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SSCC 2015

Posters abstracts

SSCC2015 - Posters abstracts

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However, the location of the posters in the posters area follows the poster number (Pnn).
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SSCC2015 Abstract 01

Rapid screening of different groups of steroids by multiple selected ion monitoring in biological fluids

Bernhard Dick (1), Bruno Vogt (1), Geneviève Escher (1)

1. Department of Nephrology, Hypertension and Clinical Pharmacology, Inselspital, Bern

Introduction: Steroid hormones are cholesterol derivatives that regulate various biological processes such as salt and water balance, metabolism, sexual development and function, inflammation, immunity and stress responses. Quantitative assessment of urinary steroids is routinely used in clinical practice.for the diagnostic of specific disorders. Measurements can additionally be performed in serum, plasma, cell culture supernatants and various tissues.
Since different groups of steroids can interact with each other, it is essential to quantify the maximum number of steroids with one analytical procedure.
Methods: Methods for quantitative measurement of 64 steroid including androgens, estrogens, progesterone (P), mineralocorticoid and glucocorticoid metabolites, as well as 9 bile acids, 7 oxysterols, 4 synthetic glucocorticoids and glycyrrhetinic acid have been established in our laboratory. After extraction, hydrolysis and specific derivatisation, steroids are analysed by gas chromatography-mass spectrometry. All the methods are based on the principle of multiple selected ion monitoring (SIM) with one specific ion attributed to each steroid. A standard containing a mixture of known amounts of each substance is used as calibration on a regular basis.The values obtained in the sample are determined relatively to this calibration. For each steroid the intensity of the “ion-peak” is correlated to an internal standard and a recovery standard.
Preliminary data: Elevated circulating bile acid levels in patients with cholestasis caused by obstructed bile duct led to oxidation’s inhibition of cortisol (F) into cortisone (E). The F/E ratio, an indicator of 11β-hydroxysteroid dehydrogenase (11β-HSD) 2, was significantly lower following surgical removal of the obstruction. Since cortisol can activate the mineralocorticoid receptor, increased F levels during cholestasis induce sodium and water retention, potassium loss and hypertension.
Under normal conditions 7-oxo-cholesterol is rapidly reduced to 7β-hydroxy-cholesterol by 11βHSD1 and plasma and tissue levels of 7-oxo-cholesterol are low. This is an essential metabolic step to overcome the toxic effects of 7-oxo-cholesterol. Upon inhibition of 11βHSD1 by bile acids, increased levels of 7-oxo-cholesterol levels exert toxic effects.
In another study, we demonstrated that 27-hydroxycholesterol, the oxysterol produced from the hydroxylation of cholesterol by the enzyme sterol 27-hydroxylase (CYP27A1) regulates P metabolism by inhibiting the enzyme 20α-hydroxysteroid dehydrogenase which metabolizes P into 20α-dihydroprogesterone (20α-DHP). P and 20α-DHP were quantified in urines of CYP27A1 gene knockout (KO) mice and their control wild-type (WT) littermates. In CYP27A1 KO mice, urinary P concentrations were decreased, 20α-DHP increased, and the P/20α-DHP ratio decreased threefold when compared to WT.
Synthetic glucocorticoids such as prednisone, prednisolone, dexamethasone and betamethasone were shown to suppress endogenous cortisol production. As a consequence, the F/E ratio is markedly increased and the urinary excretion of glucocorticoid metabolites decreased. Upon prescription of synthetic glucocorticoids over a long period of time patients start to develop symptoms of Cushing’s disease.
Glycyrrhetinic acid inhibits both11β-HSD1 and 2, causing sodium and water retention and hypertension both in patients and in rodents.
Novel aspects: Screening multiple steroid metabolites in biological fluids allows to study the interaction between the different groups of steroids.

bernhard.dick@insel.ch

SSCC2015 Abstract 02

Fully automated Multi-Method LC-MS: application in clinical Laboratory

M Cantù (1); F Keller (1)

1. Dipartimento di Medicina di Laboraotrio EOLAB, Ente Ospedaliero Cantonale, Bellinzona

Clinical mass spectrometry laboratory could be ideally divided into 2 main groups: big laboratories who perform more than 20’000 test/year per method, and medium-little laboratories who perform less than 20’000 test/year per method.
If the former group could use one LCMS per one method, the second need to apply different methods on the same LCMS in order to optimize the time machine and cost.
In the last years several LCMS homemade methods or IVD kits became available into the market but, unfortunately, each one define their own configuration: Isocratic vs Gradient, single vs binary pumps, different connections, one analytical column vs online SPE, etc.
This is a big problem for medium-little laboratories in order to: identify the best instrument configuration when buy an LCMS, and instrument management during routine due to intra-day changes. So, it’s easy to understand that the main request into medium-little clinical mass spectrometry laboratories, it is the instrument flexibility.
In the last 4 years we setup a flexible configuration able to do different methods on a single LCMS. This configuration is able to load simultaneously: up to 24 solvents into the primary pump, up to 4 solvents into the secondary pump and up to 15 columns, divided into 2 independents thermostats.
Thanks to this configuration, this LCMS is able to work with different approaches without any hardware changes: direct injection (e.g. new-born screening or MS parameters optimization), single analytical column method (e.g. typical home-made or some TDM IVD kits), online SPE (e.g. typical IVD kits for Vitamin D or Immunosuppressants) and method development (test different columns or different mobile phases).
This system is able to manage all instrument changes (solvent line selection, column selection, etc.) directly from the work-list. That mean a really easy work for technicians and a more stable-robust instrumentation and analysis value.

marco.cantu@eoc.ch

SSCC2015 Abstract 03

Anti-Apolipoprotein A-1 autoantibodies as active modulators of atherothrombosis

S Pagano (1,2); F Carbone (3); F Burger (3); A Roth 3); M Bertolotto (4); B Pane (5); G Spinella (5); D Palombo (5); A Pende (4); F Dallegri (4); N Satta (1,2); J Virzi (1,2); F Mach (3); F Montecucco (3,4); N Vuilleumier (1,2)

1 Division of Laboratory Medicine, Department of Genetics and Laboratory Medicine, Geneva University Hospitals, 4 rue Gabrielle-Perret-Gentil 1205 Geneva, Switzerland.2 Department of Human Protein Sciences, Faculty of medicine, 4 rue Gabrielle-Perret-Gentil 1205 Geneva, Switzerland.3 Division of Cardiology, Geneva University Hospitals, Faculty of Medicine, Foundation for Medical Researches, 64 avenue de la Roseraie 1211 Geneva, Switzerland. 4 First Medical Clinic, Laboratory of Phagocyte Physiopathology and Inflammation, Department of Internal Medicine, University of Genoa, 6 viale Benedetto XV 16132 Genoa, Italy.5 Vascular and Endovascular Surgery Unit, Department of Surgery, San Martino Hospital, Genoa, 10 Largo Rosanna Benzi 16132 Genoa, Italy.

Introduction: Humoral autoimmune-mediated inflammation plays a role in atherogenesis, and potentially in atherothrombosis. Anti-apolipoprotein A-1 (apoA-1) IgG have been reported to represent emergent mediators of atherogenesis trough Toll-like receptors (TLR) 2,4 and CD14 signaling. Aims: to investigate if and how anti-apoA-1 IgG could promote atherothrombosis by modulating the expression and activity of Tissue Factor (TF), a key coagulation pathway regulator involved in atherothrombosis. Methods: serum levels of anti-apoA-1 IgG were measured by ELISA. Atherothrombosis features were determined by immunohistochemical TF staining of human carotid biopsies derived from patients with severe carotid stenosis undergoing elective surgery (n=176), and on aortic roots of different genetic backgrounds mice (ApoE-/-; TLR2-/-ApoE-/- and TLR4-/-ApoE-/-) exposed to passive immunization with anti-apoA-1 IgG. On human-monocytes-derived-macrophages (HMDM) the anti-apoA-1 IgG-induced TF expression and activity was analyzed by FACS and chromogenic assays in presence of different pharmacological inhibitors. Results: Significant associations were retrieved between anti-apoA-1 IgG circulating levels and intraplaque TF expression in human carotid biopsies. On HMDM, anti-apoA-1 IgG induced a TLR2,4/CD14-dependent increase in TF expression/ activity, involving NF-kappaB and AP-1 transcription factors, after JNK activation. In ApoE-/- mice, anti-apoA-1 IgG passive immunization significantly enhanced intraplaque TF expression when compared to control IgG. This effect was lost in both TLR2-/-ApoE-/- and TLR4-/-ApoE-/- backgrounds. Conclusions: These results extend previous findings by demonstrating that anti-ApoA-1 IgG are associated with higher propensity to atherothrombosis, and could per se induce TF expression in human macrophages, supporting a possible causal link between anti-ApoA-1 IgG and atherothrombosis.

Sabrina.Pagano@hcuge.ch

SSCC2015 Abstract 04

Comparison of Glycation Gap Value with selected laboratory analytes in Healthy, Prediabetic and Diabetic Swiss Senior Citizens

P Medina Escobar (1), C Grebhardt (1), U Nydegger (1), Z Stanga (2), M Risch (3), L Risch (1)

1. Labormedizinisches Zentrum Dr. Risch, Bern-Liebefeld,2. Inselspital/Universitätsspital Bern, 3. Kantonsspital Graubünden, Chur

Introduction: Glycation Gap (GG), a difference between measured glycosylated haemoglobin (HbA1c) and HbA1c predicted from fructosamine (FA), quantifies glycemic differences in formation of HbA1c between individuals. With the SENIORLAB study, we explore (i) how GG values do evolve in senescence, (ii) how they relate to insulin resistance (HOMA-IR) and to low grade inflammation value (hsCRP). (iii) The study also compares GG to triglyceride (TG) and kidney function (eGFR) .
Subjects and Methods: 1432 (healthy=547/prediabetic=701/diabetic=184) subjects were included in the study. Statistical analyses were performed using MedCalc for Windows, version 15.6.1 (MedCalc Software, Ostend, Belgium). The GG was evaluated and assigned to the following three categories: <-0.5 , ≥-0.5 to ≤0.5, and >0.5.
Results: In healthy elderly, HOMA-IR ($p<0.01$), hsCRP ($p<0.001$) and TG ($p=0.02$) values tended to increase with GG categories and were highest for GG>0.5. In prediabetic participants, HOMA-IR, hsCRP and TG tended to increase with GG categories, being highest in the GG category >0.5; significant differences ($p<0.01$) between GG categories were seen in comparison to hsCRP concentrations. In diabetic participants, HOMA-IR, TG and eGFR values tended to decrease along GG category up to GG ≤0.5 but behaved inversely in the pathological GG category >0.5. Correspondingly, hsCRP values tended to increase up to GG≤0.5 and behaved inversely, after exceeding a pathological >0.5. The percentage of participants with diabetes increased from 2% in the GG category < -0.5, to 76% in the GG category > 0.5; the percentage of healthy participants fell from 85% to 7%.
Discussion: This is the first time that a direct comparison of healthy, prediabetic and diabetic subjects, assessed under identical conditions and using identical methods clearly expressed a different GG pattern. Albeit more studies are needed to confirm us, we thus present evidence that GG might be of interest for the care of diabetic patients.

