01–50 µg/mL (N = 5). Coefficients of variation for inter- and intra-assay imprecision for low (1.94 µg/mL), mid (2.35 µg/mL), high (5.5 µg/mL) QC samples were all < 10% (N = 25, days = 5). Method comparison using patient samples previously analyzed with a validated LC/MS/MS assay (N = 50) was described by the Deming equation y = 0.99x-0.01. Compared with conventional one-dimensional chromatography, matrix effects were reduced by the online SPE as determined qualitatively by targeted multiple reaction monitoring of phospholipids and post-column infusion of analytes.

Conclusions: We have successfully quantified mycophenolic acid utilising automated online SPE with UPLC/MS/MS. The assay demonstrates good linearity, precision and accuracy with minimal ion suppression.

*NOTE: The MassTrak Online SPE Analyzer is under development

P21-08

Automated online solid phase extraction UPLC/MS/MS for simultaneous analysis of immunosuppressants

Eastwood M, Balloch S, Cooper D, Calton L

Waters Corporation, Clinical, Manchester, United Kingdom

Corresponding author: martin_eastwood@waters.com

Background: Therapeutic drug monitoring of immunosuppressants is an important requirement for the management of transplant patients. To streamline workflow there is a demand for the simultaneous measurement of multiple analytes. Here we evaluate the potential of online solid phase extraction (SPE) coupled to UltraPerformance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) for the automated sample preparation and simultaneous analysis of cyclosporin A (CsA), tacrolimus, sirolimus and everolimus.

Materials and methods: Commercially available kits were used for calibrators and QC material. Samples obtained from the ASI Ltd International Proficiency Testing Scheme (IPT) were used to assess accuracy. All samples were pre-treated with zinc sulphate and acetonitrile. Automated online extraction was carried out with a Waters® ACQUITY UPLC coupled to a Waters MassTrak Online SPE Analyzer* and analysed using a Waters ACQUITY® TQD mass spectrometer.

Results: The assay was linear from 24.8–1515 ng/mL for CsA, 1.0–31.6 ng/mL for tacrolimus, 0.9–30.1 ng/mL for everolimus and 0.9–27.7 ng/mL for sirolimus, with r2 values > 0.997 (N = 5). Inter- and intra-assay imprecision for low, mid, high QCs were all < 10% CV (N = 5 *per* analyte). IPT samples were all within 10% of expected values (N = 5 per analyte). Compared with conventional one-dimensional chromatography, matrix effects were reduced as determined qualitatively by targeted multiple reaction monitoring of phospholipids and post-column infusion of analytes.

Conclusions: We have successfully quantified CsA, tacrolimus, sirolimus and everolimus simultaneously utilising automated online SPE with UPLC/MS/MS. The assay demonstrates good linearity, precision and accuracy with minimal ion suppression.

*NOTE: The MassTrak Online SPE Analyzer is under development

P21-09

Development of a LC-MS/MS method for the measurement of propofol and propofol glucuronide

Pitkin S, Zaman M, Benton S

Barts Health NHS Trust, Department of Clinical Biochemistry, London, United Kingdom

Corresponding author: sarah.pitkin@bartshealth.nhs.uk

Background: Propofol is an intravenous hypnotic agent used for sedation in intensive care units. A potentially fatal adverse effect is 'propofol-related infusion syndrome' (PRIS). There is interest in whether risk factors for PRIS relate to changes in metabolism of propofol. The aim was to develop and validate an LC-MS/MS method for the measurement of propofol and propofol glucuronide in whole blood, suitable for pharmacokinetic studies.

Materials and methods: Freeze-thawed whole blood was spiked with internal standards (propofol-d17; propofol glucuronide-d17) and proteins precipitated using acetone. Supernatant was heated for 10mins at 60 °C with dansyl chloride (2.7 mg/mL) and ammonium hydroxide (0.03N). The reaction mixture was injected on to a Waters Acquity UPLC and Quattro Premier XE tandem mass spectrometer. Gradient elution was followed by quantification by electrospray ionisation mass spectrometry in multiple reaction monitoring mode.