pedro.medina@risch.ch

SSCC2015 Abstract 05

The Glycation Gap (GG) Value may be used as a Predictive Factor for T2DM Diagnosis: Results of the SENIORLAB Study

P Medina Escobar (1), C Grebhardt (1), Z Stanga (2), U Nydegger (1), M Risch (3), L Risch (1)

1. Labormedizinisches Zentrum Dr. Risch, Bern-Liebefeld 2. Inselspital/Universitätsspital Bern 3. Kantonsspital Graubünden, Chur

Introduction: GG reflects difference between measured level of HbA1c and the predicted HbA1c level derived from fructosamine level. Recently, GG has been used to improve good medical practice in the care of patients diagnosed with type 2 diabetes mellitus (T2DM), as it may be a useful clinical research tool for evaluating physiologic sources of variation in diabetic complications beyond glycemic control.
Subjects and Methods: Of all samples from subjectively healthy study participants 1248 subjects were included in this study. Statistical analyses were performed using MedCalc for Windows, version 15.6.1 (MedCalc Software, Ostend, Belgium). Our investigation is based on the TOPICS 4 study which evaluates HbA1c/Fasting Plasma Glucose ratios to elucidate stages of progression to diabetes. The GG was evaluated and assigned to the following three categories: <-0.5, ≥-0.5 to ≤0.5, and >0.5.
Results: We investigated GG value in subjectively healthy participants, according to the baseline classifications of pre-diabetes. We found a statistically significant trend to higher GG levels, as the cumulative risk from TOPICS 4 to develop T2DM increased ($p<0.001$). In the lowest risk group of ~10% to develop T2DM within 5.3 years, GG levels fell in the below-zero range. Already at a ~50% risk GG levels switched from below- to above–zero (~ 0.1) to exceed, at 80% risk, above GG 0.25. Finally, with TOPICS 4-cumulative risk of 100%, the GG values were the highest seen in our data base.
Discussion: Separate consideration of GG in three categories, borrowed from earlier studies to draw an error grid as used for TOPICS 4, are supported by this study: incremental values from negative to positive indicate progression from healthy to possibly diabetic metabolism. This finding presents evidence that GG comprising glycation of both short- and long-lived proteins would display current and passed glycation history.

pedro.medina@risch.ch

SSCC2015 Abstract 06

CYP3A5 genetic variation influences everolimus maintenance dose requirement in heart transplant patients

D Lesche (1); V Sigurdardottir (2); R Setoud (2); L Englberger (3); GM Fiedler (1); CR Largiadèr (1); P Mohacsi (2); J Sistonen (1)

1. University Institute of Clinical Chemistry, University Hospital (Inselspital Bern), and University of Bern, Bern, Switzerland; 2. Departments of Cardiology and 3. Cardiovascular Surgery, Swiss Cardiovascular Centre, University Hospital (Inselspital Bern), Bern, Switzerland.

Background: The immunosuppressive drug everolimus (ERL) has become an alternative to nephrotoxic calcineurin inhibitors (CNI) in transplant patients due to its antiproliferative properties and renal-sparing mode of action. However, ERL therapy is associated with a large variety of adverse events owing to its narrow therapeutic window combined with substantial variability in response. Mechanisms underlying this inter-individual variability are poorly characterized. We aimed to evaluate the effect of clinical factors and genetic variation in ERL pharmacokinetic pathways on ERL maintenance dose requirement in heart transplant (HTx) patients.
Methods: This pilot study comprised of 37 patients recruited at the Bern University Hospital who were treated with CNI-free ERL therapy for at least three months. Variants in CYP3A5, CYP3A4, CYP2C8, POR, NR1I2, and ABCB1 were genotyped and clinical data were retrieved from patient charts.
Results: Although ERL trough concentration (C0) was within the targeted range for most patients, over 30-fold variability in the dose-adjusted ERL C0 was observed. Regression analysis revealed a significant effect of the common splice-site variant CYP3A5*3 on the dose-adjusted ERL C0 ($P = 0.031$). Patients carrying the CYP3A5*1/*3 genotype required 0.02 mg/kg/day higher ERL dose compared to patients with CYP3A5*3/*3 genotype to reach the targeted C0. Additionally, ERL therapy substantially improved estimated glomerular filtration rate (28.6 ± 6.6 ml/min/1.73m2) in patients starting ERL therapy due to kidney dysfunction.
Conclusion: Our preliminary data indicates that ERL pharmacokinetics in HTx patients is highly variable and that CYP3A5 genetic variation may contribute to this variability.

dorothea.lesche@insel.ch

SSCC2015 Abstract 07

Preanalytical and analytical factors of PTH measurements before, during and after parathyroidectomy

Stampfli A (1), Thomann R (2), Schmid R, Hirschi Y (1), Walter P (1)

1. Institute of Laboratory Medicine, Solothurner Spitäler AG, 2. Department of Medicine, Bürgerspital Solothurn, 3. Department of Surgery, Bürgerspital Solothurn

Introduction: Primary hyperparathyroidism is biochemically characterised by elevated intact parathyroid hormone (PTH) or high-normal PTH in combination with high serum calcium levels. PTH is measured intraoperatively to observe its decrease during resection of parathyroid adenomas and is again routinely analysed during follow-up by an endocrinologist. Repeated observation of discordant PTH levels before and elevated PTH concentrations weeks after surgery forced us to assess analytical and pre-analytical factors, which we addressed by 1st) comparison of the results obtained in the intraoperative (PTHio) and in the routine mode (PTHr) and 2nd) a study on the stability of serum and plasma samples under variable storage conditions

Methods: Analytical assessment: The intraoperative (PTHio) and routine mode (PTHr) of the Beckman Access PTH assay were compared by parallel analysis of 50 randomly selected serum samples from daily routine.
Preanalytical conditions: Serum and plasma samples of 20 patients were analysed in the PTHr modus after 0, 4 and 8 hours h at 2-8°C. Obtained values were compared with paired t-tests.

Results: Results of the PTHio mode correlated well with PTHr (R2=0.9955) and were slightly, but statistically not significantly higher (PTHio = 1.1080 x PTHr – 0.1109; p=0.129). Serum PTH levels were higher than plasma levels (+12.1%, $p<0.05$). Contrary to plasma, serum levels decreased significantly (-6.1%, $p<0.05$) within 8 h at 2-8°C.

Discussion and conclusion: PTH results did not differ between the intraoperative and routine modus of the Beckman PTH assay. Serum PTH was found to significantly decrease within 8 h, but the observed mean difference was too small to explain the discordance in repeated PTH measurements. A strict standardisation of the storage conditions or the use of EDTA plasma would reduce the preanalytic variability. These measures will not sufficiently minimize the longitudinal. Further factors that affect the robustness should be addressed.

yasmin.hirschi@spital.so.ch

SSCC2015 Abstract 08

Between-technician variation upon Estimation of Electrophoresis Peaks of Gammopathy Patients

P Medina (1); U Nydegger (1); L Risch (1); M Risch (2)

1. Labormedizinisches Zentrum Dr. Risch, Bern-Liebefeld 2.Kantonsspital Graubünden, Chur

Background Humoral consequences of plasma cell dyscrasias, e.g. monoclonal Gammopathy Disease (mGD) Syndromes are monitored using monoclonal peak expression in serum electrophoresis, free light chain ratio and also, more recently, hevylite™. Photometric electrophoresis peak and in-between peak (« valley ») profiles are used to estimate the level of abnormal gammaglobulins produced; such approach involves between-technician variation, expressed as coefficient of variation (CV).
Methods We used capillarys 2 Flex-Piercing Sebia™, Alere GmbH. Arbitrary limits using vertical demarcation (perpendicular drop method, pdm) or tangent docking (tangent skimming method, tsm) are subject to validation aleas and are here compared. With 5 patients suffering from mGD we made 5 protein electrophoresis profiles and had them evaluated by 5 different experienced technicians. The results are expressed in g/l paraprotein in serum and the coefficient of variation (CV) is SD/mean.
Results Applying tsm/pdm measurement the profiles (CV) were yielding 2.8/4.8 g/l (9.5/14), 9.0/11.4 g/l (10.3/12), 6.2/8.3 g/l (6.4/17.2), 2.5/4.8 g/l (17.4/17.2) and 5.1/6.8 (2.6/15). As can be seen, tsm produced a range of g /l concentrations between 2.5 and 9.0 whereas using pdm such range extended to as much as from 4.8 to 11.4. With each of the 5 analyses, the estimated paraprotein concentrations were lower using tsm and revealed less variability among technicians than pdm.
Conclusions With such an important CV for both methods, one should scrutinize the degree of reliance of either way to estimate an extent of gammopathy, in the present study in favour of tsm. Other means to assess for an extent of abnormal protein, calculation of area under the curve, or planimetric approach might help to improve the significance of such technology. It was shown earlier that the pdm procedure overestimates M proteins at lower concentration ranges a drawback which might fade off in higher concentration ranges. Priority to use tsm over pdm is mandatory.

pedro.medina@risch.ch

SSCC2015 Abstract 09

THE HUMAN AUTOANTIBODY RESPONSE TO APOLIPOPROTEIN A-I IS FOCUSED ON THE C-TERMINAL HELIX: A NEW RATIONALE FOR DIAGNOSIS AND TREATMENT OF CARDIOVASCULAR DISEASE?

Nathalie Satta (1,2); Sabrina Pagano (1,2); Hubert Gaertner (3); Fabrice Cerini (3); Tiphaine Mannic (1,2); Priscila C. Teixeira (4); Paul Cutler (4); François Mach (5); Nicolas Vuilleumier (1,2) & Oliver Hartley (3).

1.Department of Human Protein Sciences , Faculty of Medicine, University of Geneva; 2.Division of Laboratory Medicine, Department of Genetics and Laboratory Medicine, Geneva University Hospitals; 3.Department of Pathology and Immunology, Faculty of Medicine, University of Geneva; 4.Pharmaceutical Sciences, Pharma Research and Early Development, F.Hoffmann-La Roche; 5 Division of Cardiology, Foundation for Medical Researches, University of Geneva..

Introduction: Cardiovascular disease (CVD) is the leading cause of death worldwide, and new approaches for both diagnosis and treatment are required. Autoantibodies directed against apolipoprotein A-I (ApoA-I), represent promising biomarkers for use in risk stratification of CVD, and may also play a direct role in pathogenesis.
Aim: we investigate new approaches for diagnostic and treatment of CVD involving blocking peptides to anti-Apo-A1 antibodies.
Methods and Results: To characterize the anti-ApoA-I autoantibody response, we measured the immunoreactivity to engineered peptides corresponding to the different alpha-helical regions of ApoA-I, using plasma from acute chest pain cohort patients known to be positive for anti-ApoA-I autoantibodies. Our results indicate that the anti-ApoA-I autoantibody response is strongly biased towards the C-terminal alpha-helix of the protein, with an optimized mimetic peptide corresponding to this part of the protein recapitulating the diagnostic accuracy for an acute ischemic coronary etiology (non-ST segment elevation myocardial infarction and unstable angina) obtainable using intact endogenous ApoA-I in immunoassay. Furthermore, the optimized mimetic peptide strongly inhibits the pathology-associated capacity of anti-ApoA-I antibodies to elicit proinflammatory cytokine release from cultured human macrophages.
Conclusions: In addition to providing a rationale for the development of new approaches for the diagnosis and therapy of CVD, our observations may contribute to the elucidation of how anti-ApoA-I autoantibodies are elicited in individuals without autoimmune disease.

nathalie.satta@unige.ch

SSCC2015 Abstract 10

Guanidinoacetate-specific effects of GAMT deficiency in developing brain cells

L Hanna-El-Daher (1) ; M Loup (1) ; H Henry (1) ; L Tenenbaum (2) ; O Braissant (1)

1. Biomédecine CHUV, Lausanne ; 2. Neurosciences Cliniques CHUV, Lausanne

GAMT deficiency is the most severe of creatine deficiency syndromes, showing creatine deficiency and guanidinoacetate accumulation in CNS. So far, the brain guanidinoacetate-specific pathomechanisms occuring under GAMT deficieny were difficult to analyze due to concomitant effects of creatine deficiency.

A model of partial GAMT deficiency was developed in 3D organotypic rat brain cell cultures by AAV2-transduced GAMT knock-down, showing no creatine deficiency but guanidinoacetate accumulation. Guanidinoacetate-specific effects were confirmed by direct guanidinoacetate exposure. Cultures were transduced, respectively GAA-exposed, at DIV0 (AAV2/GAMT MOI:1000; GAA: 10;30µM), and followed during one month (DIV8;18;28).

Mild guanidinoacetate exposure (GAMT knock-down: 9.0µM; controls: 0.9µM / direct guanidinoacetate exposure: 10 and 30µM) led to axonal hypersprouting and decrease in natural apoptosis (DIV8;18). This was paralleled by dysregulation of MAPK pathways (Erk1/2;SAPK/JNK;p38) and increased expression of GABA neurotransmission-related genes (GAD;GABAAR). GAA exposure led to non-apoptotic cell death at later stages (DIV28). All guanidinoacetate-induced effects were prevented by creatine co-treatment.

Our findings demonstrate for the first time some of the specific effects of guanidinoacetate on brain cells under GAMT deficiency, and suggest new targets for its treatment.

Olivier.Braissant@chuv.ch

SSCC2015 Abstract 11

Hemoglobin disorders diagnosis using intact protein analysis by mass spectrometry and tandem mass spectrometry

Didia Coelho Graça (1), Ralf Hartmer (2), Wolfgang Jabs (2), Photis Beris (3,4), Lorella Clerici (5), Markus Meyer (2), Olivier Lassout (5), Carsten Stoermer (2), Kaveh Samii (3), Denis Hochstrasser (1,5), Yury O. Tsybin (6), Alexander Scherl (1,5), Pierre Lescuyer (1,5)

1. Department of Human Protein Sciences, Faculty of Medicine, Geneva University, Geneva, Switzerland; 2. Bruker Daltonics, Bremen, Germany; 3. Hematology Service, Geneva University Hospitals, Geneva, Switzerland; 4. Laboratoire Unilabs Coppet, Geneva, Switzerland; 5. Department of Genetic and Laboratory Medicine, Geneva University Hospitals, Geneva, Switzerland; 6. Biomolecular Mass Spectrometry Laboratory, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Hemoglobin (Hb) is a tetrameric blood protein transporting oxygen to organs and tissues. A healthy person carries 97% of HbA (α2β2), 2,5% to 3.5% of HbA2 (α2δ2) and less than 1% of HbF (α2γ2). Hemoglobin disorders are classified in two categories: qualitative disorders if a gene defect leads to the synthesis of a Hb variant and quantitative disorders resulting from an unbalance in Hb chains synthesis. The biological diagnosis of Hb disorders is based on the combination of hematological tests, protein analysis techniques and molecular biology assays. The protein analysis step relies on the combination of several techniques such as gel electrophoresis, cation-exchange liquid chromatography, and/or capillary electrophoresis. The objective of the analysis is to identify the presence of Hb variants and to quantify Hb chains. The aim of the present work was to develop an alternative protein analysis methods based on top-down mass spectrometry (MS). First, a selected reaction monitoring (SRM) assay with electron transfer dissociation (ETD) activation was developed using a low-resolution mass spectrometer, allowing to specifically identify the most clinically significant Hb variants (i.e. HbS, HbC, HbE, HbO-Arab). Second, an intact protein quantification method was developed with the same instrument. HbA2 quantification was achieved with CVs below 4%. Finally, a high-resolution top-down ETD MS method was developed to characterize uncommon Hb variants. Selected diagnostic product ions were monitored and reported with a color code strategy for fast and reliable data interpretation by non-expert MS users. Several uncommon Hb variants, including a fusion protein (Hb Kenya), were successfully analyzed. MS methods bring more specific structural information at the protein level compared to protein analysis methods currently used for hemoglobin disorders diagnosis. In order to evaluate their potential for application in clinical laboratories, a comparative study with the standard workflow used at the Geneva University Hospitals is ongoing.

didia.coelhograca@unige.ch

SSCC2015 Abstract 12

CHARACTERIZATION OF URACIL CATABOLISM VARIABILITY IN HEALTHY VOLUNTEERS AND CANCER PATIENTS

D Kummer (1,2) ; B Rindlisbacher (1) ; S Fontana (3) ; M Joerger (4) ; J Sistonen (1) ; U Amstutz (1) and C. Largiadèr (1)

1. Institute of Clinical Chemistry, Bern University Hospital, and University of Bern, Bern, Switzerland; 2. Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland; 3. Regional Blood Transfusion Service of the Swiss Red Cross, Bern, Switzerland; 4. Department of Medical Oncology and Hematology, Cantonal Hospital St. Gallen, St. Gallen, Switzerland

Background: Pharmacokinetics of 5-fluorouracil (5-FU) is mainly determined by Uracil (U) ca-tabolism pathway. Decreased activity of the first catabolizing enzyme, dihydropyrimidine dehy-drogenase (DPD), is a major predictor of 5-FU toxicity with known risk variants in the DPD gene accounting for ~30% of toxicities. Conversely, phenotypic variability in the catabolism down-stream of DPD by dihydropyrimidinase (DHP) and β-ureidopropionase (bUP) and its potential contribution to 5-FU toxicity has not been investigated. Here, we aimed to characterize the vari-ability of metabolites and metabolic ratios of uracil catabolism and to evaluate their association with genetic variation in the DHP and bUP genes (DPYS and UPB1). Methods: Plasma concentra-tions of U, 5-FU, and their metabolites were determined by LC-MS/MS and three variants in DPYS and UPB1 previously associated with 5-FU toxicity were genotyped in 320 healthy volun-teers and 27 cancer patients. Results: In healthy volunteers, we observed lower concentrations ($P \leq 0.007$) of all metabolites as well as lower β-ureidopropionic acid/dihydrouracil ratios (UPA/UH2; $P < 0.001$) in women. Among volunteers, DPYS c.265-58T>C carriers had higher UPA/UH2 ratios ($P = 0.036$). In cancer patients, only the UH2/U ratio was significantly altered during 5-FU infusion ($P < 0.001$). Conclusions: Observed changes in endogenous metabolite rati-os during 5-FU infusion support the rate-limiting role of DPD in uracil catabolism. Higher UPA/UH2 ratios in females suggest that reduced 5-Fluoro-UH2 catabolism contributes to higher 5-FU toxicity rates in women. The association of DPYS c.265-58T>C with the UPA/UH2 ratio is in agreement with reduced 5-FU toxicity previously observed in c.265-58C carriers.

dominic.kummer@insel.ch

SSCC2015 Abstract 13

Limited Evidence for Antibody Detection in a Polyreaktive Serum - a rare case report

D Buhl (1); J Sinz (2); A Duss (2)

1. Kantonsspital Luzern, Institut für Klinsche Chemie und Immunologie; 2. Kantonsspital Luzern, Nephrologie

24 year-old man urgently hospitalised with haemoptosis,
History: Congenital aortic stenosis and aneurysm of the ascending aorta; replacement and reconstruction with a mechanical valve and graft.
Suspected diagnosis: Goodpasture syndrome referring to clinically evident pulmonary haemorrhage without renal complictions in this patient.
Differential diagnosis: Pulmonary and/or renal manifestations can be encountered in various conditions, such as antineutrophilic cytoplasmic antibody (ANCA)–positive vasculitis and other autoimmune disorders. As a consequence, the identification of anti–glomerular basement membrane antibodies (anti-GBM) in the patient's serum is of paramount importance in the diagnosis of Goodpasture disease.
Confirmation of the diagnosis of this rare autoimmune disorder in our patient occurred through detection of circulating antibodies against an antigen normally present in the glomerular and alveolar basement membrane. The target antigen is the alpha-3 chain of type IV collagen.
Results: anti-GBM 200U/ml, reference value <7 U/ml.
Notable was that PR3- und MPO were also highly elevated. Because of this mismatch an analysis was made with another producer of reagents and all the results were negative, consequently the first results were false positive and the therapy based on these results inadequate.
Conclusion: Polyspecific serum appears to have reacted with a component used by the first producer, probably against the blocking agent. To prevent nonspecific binding of the antibodies in the sampling preparation the remaining binding surface must be blocked before using antibodies to detect proteins that have been dotted or transferred to a membrane. Otherwise, the antibodies or other detection reagents will bind to any remaining sites that initially served to immobilise the proteins of interest. In principle, any protein that does not have binding affinity for the target or probe components in the assay can be used for blocking and it is imporatant to do so as every manufacturer uses another mixture. It is a very rare instance that this usual approach of the assay's test performance causes an unwanted side effect, i.e. triggering a reaction instead of preventing it. Possibly the antibodies of the patient were triggered by the device of the implanted graft/ valve normally coated as well with a blocking substance.

daniela.buhl@luks.ch

SSCC2015 Abstract 14

Importance of an embedded biobank for laboratory medicine practice

Géraldine Poulain(1);Maria Carmen Vazquez(1); ; Julien Virzy (1); Alexandre Bulla (1); Pierre Lescuyer (1)

(1) Hôpitaux Universitaires de Genève

Biobanks are dedicated structures for the processing and storage of biological samples. They are now recognized as essential tools in biomedical research to guarantee the quality of samples collected for clinical studies. At the University Hospitals of Geneva (HUG), the Laboratory Medicine Service (SML) has developed since 1982 its own biobank for the management of biological fluids. The SML-Biobank plays a key role in several important aspects of laboratory medicine activity. First, the SML-Biobank is storing samples coming from clinical practice destined to delayed complementary analyses. Second, the SML-Biobank is managing samples from more than 30 clinical studies, including cohort studies and multicenter studies. In this context, the SML-Biobank ensures quality of collected samples by controlling preanalytical conditions and by the standardization of operating procedures from collection to delivery for laboratory analyses. Third, the SML-Biobank is providing biological samples to laboratories of the SML for the development and validation of analytical methods (e.g. reference intervals establishment). Fourth, the SML-Biobank carries independent research projects on preanalytical conditions and biospecimen processing in collaboration with international groups. The results should allow developing improved procedures directly applicable to the current laboratory practice.
In conclusion, the availability of a dedicated structure within the SML for biological sample processing provides a diversity of services for clinicians, researchers and laboratory professionals with an expertise that allows maximizing the quality of the collected samples and, consequently of subsequent laboratory data.