Results: Dansyl chloride derivatisation significantly increased the detection of propofol. Propofol glucuronide did not react with dansyl chloride and was quantified in its underivatised state. The run time was 11.5 mins. Standard curves were linear (r^2 > 0.99) across the calibration ranges. The intra- and inter-assay coefficients of variation were < 15% (N = 9-10) and the lower limits of quantitation for propofol and propofol glucuronide were 0.1 µg/mL and 0.25 µg/mL respectively. No ion suppression or enhancement or carry-over was observed. The parent drug and its metabolite were detectable in whole blood from patients receiving propofol infusions.

Conclusions: A novel LC-MS/MS method for the simultaneous measurement of propofol and propofol glucuronide was developed, which will be of use in the further investigation of PRIS.

P21-10

Oxidative stress/serum acetylcholinesterase in Nigeria organophosphate pesticide exposed farmers

Akinosun O (1), Adewole T (2)

(1) College of Medicine, University of Ibadan, Chemical Pathology Department, Ibadan, Nigeria
(2) University College Hospital, Chemical Pathology Department, Ibadan, Nigeria

Corresponding author: bayoakin2002@yahoo.com

Background: Organophosphate agents constitute about half of all pesticides used globally and they appear to pose the greatest risk among all the pes-

ticides. Despite significant advances in the understanding of the potential mechanism of toxicity in intentional exposures, the precise health effects following occupational exposures are yet to be completely defined.

Materials and methods: Oxidative stress status and Acetylcholinesterase activities were studied in blood samples obtained from 25 farmers in Idi Ayunre, Oluyole local government area of Oyo State, using organophosphate (OP) pesticides in spraying their cash crops (cocoa and cola nut trees) with a minimum work history of 10 years, in the age range of 35-75 years. 20 age-matched workers, who never had any exposure to OP pesticides were selected as controls in Ibadan, the capital of Oyo State. Total Plasma Peroxide (TPP) levels using FOX-2 reagent, Total Anti oxidant potential (TAP) using the ferric reducing antioxidant power (FRAP) assay were determined and oxidative stress index (OSI) an indicator of oxidative stress status was calculated. Blood acetylcholinesterase activity was measured using HPLC.

Results: Statistically significant decrease in the mean blood levels of acetylcholinesterase (IU/L) in the farmers (43.35 ± 9.07) compared to the controls(65.28 ± 7.66). TPP (umol H_2O_2/L) increased significantly in the farmers (14.32 ± 5.18) than in the controls (10.25 ± 3.60) ($P < 0.05$), while depletion of TAP (umolTroloxequiv/L) was observed in the farmers (915.65 ± 130.16) than the controls (975.80 ± 142.70). OSI(%) in farmers (1.65 ± 0.69) increased significantly than controls (1.08 ± 0.39).

Conclusion: OP pesticides users are exposed to increased oxidative stress. Assay of acetylcholinesterase activities could be a good biomonitoring index.

P21-11

Blood mercury concentrations in 3 cities in Spain

Trasobares E (1), González -Estecha M (1), Martínez García M (2), Herbello Hermelo P (3), Bermejo Barrera P (3), Guillén Pérez J (4)

(1) Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain
(2) Universidad Politécnica de Cartagena, Ingeniería Química y Ambiental, Cartagena, Spain
(3) Universidad de Santiago de Compostela, Química Analítica, Santiago de Compostela, Spain
(4) Dirección General de Salud Pública, Región de Murcia, Dirección General de Salud Pública, Región de Murcia, Cartagena, Spain

Corresponding author: montse@cmpx.net

Background: There is increasing concern about the effects of exposure to methylmercury in adults. The aim of this multicenter study is to measure blood mercury concentrations in an adult population in 3 cities in Spain.