geraldine.poulain@hcuge.ch

SSCC2015 Abstract 15

Therapeutic Drug Monitoring of newer anti-epileptics in saliva: an attractive, minimally invasive approach for the management of epilepsy treatment and prevention

C Stampfli (1) ; J Novy (2) ; P André (1) ; LE Rothuizen (1) ; C Csajka (1) ; T Buclin (1) ; AO Rossetti (2) ; LA Decosterd (1)

1. Laboratory and Division of Clinical Pharmacology, Service of Biomedicine. 2. Department of Clinical Neurosciences, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Switzerland

Optimization of the pharmacological treatment of epilepsy possibly benefits from therapeutic drug monitoring (TDM) based on total (Ptotal), more rarely free (Pfree) – unbound– plasma concentrations of anti-epileptic drugs (AEDs). Simple, less invasive monitoring methods are needed to facilitate the monitoring of antiepileptic treatment in special populations (pediatrics, geriatrics, behavioural disturbances). Saliva (S) may represent an attractive surrogate fluid for TDM of AEDs, because its drug concentration is thought to reflect the unbound, pharmacologically active fraction. An assay by ultra-performance liquid chromatography tandem-mass spectrometry has been developed for the multiplex analysis of the AEDs lacosamide (LCM), lamotrigine (LTG), levetiracetam (LEV), topiramate (TPM) and zonisamide (ZNS) in saliva. This technique was applied to 39 paired plasma-saliva samples collected simultaneously in patients, allowing for comparison between, S, Ptotal and Pfree levels.
Fair correlations were observed between S and either Ptotal or Pfree concentrations ($r>0.85$ and $r>0.83$, respectively). The S/Ptotal ratios were 1.42, 0.48±0.10, 1.74±0.55, 1.27±0.51, 0.7±0.18, for LCM, LTG, LEV, TPM and ZNS, respectively. S/Pfree ratios were 2.2, 1.83±0.42, 1.99±0.51, 2.75±1.08 and 1.51±0.19. S/Ptotal values compare well with literature for LTG, but were overall higher than reported for LCM, LEV and TPM (no data for ZNS). The S/Ptotal and S/Pfree ratios showed large inter-patient variability (CV> 40% for TPM) without influence from AEDs concentrations in plasma nor from delay between last intake and sampling. The S/Pfree ratios were above the expected value of 1, suggesting active drug secretion into saliva or pH-driven gradients, possibly modulated by saliva production rate. Thus, while clearly correlated, S levels were 1.5 to 2.8-fold higher than corresponding Pfree. Variability in S/P ratios might limit the accuracy of salivary monitoring. This investigation should be extended with population pharmacokinetic analyses, allowing for extrapolation of AEDs plasma concentrations based on salivary determination and thorough assessment of its potential usefulness for TDM.

Camille.Stampfli@etu.unige.ch

SSCC2015 Abstract 16

17-OH Progesterone for congenital adrenal hyperplasia by immunoassay and LC-MS: a case report study

SJ Bruce (1); D Werner (1); F Rey (1); PA Binz (1); and H Henry (1)

Laboratoire de chimie clinique, centre hospitalier universitaire vaudois (CHUV)

Congenital adrenal hyperplasia (CAH) is one of the most common inherited metabolic disorders (1/10'000 births). It comprises a group of autosomal recessive disorders caused by various deficiencies of steroidogenic enzymes involved in cortisol synthesis. More than 90 % of cases are caused by P450c21 deficiency, producing high blood levels of 17-OH-Progesterone (17-OHP4). A portion of the remaining cases can be attributed to P450c11□ deficiency which generates high levels of 11-Deoxycortisol (S).

In basal conditions, a five month old female patient was found to have an elevated 17-OHP4 of 36.1 nmol/l, determined by the Cisbio radioimmunoassay (OHP-CT) and a cortisol (F) of 198 nmol/l determined by ECLIA on a cobas® e602 system (Roche Diagnostics). The 17-OHP4 and F both remained at the same levels after administration of Synacthen (86 µg iv). When the same samples were analysed using an in-house LC-MS method for steroid profiling, we observed lower 17-OHP4 levels of 5.1 (T = 0 min), 6.0 (T = 30 min), and 5.7 (T = 60 min) nmol/l with decreased F levels of 54.0 (T = 0 min), 58.2 (T = 30 min) and 48.7 (T = 60 min) nmol/l. Interestingly, within the LC-MS steroid profile, the [S] was observed to be elevated (140.0, 174.0 and 146.3 nmol/l for the same respective time points) suggesting a P450c11□ deficiency rather than a P450c21.

Our results show 17-OHP4 analysed by the OHP-CT method can be an indirect marker for P450c11□□deficient CAH due to cross reactivity, seemingly in this example with S. Additionally, using highly specific LC-MS methods targeting only 17-OHP4 in cases of CAH would only determine a P450c21 deficiency, potentially leading to misdiagnoses without further S or F measurements.

In conclusion the increase in specificity of LC-MS methods requires further criteria regarding clinical diagnoses founded on some non specific immunoassay based methods. We recommend analysing steroids via a targeted panel (e.g. for CAH at least 17-OH-P4, F and S) or a profile style approach rather than via isolated metabolites.

Steve.Bruce@chuv.ch

SSCC2015 Abstract 17

DEVELOPMENT OF A LC-MS/MS METHOD FOR THE QUANTITATION OF PURINE AND PYRIMIDINE METABOLITES IN HUMAN URINE

A Cremonesi (1); N Perkins (1); B. Fowler (2); J. Häberle (2); M. Hersberger (1)

1. Abteilung Klinische Chemie und Biochemie, Universitäts-Kinderspital Zürich; 2. Abteilung Stoffwechselkrankheiten, Universitäts-Kinderspital Zürich

Purines (Pu) and Pyrimidines (Py) are biologically ubiquitous compounds involved in a multitude of biochemical processes: they are the building blocks of DNA and RNA, participate in signal transduction, drive many energy-requiring reactions and are the structural component of many coenzymes. Their physiological relevance is underlined by the fact that nearly all cells can synthesize them and, if errors in their metabolism manifest, if not lethal, can often lead to very debilitating diseases. Typical clinical manifestations caused by genetic aberration in the metabolism of Pu/Py could be neurological, immunological and renal impairments as well as cancer.

To date, more than 30 enzyme defects in the metabolism of Pu/Py have been characterized. It is therefore crucial to accurately quantify these metabolites for diagnostics as well as therapeutic purposes. In this study we present the development and validation of a LC-MS/MS method for the quantification of 32 clinically relevant Pu/Py metabolites in human urine. The resulting validated LC-MS/MS method has then been used to screen clinical samples for these rare diseases and has already enabled to identify patients with ADSL- and APRT deficiency, Lesch-Nyhan Syndrome and MNGIE (Mitochondrial Neurogastrointestinal Encephalomyopathy). For some of them, data will be presented showing the benefits of an accurate diagnosis, which allowed to start an appropriate therapy leading to a normalization of the affected metabolite concentrations as well as an improvement in the clinical outcome.

alessio.cremonesi@kispi.uzh.ch

SSCC2015 Abstract 18

Performance characteristics of HemoCue® Glucose 201+ vs Bayer Contour® XT

A Stampfli (1); G Rudofsky (2); Y Hirschi (1); P Walter (1)

1. Institute of Laboratory Medicine, Solothurn Hospitals; 2. Department of Internal Medicine, Kantonsspital Olten

Introduction: Near patient testing of blood glucose plays an increasingly important role in various settings in the hospital where low turnaround times and lean process management are critical. The use of blood glucose meters in clinical practice requires adaequate reliability for patient monitoring and diagnosis. HemoCue has been mentioned by the Swiss Society of Gynaecology and Geburtshilfe (SGGG) as well as by the Swiss Society of Endocrinology and Diabetology (SGED) to be the only portable device with « laboratory accuracy ». At our hospital, the HemoCue® Glucose 201+ was proposed for the monitoring of hypoglycemic newborns, especially at levels < 2.00 mmol/l. We evaluated performance characteristics of Bayer Contour XT (CX) and HemoCue Glucose 201+ (HC) in comparison to Beckman UniCel DxC (DxC).

Methods: Interday imprecision was calculated from 10 repeated measurements of low and normal controls (HC) and 10 low controls (CX). Agreement with UniCel DxC was calculated from 20 parallel glucose measurements of heparinised and partly glycolysed capillary samples on HC, CX and DxC.

Results and discussion: Interday precision of CX (VC=3.3% at 1.47 mmol/l) was slightly superior to HC (2.7% at 6.33 mmol/l resp. 4.8% at 2.77 mmol/l) at low concentrations and close to DxC (VC=2.7% at 2.96 mmol/l, 1.3% at 6.44 mmol/l). Both blood glucose monitors were in excellent agreement with the routine method in the central laboratory.

Conclusion: CX and HC both met the precision requirements postulated by the SGGG (< 3%). CX seems to be at least equally qualified for the monitoring of hypoglycemic newborns and was slightly more precise in the low range. With regard to the diagnosis of gestational diabetes, limitations arise only from the use of capillary samples, but not from the analytical performance of the two blood glucose meters.

yasmin.hirschi@spital.so.ch

SSCC2015 Abstract 19

Suitability of a novel urine collection tube for microbial testing

D Leichtfried (1); J Böttcher (2)

Greiner Bio-One; Kremsmünster

Background: Reliable urine testing results are of utmost importance for diagnosis, monitoring and therapy of patients with urinary tract diseases. Particularly with regard to delays in delivery to the laboratory, an increase in microbial counts due to a missing preservative or too high transport temperatures may lead to false results. The VACUETTE® Urine CCM Tube contains a novel preservative stabilizing urine samples at room temperatures (20-25°C) for up to 48 hours in order to offer a urine tube for collection, transport, storage and urine culture in the laboratory.
Materials: A study was designed to evaluate urine samples (total n= 170, partly spiked) from clinically inconspicuous as well as conspicuous (nitrite and leucocyte positive with dipstick urinalysis) urine specimens. Those samples were collected in the new VACUETTE® Urine CCM Tube. The microbiological cultures for bacterial counting were generated at the same day within 2 h after sample tube filling, after 24 h and 48 h. All specimens were stored at room temperature (20 – 25°C) between the sampling time points. The samples were tested regarding stability of the following pathogenic organisms: Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Proteus mirabilis, Candida albicans.
Results: According to the performance criteria, that the starting values do not differ significantly from the reference tube and the results after storage for 48 hours at room temperature do not differ significantly (one log step) from the 0-2 hour results, the stability of the pathogens could be demonstrated without significant differences in comparison to the reference tube.
Conclusion: On the basis of these results, the suitability of the VACUETTE® Urine CCM Tubes for microbial testing has been demonstrated. This tube stabilizes the tested organisms being responsible for urinary tract infections for 48h at room temperature. The VACUETTE® Urine CCM tube is a urine sampling and transport system suitable for microbiologic diagnostics and is found to be useful in improving preanalytics in urine culture testing.

sirid.griebenow@gbo.com

SSCC2015 Abstract 20

Free light chain analysis in cerebro-spinal fluid

W Fierz (1); B Walz (2)

1. labormedizinisches zentrum Dr Risch, Schaan; 2. Kantonsspital Graubünden, Chur

Background
45 years ago already, it has been reported that in the cerebro-spinal fluid (CSF) of patients with multiple sclerosis (MS) markedly higher kappa/lambda ratios of light chains are observed, this in contrast to findings in serum and CSF of patients with other CNS diseases like neuro-syphilis. Later work has confirmed these findings and extended it to free light chains (FLC). Furthermore, kappa FLC in CSF already appear in early (mono-symptomatic) MS before development of oligoclonal bands and their presence correlate well with changes in MRI.
Objective
The study reports on FLC analysis in CSF and serum in a general laboratory. All specimens referred to the lab for analysis of oligoclonal bands were subjected to FLC analysis. The aim was to define cut-offs for free kappa synthesis in CSF in relation to blood brain barrier (BBB) disturbance.
Methods
Kappa and lambda FLC were measured in addition to albumin, IgG, IgA, and IgM (Binding Site) in CSF and serum on a SPAPLUS device. CSF/serum ratios, intrathecal synthesis rates and kappa/lambda ratios were calculated and results displayed in form of hyperbolic “Reiber”-type diagrams.
Results
Based on BBB barrier measurements and oligoclonal band findings a hyperbolic cut-off for FLC kappa CSF/serum ratios was defined that well separated cases with signs of intrathecal immunoglobulin synthesis depending on BBB disturbance. This cut-off exactly matches the cut-off recently published in a multicentre study describing kappa FLC as a diagnostic biomarker in MS and clinically isolated syndrome. Various cases with individual kappa/lambda signatures were compared with clinical findings.
Conclusions
FLC analysis in CSF and serum is a valuable addition to the current analysis of albumin, immunoglobulins and oligoclonal bands and might be useful to detect early MS cases and to estimate their prognosis.

walter.fierz@risch.ch

SSCC2015 Abstract 21

Comprehensive multidimensional reporting of HLC and FLC results in gammopathy follow-up

W Fierz (1); B Walz (2)

1. laboratorium Dr Risch, Schaan; 2. Kantonsspital Graubünden, Chur

Background
A new dimension in evaluating the course of clonal gammopathies has been reached with the measurement of heavy/light chains (HLC), i.e. the separate quantification of IgG□, IgG□, IgA□, IgA□, IgM□ und IgM□, in addition to total free light chain (FLC) evaluation. This allows a better quantification of the clonal immunoglobulins than protein electrophoresis. In addition, the valuation of so called pair-suppression, i.e. suppression of the non-involved □/□ partner, and suppression of non-involved immunoglobulins help to detect the beginning of bone marrow suppression by the developing multiple myeloma (MM). These parameters are of higher prognostic value than other criteria.
Objective
The challenge of reporting electrophoresis, immunofixation, HLC and FLC results to the clinician is to create an easy to read, comprehensive cumulative overview over the various results. A graphical display rather than a list of numerical results had to be developed.
Methods
HLC and FLC (Binding Site) are measured on a SPAPLUS device in addition to electrophoresis and immunofixation. The time course of IgG□, IgG□, IgA□, IgA□, IgM□ and IgM□ are displayed in 2-dimensional multicolour diagrams with FLC values being indicated by the weights of the symbols representing individual time points of measurements. To complete the picture, electrophoresis curves with values of albumin, □1, □2, □1, □2 and □ globulins as well as an estimation of protein quantity in the m-gradient, and the graph of the most recent immunofixation are included. On top, a summary of the results and an interpretation are given.
Results and Conclusions
A comprehensive multidimensional view of multiple results and their time-course can be displayed on an easy to read single A4-page. This approach is of importance for staging and the evaluation of the prognostic risk of patients with gammopathies. Positive feedbacks from oncologists confirmed that the approach is of practical value.

walter.fierz@risch.ch

SSCC2015 Abstract 22

UHPLC high-resolution Mass Spectrometry for the evaluation of steroid perturbation: focus on feature identification

D Tonoli (1,2); P Strajhar (2,3); J Boccard (1); GM Randazzo (1); F Jeanneret (1,2); D Kratschmar (3); D Hochstrasser (4); A Odermatt (2,3); S Rudaz (1,2)

1. University of Geneva, University of Lausanne; 2. Swiss Centre for Applied Human Toxicology; 3. University of Basel; 4. Geneva University Hospitals

Alterations in steroidogenesis are associated with cancer, metabolic syndrome, cardiovascular diseases, immune disorders, and developmental dysfunctions. Therefore, the identification of endocrine disruptors and the understanding of the underlying mechanisms are of great interest. The development of efficient methods to screen environmental chemicals, drugs, and cosmetics to evaluate their impact on human steroidogenesis is also pursued by regulatory agencies.
UHPLC coupled to high-resolution MS has been proven as a useful tool to measure steroid alterations. However, the identification of unanticipated steroids remains tedious. Therefore, the combined use of retention time prediction by dedicated software and biological rational is often mandatory. To highlight some issues regarding systematic identification of steroid candidates the putative endocrine disruptor triclocarban was studied. H295R cells were incubated with increasing concentrations of triclocarban for 48 h according to the OECD guidelines. Steroids were measured in the culture supernatants.
After data acquisition and extraction, chemically-driven feature selection with database matching using a home-made database followed by a Consensus OPLS-DA analysis were performed to extract simultaneously data acquired in positive and negative mode. These steps led to a selection of the 30 most important steroids perturbed by triclocarban; among them, 14 (47%) were straightforwardly identified with authentic chemical standard comparison and quantified. Afterwards, two concomitant strategies were applied to improve the throughput of identification; i) the results obtained based on well-known endocrine disruptors (positive controls) helped to reduce the number of possible identifications by considering selectively sub-pathways of the steroidogenesis, and ii) the modelization of retention times was also pursued to decrease candidates possible identifications. Using this analytical workflow, it was possible to determine four additional steroids that were not reported so far in H295R cell cultures.

david.tonoli@unige.ch

SSCC2015 Abstract 23

Metabolomic analysis of immature and mature 3-dimensional (3D) rat brain cell cultures exposed to the herbicide paraquat

F Jeanneret (1,2), D Tonoli (1,2), J Sandström von Tobel (2,3), F Monnet-Tschudi (2,3), J Boccard (1), S Rudaz (1,2)

1. University of Geneva, University of Lausanne 2. Swiss Centre for Applied Human Toxicology 3. University of Lausanne

Epidemiological studies have linked exposure to Paraquat (PQ), a neurotoxic herbicide, with an increased risk of developing Parkinson's disease. 3D rat brain cell cultures exposed to PQ during an early developmental stage displayed delayed neuroinflammation and neurodegeneration, as previously assessed using gene expression, proteomic, and morphological analyses. The current metabolomic study aims at investing changes in metabolic profiles upon PQ-exposure of 3D-cultures at two different maturation stages. Particularly monitored are amino acids (AA) and neurotransmitters (NT), such as glutamate and γ-amino butyric acid, which are crucial metabolite classes both for neuronal development and function.
3D cultures were exposed for ten days to two non-cytotoxic concentrations of PQ, at an immature (day in vitro (DIV) 5-15) and a mature (DIV 25-35) stage. After metabolite extraction in acidic conditions, the samples were analyzed by UHPLC, in reverse phase (RP) and in hydrophilic interaction liquid chromatography (HILIC) modes, coupled to high-resolution MS.
Both chromatographic modes were investigated to obtain a large range of metabolite polarities. Multivariate data analysis of RP mode displayed a perturbed metabolic profile depending on the PQ concentration and on the maturation stage. Analysis of the HILIC acquisition displayed different patterns in mature and immature cultures, but no differences due to PQ exposure. Some metabolites were identified as AA and NT by comparison with authentic chemical standards, such as taurine or choline.
This metabolomic approach characterized two distinct patterns of metabolites after PQ exposure, depending on the developmental stage of the 3D cultures; this could indicate differences in the defense mechanisms due to brain cell maturity, as suggested by complementary analyses. Future work will be done on sample preparation to improve both metabolite extraction and identification, with the aim to better understand the events leading to toxicant-induced neuroinflammation and neurodegeneration.

fabienne.jeanneret@unige.ch

SSCC2015 Abstract 24

Iron disorders: Hepcidin might be the key to differential diagnosis

C Steuer, S Brunner-Agten, L Bernasconi, M Kaeslin-Meyer, AR Huber

Kantonsspital Aarau, Center of Laboratory Medicine

Hepcidin (Hep) is the key regulator for iron homeostasis in the human body and is mainly produced in the liver. Hep is a 25 amino acid containing well-structured β-hairpin peptide molecule. The molecular target of Hep is ferroportin, a transmembrane protein which is the only known iron exporter in vertebrates. Hep binds to its receptor inducing its degradation in the basolateral membrane of the intestine, macrophage and hepatic cells. Hep is feedback regulated by iron concentrations in plasma and the liver and by erythropoietic iron demand. The determination of Hep concentrations is essential for the understanding of iron metabolism in various diseases. Recent studies demonstrated considerable evidence that Hep can be used as a diagnostic marker for disorders like anemia of chronic inflammation, chronic kidney disease, hereditary hemochromatosis, thalassemias, various liver diseases and malignancy. Furthermore, Hep might be also of prognostic value in the prediction of erythropoietin response and to guide treatment with erythropoietin and intravenous iron. Herein we describe an isotope dilution LC-MS/MS assay for the accurate detection of Hep in human serum samples. Our data confirm the excellent performance of Hep for the discrimination between iron deficiency anemia (IDA) and anemia of chronic diseases (ACD). The corresponding positive predictive value of Hep is 82% (Ferritin 59%, TSAT 56%). In addition we could show that ferritin is the primary correlate of Hep. Together, Hep will be helpful in differentiation of various anemias, especially when combinations of IDA and ACD are present.

christian.steuer@ksa.ch

SSCC2015 Abstract 25

Inhibition screening method of UGTs using the cocktail approach

S Romand (1); J Gradinaru (1); PA Carrupt (1); D Spaggiari (1); S Rudaz (1)

1. School of pharmaceutical sciences, University of Geneva, University of Lausanne, Boulevard d'Yvoy 20, 1211 Geneva 4, Switzerland