Materials and methods: We recruited 792 employee volunteers from 2 hospitals and one University in Madrid, Cartagena and Santiago de Compostela. Blood mercury concentration (µg/L) was measured in Madrid by cold vapour atomic absorption spectrometry in a Perkin Elmer FIMS 400 and in Cartagena in a direct manner in a DMA-80 Millestone based on the EPA 7473 method. Evaluation of concordance by Bland Altman plot was performed between these two methods. Blood mercury in Santiago de Compostela was determined by Cold Vapour Atomic Absorption Spectrometry in a Perkin Elmer 4100 equipped with a FIA Perkin Elmer 400.

Results: Upon evaluation of concordance between Madrid and Cartagena methods, almost all the measurements concorded and were included in the 95% confidence interval of the mean of the differences. The medians of blood mercury (µg/L) obtained were: Madrid (7.9; IQR: 5.2-11.5); Cartagena (8.95; IQR: 6.7-13.8) and Santiago de Compostela (15.1; IQR: 10.2-19.9). A statistically significant difference was observed among blood mercury concentrations in the 3 cities ($P < 0.001$). We also observed statistically significant differences between Madrid

and Cartagena (P = 0.004); Madrid and Santiago de Compostela (P < 0.001) and between Cartagena and Santiago de Compostela (P < 0.001).

Conclusions: Higher blood mercury concentrations were found in Spain than those previously reported in other European countries, probably due to the higher fish consumption in Spain.

P21-12

Influence of immunosuppressive regimen change on renal function in liver transplant recipients

Ožvald I (1), Mrzljak A (2), Radisic Biljak V (1), Perkov S (1), Flegar Mestric Z (1)

(1) University Hospital Merkur, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) University Hospital Merkur, Department of Gastroenterology, Zagreb, Croatia

Corresponding author: iozvald@gmail.com

Background: Liver transplantation is accepted treatment of choice for many liver diseases. Long–term survival is limited by toxicity of immunosuppressive agents, sub-clinical as well as cronic rejection. Newer classes of immunosuppressive agents, including calcineurin inhibitors CNI (cyclosporine and tacrolimus) and mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) have potential to improve long-term outcomes. Many long-term survivors face a considerable risk of renal dysfunction due to CNI. The aim of the study was to determine benefits of sirolimus compared to CNI towards kidneys toxicity.

Materials and methods: We have monitored nine orthotopic liver transplantation patients (OLT) who underwent conversion of immunosuppressive regiment from CNIs to sirolimus. Creatinine concentrations were measured with the creatinine enzymatic assay. Measured concentrations of serum creatinine were used to estimate renal function, after dosing CNIs and converting to sirolimus, each one, three and six months after the dose.

Results: Referring to the long-term outcomes of OLT patients following results could be seen: Among all patients switched to sirolimus, the levels of serum creatinine (80.3 µmol/L ± 12.8 *vs*. 76.0 µmol/L ±12.7) remained stable in three of them, whereas the levels of serum creatinine started to decrease (119.4 µmol/L ± 29.8 *vs*. 83.2 µmol/L ± 26.3) after administration of sirolimus in six of the patients.

Conclusion: These preliminary results have shown that sirolimus is effective in preventing rejection in OLT recipients and it is associated with improved renal function. mTOR inhibitors might have a role as an early alternative to CNIs in patients with CNI nephrotoxicity.

P21-13

Multi-drug intoxication fatality involving atorvastatin: a case report

Cibicek N (1), Caran T (2), Pribyl P (3), Dobias M (4), Ondra P (4), Vorisek V (5)

(1) Palacky University Olomouc, Faculty of Medicine and Dentistry, Department of Medical Chemistry and Biochemistry, Olomouc, Czech Republic
(2) Hospital Hranice, Department of Anaesthesiology and Resuscitation, Hranice, Czech Republic
(3) Hospital Hranice, Department of Internal Medicine, Hranice, Czech Republic
(4) University Hospital Olomouc, Institute of Forensic Medicine and Medical Law, Olomouc, Czech Republic
(5) Charles University in Prague, Faculty of Medicine and University Hospital in Hradec Kralove, Institute of Clinical Biochemistry and Diagnostics, Hradec Kralove, Czech Republic

Corresponding author: norbert.cibicek@upol.cz

Background: Mixed antihypertensive drug intoxication poses a significant risk for patient mortality. In tandem to antihypertensives, hypolipidemic medicines (especially statins) are often prescribed. Among their adverse effects belongs rhabdomyolysis.