During drug development process, early determination of ADME behaviour (absorption, distribution, metabolism and excretion) is needed to avoid rejection of new chemical entities. UDP-glucuronosyltransferases (UGT) are the main enzymes involved in phase II metabolism of many endogenous (e.g., bilirubine, hydrosteroids) and exogenous compounds including drugs (e.g., nonsteroidal anti-inflammatory drugs, opioids). The UGT superfamily is comprised of 2 families and 3 subfamilies with isoforms exhibiting distinct but overlapping substrate and inhibitor selectivities. In vitro systems are commonly used to predict drug-drug interactions (DDI), but they require the use of specific probes. Using recombinant UGTs, a cocktail of specific substrates was selected for measuring relative activity of the 10 main hepatic UGTs. This cocktail was incubated in human liver microsomes and then the substrates and their glucuronides were analysed by HPLC-ESI-TOF/MS allowing the metabolite to substrate area ratio determination. Various inhibitors were incubated with the cocktail or morphine (individual substrate highly glucuronidated by UGT 2B7). UGT activities were reduced in accordance with literature, thereby confirming the ability of the cocktail to evaluate potential DDI. The reduction of morphine glucuronidation activities was similar to that observed for UGT 2B7 probe substrate. Therefore, the developed cocktail approach proved to be reliable for the characterization of the relative activities of 10 hepatic UGTs and rapidly identifying potential inhibitors.

serge.rudaz@unige.ch

SSCC2015 Abstract 26

Polymorphisms in MIR27A associated with early-onset toxicity in fluoropyrimidine-based chemotherapy

U Amstutz (1), SM Offer g(2), J Sistonen (1), M Joerer (3), RB Diasio (2), CR Largiadèr (1)

1. University Institute of Clinical Chemistry, Inselspital Bern University Hospital and University of Bern, Switzerland; 2. Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic Cancer Center, Rochester, MN, USA; 3. Department of Medical Oncol- ogy, Cantonal Hospital St. Gallen, St. Gallen, Switzerland

Background: The microRNA miR-27a was recently shown to directly regulate dihydropyrimidine dehydrogenase (DPD), the key enzyme in fluoropyrimidine catabolism. A common polymorphism (rs895819) in the miR-27a genomic region (MIR27A) was associated with reduced DPD activity in healthy volunteers. Here, we assessed the association of MIR27A variants with early-onset FP toxicity in cancer patients.

Methods: MIR27A was sequenced in 514 cancer patients receiving fluoropyrimidine-based chemotherapy. Associations of MIR27A polymorphisms with fluoropyrimidine toxicity in the first two chemotherapy cycles were assessed in the context of known risk variants in the DPD gene (DPYD) and other covariates associated with toxicity.

Results: An association of rs895819 with early-onset fluoropyrimidine toxicity was observed, which was strongly dependent on DPYD risk variant carrier status (interaction p=0.0025). In patients carrying DPYD risk variants, rs895819G was associated with a strongly increased toxicity risk (OR: 7.6; 95% CI: 1.7-34.7; p=0.0085) with 71% (12 of 17) of carriers of both rs895819G and a DPYD risk variant experiencing severe toxicity. Conversely, an opposite effect was observed in patients without DPYD risk variants (OR: 0.62; 95%CI: 0.43-0.9; p=0.012).

Conclusions: Our results indicate a clinically relevant role of miR-27a for fluoropyrimidine toxicity risk stratification in carriers of DPYD risk variants and that direct suppression of DPD by miR-27a may predominate in DPYD risk variant carriers with reduced DPD activity. In patients with normal DPD activity, this effect may be outweighed by miR-27a regulation of additional targets involved in drug transport or apoptosis regulation, explaining the negative association with fluoropyrimidine toxicity.

ursula.amstutz@insel.ch

SSCC2015 Abstract 27

Endocannabinoid and steroid profiles in blood and seminal fluid as putative new biomarkers of male fertility

F. Zufferey (1), N. Donzé (1), S. Adamski (2), F. Jeanneret (3,4,5), D. Tonoli (3,4,5), J. Boccard (3), J. Escoffier (6), S. Nef (6) , S. Rudaz (3), N. Schneider (2), MF. Rossier (1,4)

(1) Service de Chimie clinique & Toxicologie, ICH, Hôpital du Valais (2) Unité de Stérilité, Service de Gynécologie et Obstétrique, CHVR, Hôpital du Valais (3) Section des Sciences Pharmaceutiques, Universités de Genève et Lausanne (4) Département de Science des Protéines Humaines, CMU, Université de Genève (5) Swiss Centre for Applied Human Toxicology, Suisse (6) Département de Médecine génétique et Développement, CMU, Université de Genève

Sperm examination is crucial in the evaluation of male fertility. While the methodology for analyzing sperm quantity and quality is well established, the biochemical composition of the seminal fluid is still poorly investigated. Given the role of androgen steroids in spermatogenesis and in the function of sexual accessory organs, the analysis of the profile of these hormones in the seminal fluid could bring some additional information about the chance of success in medically assisted procreation. Moreover, endocannabinoids (ECB), because of their action on human sperm and Leydig cells, have been recently considered as key players in male reproduction.
The main purpose of our project is to identify new biochemical markers of male fertility, by analyzing relationships between specific steroids, ECB compounds, spermogram parameters and fertility outcomes of patients addressed to the clinic of Fertility in the Hospital of Sion. Multivariate data analysis was performed to characterize correlations between these parameters, retrospectively in a cohort of 150 patients with spermograms only, and prospectively in 7 patients with, in addition, an extended blood and semen analysis of steroids and ECB by LC-MS/MS. Urines from the later patients were also analyzed by a steroidomic untargeted approach to extend the number of screened steroids to putatively detect additional markers.
Preliminary analyses revealed for example a significant correlation between blood N-arachidonoyl-dopamine (NADA) and sperm velocity (Rs=0.818, p=0.024). The functional relationship is currently confirmed in vitro by exposing freshly prepared sperm cells to various NADA concentrations. The distribution of each patient according to PCA scores is also compared to their time-to-pregnancy, a final index of fertility.
Our pragmatic and combined approach should help us to discover and functionally confirm new biochemical markers of male fertility, helpful not only in fertility clinics, but also to monitor changes in blood and seminal fluid after exposure to environmental endocrine disruptors.

fanny.zufferey@hopitalvs.ch

SSCC2015 Abstract 28

High Participation Rate in a Systematic, Hospital-Based Biobank with Broad Consent

Christine Currat (1); Murielle Bochud (2); Laurence Chapatte (1); Cindy Roth (1); Vincent Mooser (1)

1. Service de Biomédecine; 2; Institut de Médecine Sociale et Préventive, CHUV et UNIL, Zurich

BACKGROUND
Large, high-quality, secured, standardized biobanks are essential for the discovery and development of novel biomarkers, including clinical chemistry and genomic markers of diseases. Little is known about the interest of hospitalized patients to participate in such biobanks.

METHODS
Within the framework of the Lausanne University Hospital Institutional Biobank (BIL), CHUV inpatients are systematically invited to grant researchers access to their clinical data and to donate blood for future clinical chemistry (on plasma) and genomic analyses (on DNA extracted from buffycoats). Additionally, participants who sign this general consent are offered the options to be re-contacted in case of incidental findings and to receive an electronic newsletter. Multivariable logistic regression analysis was used to identify personal factors associated with willingness to participate in BIL and with interest in these options. Analyses were restricted to the initial 11099 invited patients for whom full dataset was available, and were stratified by age.

RESULTS
Overall participation rate was 82.4% (9141/11099) and was higher in the < 64-year old group (odds ratio [OR] 1.70; 95% CI 1.53 to 1.90). In the ≥ 64-year old group, participation was lower among women (OR 0.77; 95% CI 0.68 to 0.89), among non-Swiss citizens (OR 0.66; 95% CI 0.55 to 0.79) and those with emergency admissions (OR 0.59; 95% CI 0.51 to 0.69). A total of 8576 (93.8%) and 3020 (33.0%) participants were willing to be re-contacted for incidental findings and to receive the newsletter, respectively.

CONCLUSIONS
A large proportion of patients are willing to actively participate in this hospital-based biobank. Hospitals adopting broad consent represent an efficient setting to recruit participants into studies designed to identify and validate novel markers of diseases.

Christine.currat@chuv.ch

SSCC2015 Abstract 29

Extensive CYP450-based cocktail approach for toxicological in vitro inhibition screening: example for pesticides

Dany Spaggiari (1); Rossana Canavesi (2); Youssef Daali (3); Serge Rudaz (1)

1. Université de Genève; 2. Università di Novara; 3. Hôpitaux universitaires de Genève

The exposure to environmental toxicants could strongly affect the metabolic activity of human cytochrome P450 (CYPs), i.e., the major phase I enzymes involved in the oxidative biotransformation of drugs, xenobiotics and endogenous compounds. As a consequence, the risk of interaction with current medication could be increased, modifying the pharmacokinetic of the drug and thus the clinical outcomes. Because toxic agents could be not administrated in vivo, in vitro approaches are recommended in the 3Rs principle to evaluate and/or anticipate the impact on CYPs activities.
An extensive cocktail mixture was elaborated and validated for in vitro CYPs inhibition studies (IC50 assay). The cocktail comprised several specific probe substrates to simultaneously assess the activity of major CYPs involved in drugs biotransformation, namely 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 2J2 and 3A subfamily. The high selectivity and sensitivity of the developed UHPLC-MS/MS method were critical for the success of this cocktail approach, whose main advantages are: (i) the use 11 probe substrates with minimized interactions, (ii) low HLM concentration, (iii) fast incubation (5 min) and (iv) fast analysis (7 min). By using CYP specific inhibitors, the cocktail approach was successfully validated by comparing the obtained IC50 values with those generated with the classical methodology. Accordingly, reliable inhibition values could be generated about 10 times faster with less amount of HLM, which is required for screening strategies. After cocktail incubation in optimized conditions and estimation of the “control” CYP-dependent biological activity, the system was individually exposed to pesticides to estimate the nature and the impact of the toxicological interference on CYP activities. A mixture of low-dosed pesticides was also incubated and an additive inhibitory effect was observed.
Lastly, this cocktail approach could be a potential tool to facilitate the risk assessment of a high number of chemicals in a relatively short time.

serge.rudaz@unige.ch

SSCC2015 Abstract 30

Human Whole Blood storage conditions for Genomic DNA extraction

A Bulla (1), BD Witt (2), W Ammerlaan (2), F Betsou (2), P Lescuyer (1)

1.Sérothèque Centrale, Département de Médecine Génétique et de Laboratoire, Hôpitaux Universitaires de Genève, Geneva, Switzerland ; 2. Integrated Biobank of Luxembourg, Luxembourg

Optimal storage conditions and standardization of extraction procedures are critical to obtain genomic DNA with sufficient quantity and quality, which allows the broadcast range of downstream molecular biology applications. The objective of the present study was therefore to evaluate the impact of temperature and time of storage of human whole blood on genomic DNA extraction. We compared the yield and quality of DNA extracted from human whole blood samples exposed to seventeen different combinations of storage time (0, 24h, 48h, 7 and 14 days, 6 and 12 months) and temperature (RT°, 4°, -20° and -80°C). We also assessed the protective effect of Biomatrica DNAgard, a DNA stabilizing agent, added just before toweling to the whole blood samples stored at -20° and -80°. At the end of the each storage period, genomic DNA extractions were performed on Autopure LS and QIacube, two automated robotic workstations (QIAgen). Data obtained by both QIAcube and Autopure LS extraction systems display similar relative extraction yield patterns. DNA concentrations measured by UV spectrophotometry were used to calculate DNA extraction yields. DNA integrity was controlled by agarose gel electrophoresis and long-range PCR. Results showed that the DNA extraction yield decreased rapidly from T0 to day 14 for samples stored RT. A much lower rate of decrease was observed for storage at +4°C. A relatively stable DNA extraction yield during the 12 months of samples storage was observed at -80°C while it dropped at -20°C. Interestingly, adding DNAgard solution before thawing samples stored at -20° and -80°C maintained the relative extraction yield above 90% over one year of storage. While some storage conditions have a significant impact on the DNA extraction yield, little or no effect on DNA integrity was observed. Therefore, this study will support evidence-based guidelines for the storage of blood samples before DNA extraction.

alexandre.bulla@hcuge.ch

SSCC2015 Abstract 31

Serum free light chains: If automated antigen excess detection fails, the laboratories fail as well

C Schild (1); F Egger (1); JM Nuoffer (1)

1. University Institute of Clinical Chemistry, Inselspital – Bern University Hospital, Bern, Switzerland

Introduction:
Measurement of serum free light chains (sFLC) is challenging due to the individuality of patients monoclonal sFLCs and analytical problems like antigen excess, non-linearity, polymerization of sFLCs and lot-to-lot variation of assays. According to literature, sFLC methods with in-built antigen excess check obviate the need for control dilutions.

Method:
Comparability of sFLC assays in an External Quality Assessment Scheme (EQAS, Instand e.V.) was evaluated in a 3-year-period.

Results:
For 9 out of 24 EQAS sera, consensus target values for sFLC of one method deviated at least four times relative to the mean of all methods. Since the results are evaluated by consensus values, the respective results are accepted. For two out of 24 EQAS sera, one or more methods was unable to correctly type the monoclonal light chains. One example is presented below:
We measured sFLC in the EQAS serum by Freelite assay on Cobas Integra (Roche) in standard dilution: kappa=14.5mg/L; lambda=81.5mg/L. Calculated kappa/Lambda ratio was 0.28mg/L and therefore borderline normal (reference range 0.26-1.65). However, immunofixation showed monoclonal IgA lambda plus monoclonal light chains lambda. Because sFLC lambda of 81.5mg/L are not expected to be visible in the immunofixation, we tested for antigen excess. sFLC results for lambda were >1525mg/L in 1:10 prediluted serum and 2718mg/L in 1:100 prediluted serum. 67% of EQAS participants of the Roche group (N=12) successfully passed the EQAS with false results for sFLC lambda between 31.8-59.2mg/L.

Conclusion:
• Here we present a case were the in-built antigen excess of Cobas Integra failed.
• When sFLC results are incompatible with other clinical or laboratory findings, serum should be tested for antigen excess.
• Variation between methods and within methods show, that sFLC assays are not robust.
• EQAS should critically review consensus target values.

christof.schild@insel.ch

SSCC2015 Abstract 32

Characterization of CDA activity in healthy volunteers using Roche/Hitachi analyzers

S Hamzic (1) ; J Sistonen (1) ; S Brunner (1) ; S Fontana (2) ; U Amstutz (1) ; and CR Largiadèr (1)

1. Institute of Clinical Chemistry, Inselspital, Bern University Hospital, and University of Bern, Bern, Switzerland 2. Regional Blood Transfusion Service of the Swiss Red Cross, Bern, Switzerland

Background:
The enzyme cytidine deaminase (CDA) is involved in the metabolism of many antimetabolite drugs. Mutations in the CDA gene (CDA) were suspected to explain therapy-related toxicities in some cases. Direct measurement of CDA activity in plasma provides an alternative approach to investigate its role in antimetabolite-related toxicities.

Aims:
Our aim was to study the variability of CDA activity in healthy volunteers and to evaluate genetic variants in CDA, associated with differences in activity.

Methods:
CDA activity was quantified in plasma samples from 300 healthy blood donors by measuring the amount of ammonia generated during the conversion of cytidine to uridine, using the Roche/Hitachi Modular P800 and compared to the commonly used Berthelot method.
Coding, promoter and exon-flanking intronic region of CDA were sequenced in 10 and 13 individuals with very low or high CDA activity, respectively, and 10 controls with average CDA activity.

Results:
Both, the Roche/Hitachi assay and the Berthelot method yielded comparable results r2=0.89. Passing-Bablok analysis showed a small but tolerable systematic bias between the methods: y = 1.01x -2.35 (95% CI: 0.86x-1.18x and - 4.5 to -0.96), suggesting that the automated method is a suitable alternative to previously reported protocols. Plasma CDA activity in healthy volunteers showed wide variability (range: 1.2-52.8 U) and significantly higher activity in men compared to women (P=0.005). Genetic analysis revealed no variants associated with extreme CDA phenotypes.

Conclusions:
These results show that CDA activity is very variable and its regulation is not explained by CDA genotype, and that other factors are the main regulators of CDA activity.

seid.hamzic@insel.ch

SSCC2015 Abstract 33

The diagnostic value of the plasma vitamin B6 profile in vitamin B6 dependent epilepsy

L Abela (2); M Albersen (3); C Bürer (4); H Hartmann (5), M. Hersberger (1); NM Verhoeven-Duif (3); B Plecko (2)

1. Division of Clinical Chemistry and Biochemistry, University Children's Hospital Zurich, CH; 2. Division of Child Neurology, University Children's Hospital Zurich, CH; 3. Department of Medical Genetics, University Medical Center (UMC) Utrecht, NL; 4. Division of Metabolism, University Children's Hospital Zurich, CH; 5. Department of Pediatrics, Hannover Medical School, D

Introduction: Inborn error of metabolism (IEM) leading to primary or secondary intracellular pyridoxal-5'-phosphate (PLP) deficiency can cause epileptic encephalopathies that are responsive to vitamin B6. Diagnoses of some of those IEM are often delayed due to the lack of specific biomarkers. Here, we measured the plasma B6 vitamer profiles in patients with IEM of vitamin B6 metabolism including Antiquitin, PNPO and TNSALP deficiency (congenital hypophosphatasia) in order to evaluate its diagnostic value.

Methods: The vitamin B6 vitamers pyridoxal-5'-phosphate (PLP), pyridoxal (PL), pyridoxamine (PM), pyridoxine (PN) and the degradation product pyridoxic acid (PA) were quantified by LC-MS/MS in plasma of control children (n=50) and of children with Antiquitin (n=18), PNPO (n=6) and TNSALP (n=1) deficiency.

Results: The vitamin B6 profiles of patients with PNPO deficiency (before supplementation or on PN/PLP) had a clear elevation of PM when compared to controls and to patients with other inborn errors of vitamin B6 metabolism, thus making PM a specific biomarker candidate for PNPO deficiency. A clear elevation of PLP was found in the plasma of a patient with congenital hypophosphatasia (TNSALP deficiency, off supplementation). However patients with Antiquitin deficiency had an unspecific profile with an increase of PLP, PL, PA and in some patients PN when on PN supplementation.

Conclusion: These results suggest a diagnostic value of the plasma vitamin B6 profile for patients with suspected PNPO deficiency and with congenital hypophosphatasia, but not for Antiquitin deficiency. Measuring plasma vitamin B6 profiles may help in the differential diagnosis of patients with vitamin B6 dependant disorders.

deborah.mathis@kispi.uzh.ch

SSCC2015 Abstract 34

Mitochondrial diagnostics: A Retrospective Analysis of 375 Patients

D Hahn (1); A Schaller (2); S Gallati (2); JM Nuoffer (1)

1 University Institute of Clinical Chemistry, Inselspital Bern; 2. Division of Human Genetics, Inselspital Bern

Background: Mitochondrial disorders are a heterogeneous group of diseases affecting 1/5000 individuals. They may present at any age, as a multisystemic disorder or affect a single organ, with any patterns of mendelian or maternal inheritance. The diagnostic process still remains a challenge, starting with the clinical suspicion to the definitive biochemical and/or genetic diagnosis. As Swiss Mito reference centre we analysed nearly 400 patients (600 tissue samples) in the past 10 years with standardised biochemical analysis.
Objectives: Analysis of clinical, biochemical and genetic findings of patients with suspected mitochondrial disorders referred to our institute during the last 10 years.
Methods: We performed a retrospective analysis of all patients referred with suspected mitochondrial defects. Diagnostic methods used were spectrophotometric specific respiratory chain complex assays and/or oxygen consumption measurements. The analysis includes clinical characteristics (age and symptoms), frequency of specific enzymatic defects, number of genetically confirmed defects and the incidence of defects in various tissues.
Results: Patients (N=375) (161 adults; 214 children). Enzymatic defects were found in 32 % (44 adults; 76 children). Complex I and combined respiratory chain defects were the most frequent, with 28 % and 32 % respectively, followed by Complex IV (12 %) and Complex V (11 %) defects. 79 patients were further analysed genetically and 46 of them were confirmed.
Conclusion and outlook:
• Referred patients have a high positive diagnostic rate with enzymatic and oxymetric analysis (32 %).
• Positive diagnosis was more frequent when combined tissues (39 %; muscle and fibroblasts) were analysed compared to single tissue (muscle: 20 %; fibroblasts: 27%).
• 58 % of the enzymatic defects were genetically confirmed. Biochemical diagnosis was missed in 19%, all patients with heteroplasmic mtDNA deletions.
• Diagnostic strategies may have to be reconsidered in the view of the clinical Variability of mitochondrial presentations.

dagmar.hahn@insel.ch

SSCC2015 Abstract 35

Optimizing the Prescription of Clinical Chemistry Tests in a University Hospital

Greutert M (1), Jacquemont N (1), Coti Bertrand P (2), Khalatbari-Soltani S (3), Lamy O (3), Marques-Vidal P (3), Monti M (3), Sahli S (4), Wasserfallen JB (5), Waeber G (3), Mooser V (1), Boulat O(1)

1. Service of Biomedicine, Laboratories Department; 2.Clinical Nutrition, Department of Endocrinology, Diabetology and Metabolism; 3. Service of internal medicine, Department of Medicine; 4. Directorate of the Department of Medicine; 5. Medical Directorate // All: University Hospital of Lausanne (Centre Hospitalier Universitaire Vaudois CHUV), Switzerland