Case description: We report a case of fatal multidrug overdose in a 65-year-old female alcoholic. The woman was admitted to a municipal hospital unconscious. Empty blister packs indicated the abuse of 250 tablets of urapidil, 42 tablets of vera-

pamil/trandolapril, 50 tablets of moxonidin, 80 tablets of atorvastatin and 80 tablets of diacerein. Standard measures (gastric lavage, mechanical ventilation, massive doses of vasopressors, volume expansion, diuretics and alkalinisation) failed to provide adequate drug elimination and hemodynamic support. The patient deceased on the fourth day.

Results: Dramatic elevations of serum myoglobin (34880 ug/l) and creatine kinase (281 ukat/l) were accompanied by rise in cardiac troponin I and creatinine. Gas chromatography revealed ethanol 1.17 g/kg (blood) and 2.81 g/kg (urine). Thin layer chromatography and gas chromatography of gastric content and urine verified verapamil, moxonidin and urapidil fragment (diacerein method was unavailable). Atorvastatin and trandolapril concentrations (LC-MSn) equaled 277.7 ug/L and 57.5 ug/L, resp. (serum) and 8.15 ug/L and 602.3 ug/L, resp. (urine). Histology confirmed precipitates of myoglobin with acute necrosis of proximal renal tubules in association with rhabdomyolysis of striated muscle and myocardial dystrophy.

Conclusions: Distributive and cardiogenic shock in conjunction with acute renal failure due to the combined self-poisoning with vasoactive agents and atorvastatin were determined to be this decedent's immediate cause of death. The manner of death was assigned to be suicidal.

P21-14

Pharmacokinetics of mycophenolic acid in renal allograft recipients: role of ABCC2 polymorphisms

Lalić Z (1), Božina N (1), Nađ Škegro S (2), Trkulja V (3), Lovrić M (1), Granić P (1)

(1) University Hospital Center Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) University Hospital Center Zagreb, Department of Urology, Zagreb, Croatia
(3) Zagreb University School of Medicine, Department of Pharmacology, Zagreb, Croatia

Corresponding author: lovricm@kbc-zagreb.hr

Background: Mycophenolic acid (MPA) displays large between- and within-subject pharmacokinetic variability. MPA is metabolized by UGTs to inactive 7-O-MPA-glucuronide (MPAG). MPA and MPAG are subject to enterohepatic recirculation. Biliary and kidney excretion of MPA/MPAG involves several transporters, including multidrug resistant protein-2 (MRP-2) coded by polymorphic *ABCC2*, which can influence MPA pharmacokinetics. The objective of this study was to perform MPA pharmacokinetics et steady state conditions during one dosing interval (12 h), in 68 renal allograft recipients. Pharmacokinetic variability in relation to donor and recipient *ABCC2* genotypes is estimated.

Patients and methods: Blood samples were drawn at 0, 0.5, 1, 2, 3, 8, and 12 h after the morning dose. Genotyping of *ABCC2* C-24T and G1249A was performed using TaqMan-based allele-specific PCR assay. Plasma concentrations of MPA were determined using validated HPLC method.

Results: Pharmacokinetic parameters: Cmax,ss (mg/L) 12.3 ± 6.7 ; Tmax (hrs) 2 (0.2-12); AUCt,ss (mg*h/L) 39.9 ± 20.7; Cmin,ss (mg/L) 1.3 ± 1.2; Trough 1 (time 0) (mg/L) 2.7 ± 2.1; Trough 2 (time 12) (mg/L) 1.9 ± 1.9. Considering *ABCC2* genotypes associations were found between: donor C-24T and lower through1 concentrations in T allele carriers ($P = 0.003$); G1249A variants and lower Cmax/dose in A allele carriers ($P < 0.05$), -24T allele carriers and % of concentration swing (OR 1.88, 95%CI 1.09-3.23). For recipient genotypes correlations were between: 1249A allele and lower Cmin and trough2 concentrations, and higher % of concentration swing (OR 1.85 95%CI 1.05-3.28).

Conclusion: The pharmacokinetics of MPA is affected by the *ABCC2* polymorphisms.

P21-15

HPLC of carbohydrate-deficient transferin and more sialylated transferrin glycoforms in children

Bianchi V (1), Raspagni A (2), Arfini C (3), Vidali M (4)

(1) SSS Antonio e Biagio Hospital, Toxicology Laboratory, Alessandria, Italy
(2) Insubria University, School of Medicine, Varese, Italy
(3) SS Antonio e Biagio, Clinical Laboratory, Alessandria, Italy
(4) Maggiore della Carità Hospital, Clincal Chemistry Unit, Novara, Italy

Corresponding author: vbianchi@ospedale.al.it

Background: The evaluation of age-specific distribution of transferrin glycoforms in paediatric patients may help in defining reference intervals which are critical for an improved and earlier diagnosis.

Materials and methods: Serum samples from 224 children (age: 2 months-14 years) were analyzed by HPLC (CDT by HPLC kit, Bio-Rad, Munich, Germany) and glycoforms expressed as percentage of the total area of transferrin (Tf).

Results: Asialo- and Monosialo Tf were not detectable in any patient. Median (IQR) were respectively 0.92% (O.80-1.04%) for DisialoTf; 3.47% (2.69-4.18%) for Trisialo-Tf; 82.54% (81.32-83.53%) for Tetrasialo-Tf; 12,73% (11.91-14.09%) for Pentasialo-Tf. Statistically significant differences in Trisialo-Tf ($P < 0.001$), Tetrasialo-Tf ($P = 0.001$), Pentasialo-Tf ($P < 0.001$), but not in Disialo-Tf, were observed between the age groups.

Conclusions: Age-specific Diaisialo-Tf cut-offs are not necessary. In children 1.3% and 6.4% may be suggested as upper limits of normal range to detect increases of Disialo- and Trisialo-Tf. The presence of Asialo- and Monosialo-Tf should be considered an abnormal finding and prompt further investigations.

P22 - Vitamin D - PTH

P22-01

Comparison of total 25-OH vitamin D automated immunoassays versus LC-MS/MS

Bausela C, Gaviña B, Ortega I, Torrejón M, Arroyo M

Hospital Clínico San Carlos, Análisis Clínicos, Madrid, Spain

Corresponding author: cris_bausela@hotmail.com

Background: Vitamin D has an important role in both calcium homeostasis and bone metabolism .The best serum marker to determine nutritional status is Total 25-OH Vitamin D. The great methodological variability and the lack of international 25-OH Vitamin D standards for immunoassays, take to wrong classifications, treatments and monitoring. The aim of this study is to compare three automated immunoassay methods for the measurement of Total 25-OH vitamin D (Abbot, Roche and DiaSorin) with LC-MS/MS.

Material and methods: Human serum samples (N = 150) were measured with the followings methodologies:

- DiaSorin LIASION 25-OH VITAMIN D TOTAL CLIA®
- Abbot Diagnostic ARCHITECT 25-OH VITAMIN D CMIA®
- Roche ELECSYS 25-OH VITAMIN D TOTAL ECLIA®
- LC-MS/MS ASSAY

Results obtained by the three immunoassays were compared with LC-MS/MS by linear correlation plots and concordance correlation coefficients. Weight Kappa Coefficient was used to determine the agreement between different assays. All data were analyzed with SPSS software (version 15.0).

Results: Spearman´s Rho Coefficients: DiaSorin = 0.840, Abbot = 0.848 and Roche = 0.817. Intraclass Correlation Coefficients, ICC: DiaSorin = 0.931, Abbot = 0.913 and Roche = 0.897. Weight Kappa Coefficients: DiaSorin = 0.701, Abbot = 0.734, Roche = 0.718.

Conclusions: Correlation and concordance coefficients between the three immunoassays and LC-