Background: Optimizing the prescription of laboratory tests is critical, yet challenging, to improve the quality of health care, contain costs and properly train healthcare providers. The aim of this project was to decrease prescription of over-utilized tests and increase prescription of under-utilized tests.
Methods: We performed a detailed analysis of the ordering pattern of laboratory tests within the 1400-bed CHUV University Hospital, with a particular focus on the 279 analyses performed by the Clinical Chemistry Laboratory, which totalize approximately 2.2 million tests per year. A previous educational intervention on junior staff conducted by the service of internal medicine led to an improvement in the prescription of clinical tests, but the effect waned due to staff turnover. A literature review was performed and a series of discussions with senior physicians from the hospital was conducted.
Results: Five actions to improve prescription were identified: 1) Regular feedback to services regarding the number of laboratory test prescriptions; 2) Tailor-made training of junior staff regarding adequate prescription of selected laboratory tests; 3) Participation to the writing and updating of laboratory diagnostic and monitoring algorithms; 4) Use of computer-based entry systems to implement best-practice alerts; 5) Design of intervention studies in specific services. A reporting template was designed, to be sent to the services on a quarterly basis for feedback on their laboratory prescription rates. The service of internal medicine was identified as the largest prescriber of laboratory tests; physicians within this service agreed on an educational and feedback intervention aiming at raising awareness of junior staff on demand management in healthcare. An analysis is under way to quantify the impact of this intervention on prescription adequacy.
Conclusion: Several issues have been identified to improve the prescription of clinical chemistry laboratory tests. Joint efforts of laboratory experts and clinicians are required to improve prescription.

maude.greutert@chuv.ch

SSCC2015 Abstract 36

Urine Drug testing

P Medina Escobar (1), U Nydegger (1), L Risch (1), M Risch (2)

1. Labormedizinisches Zentrum Dr. Risch, Liebefeld bei Bern; 2. Zentrallabor, 2. Kantonsspital Graubünden, Chur

Background: The importance of urine drugs testing in substance abuse treatment is well acknowledged. Same sex assisted sampling by a control person is embarrassing. Therefore labelling urine through prior drinking marker-admixed potion (sugar enhanced drinks) could be a good alternative.
Methods: Drug-testing without supervision is possible using Polyethylene-glycol (PEG) as a non toxic, orally applied well tolerated kidney passing well suited for intimate urine sampling. In addition to PEG, the RUMA®-system uses sample check-procedures, i.e. detection of adulteration by fraudulent mixing the urine with chemicals, sugar recovery into the sample and creatinine assessment. Performance here reported results from international postmarketing survey.
Results: Of over 260.000 urine samples from ~26.000 ominous individuals, false-negative, incorrect marker detection came up as low as 0.00005% (n=5). Most likely involved individuals experienced a delayed clearance after the recommended 30 minutes wait between drink and sampling. needing extension to 45 minutes in the event of kidney diseases. In a cohort of 43 urine examinations, the RUMA-System allowed to improve identification of drug abusers from 2.3% □ 30.0% (opoid) and from 16.3% □ 30.0% (benzodiazepine). The marker-assisted procedure is harm less and well accepted by the majority of subjects. No unwanted outcomes are on record.
Conclusions: The RUMA® marker system has proven superior to assisted urine sampling and enjoys increasing degree of respect. Unequivocal sample attribution the involved ominous subject is asecertaiened. The medical laboratory Dr. Risch has introduced the system for its clients with considerable success.

pedro.medina@risch.ch

SSCC2015 Abstract 37

Metabolic investigations of intact fibroblasts by 1H HR-MAS NMR Spectroscopy

Eggimann S (1); Diserens G (2); Hertig D (3); Vermathen M (3); Furrer J (3); Vermathen P (2); Nuoffer JM (1)

1. Univ Inst of Clin Chem, Bern Univ Hosp, Bern, Switzerland; 2. Dept Radio and Clin Research, Univ Bern, Bern, Switzerland; 3. Dept of Chem & Biochem, Univ Bern, Bern, Switzerland

Background
Determination of small molecules in primary fibroblasts in cell culture (FB) may be clinically interesting. Here the applicability of proton high-resolution magic-angle-spinning (1H HR-MAS) NMR for metabolic profiling was investigated in control FB cell lines and few defect FB.

Methods
The variability of metabolites between control FB cell-lines (n=7) and the effect of cell storage (fresh and frozen under cryoprotective conditions) were examined. 1D-NMR spectra were acquired at 3kHz spinning rate. As a first pilot study frozen FB with a known defect (n=5) were measured with the optimized protocol. Individual peak analysis and statistical fingerprint methods were applied.

Results
Approximately 30 small molecules were analyzed. Overall metabolite variability between control cell-lines excluding the aromatic region was 20%. Variability between fresh and frozen was generally similar to that between cell-lines. However, the variability of aromatic metabolites was significantly lower ($p < 0.0001$) between fresh/frozen pairs than between cell-lines. Fresh and frozen pairs were discriminated by chemometric analysis where the main discriminative metabolites were lactate and proline. In addition to fresh/frozen separation, the different control FB cell lines could also be separated, indicating that some metabolite concentrations in FB are well preserved during freezing and are more similar than between cell lines. Therefore the preparation protocol with frozen FB was set for all future measurements. Results from the pilot study showed that fibroblast with a defect can be separated from healthy fibroblasts by chemometric analysis, suggesting metabolite differences in defect fibroblasts compared to healthy fibroblasts.

Conclusion
The results demonstrate that 1H HR-MAS NMR allows for non-destructive metabolic profiling of frozen FB and the protocol can be used to gain further insights in the metabolism of FB in cell culture. Additionally a first pilot study suggests the feasibility of the method to distinguish between control and defect fibroblasts.

sandraeggimann@hotmail.com

SSCC2015 Abstract 38

Normalization strategies for metabolomics analysis of urine samples by UHPLC-QTOF-MS

Y Gagnebin ; J Schappler ; J Boccard ; S Rudaz

School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Boulevard d'Yvoy 20, 1211 Geneva 4, Switzerland

Introduction : Metabolomics attends to analyse the most important number of metabolites (mass < 1000 Da) in a biological system. This approach constitutes a potent method for assessing phenotype modifications caused by disease or environmental influences. Thanks to its non-invasive collection and its availability in large quantities, urine represents a major biological matrix. However, one important issue remains the variability of urine concentration, which implies data normalization. This study proposes the evaluation of various normalization strategies in the context of metabolomics analysis of urine samples by UHPLC-QTOF-MS.
Methods : Urine samples were analyzed by UHPLC-QTOF-MS in negative and positive ESI mode. XCMS was used for data preprocessing and SIMCA 14 (Umetrics) for multivariate data analysis with Pareto scaling. Two data treatments, MS Total Useful Signal (MSTUS) and Probabilistic Quotient Normalization (PQN), were investigated as data normalization strategies. Creatinine concentration, osmolality and NMR measurements were used to estimate the sample concentration and calculate dilution factors.
Results : The major sources of variability observed in the data set were attributed from the analytical drift and the sample concentration. Various strategies were evaluated to circumvent both issues. MSTUS and PQN provided the best normalization strategies and reveal the intra- and inter-individual variability. With data treatments, the analytical drift was corrected but not practically removed. Multiple injections of urine contaminate the mass spectrometer and decrease the intensity of the signal during the sequence. The analytical drift is related to both the number and the concentration of samples. To remove this analytical drift, creatinine concentration, osmolality and NMR measurements were used to estimate the sample concentration and calculate appropriate dilution factors before injections. Fixing a range of concentrations during the sample preparation removed the major part of the drift and improved the analytical condition and data acquisition before the normalization by MSTUS or PQN.

yoric.gagnebin@unige.ch

SSCC2015 Abstract 39

Serum uromodulin, urinary uromodulin and estimated daily uromodulin excretion in young and healthy adults are associated with sodium concentration in urine and serum: a population based study

M. Risch (1); S.Aeschbacher (2); T. Schön (2); D. Hillmann (3); P. Medina Escobar (3); D. Conen (2); L. Risch (3)

1. Zentrallabor, Kantonsspital Graubünden, Chur; 2. Department of Medicine University Hospital Basel and Cardiovascular Research Institute Basel, University Hospital Basel; 3. labormedizinische zentren Dr. Risch, Schaan & Liebefeld

Background: Uromodulin is exclusively produced in the thick ascending limb (TAL) cells of the kidney and represents the most abundantly secreted protein in normal urine. Uromodulin concentrations in serum and urine become increasingly investigated in cardiovascular and nephrological research. The regulation of the sodium household is closely related to the occurrence of arterial hypertension.
Aim: To evaluate associations of uromodulin and markers of sodium homeostasis in urine and serum.
Methods: We consecutively included a subset of the population-based GAPP study investigating determinants of blood pressure and other cardiovascular risk factors in young and healthy study participants. Concentrations of uromodulin in urine and serum were measured with a commercially available ELISA assay (EUROIMMUN, Luzern, Switzerland). Further, urinary creatinine was assessed by the Jaffe method. Urinary creatinine excretion per 24 hours was estimated with the Ellam-equation. Daily urinary uromodulin excretion was estimated by multiplying the estimated 24 hour excretion of creatinine with the urinary uromodulin/creatinine ratio. Sodium in urine and serum as well as glucose and urea in serum were measured on a COBAS 6000 instrument (Roche Rotkreuz, Switzerland). Osmolality in serum was estimated from sodium, glucose, and urea concentrations.
Results: A total of 329 individuals (180 females, 149 males; mean age 39 + 9 years) were included into the study. There was a significant relationship between uromodulin concentrations in urine and daily uromodulin excretion and serum sodium ($r=-0.13$, $p=0.02$ & $r=-0.20$ $p<0.001$). Further, urinary sodium was significantly associated with serum uromodulin ($r=-0.25$, $p<0.001$), urinary uromodulin ($r=-0.49$, $p<0.001$) and daily uromodulin excretion ($r=-0.67$; $p<0.001$). Lastly, serum osmolality was significantly associated with serum uromodulin ($r=-0.16$;$p=0.003$), urinary uromodulin ($r=-0.22$;$p<0.001$), and daily uromodulin excretion ($r=-0.29$; $p<0.001$).
Conclusion: There is a significant association between markers of sodium homeostasis and phenotypic parameters of uromodulin metabolism. This suggests a possible link between uromodulin concentrations and the occurrence of arterial hypertension.

martin.risch@ksgr.ch

SSCC2015 Abstract 40

Comparison of HILIC and reversed-phase UHPLC-MS for non-targeted metabolic profiling of human plasma

K Freiburghaus (1); C Bovet (1); St Zahnd (2); D Lesche (1); B Rindlisbacher (1); J Sistonen (1); M Spahn (3); GN Thalmann (3); M Fiedler (1); CR Largiadèr (1)

1. University Institute of Clinical Chemistry, Inselspital, Bern University Hospital, Bern, Switzerland; 2. 2Translational Research Unit, Institute of Pathology, University of Bern, Bern, Switzerland: 3. 3Department of Urology, Inselspital, Bern University Hospital, Bern, Switzerland

Non-targeted metabolic profiling based on ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) aims to achieve broad metabolome coverage for measuring differential levels of multiple metabolites in biological matrices. The wide range of physicochemical properties of metabolites is challenging for UHPLC-MS and necessitates multi-method approaches for the discovery of diagnostic signatures and candidate biomarkers in clinical samples. Here, we evaluated two analytical procedures for profiling human plasma: the commonly applied reversed-phase (RP) chromatography and the complementary hydrophilic interaction liquid chromatography (HILIC) separation.
Non-targeted metabolomics analysis of plasma samples was performed on a UHPLC quadrupole time-of-flight mass spectrometer using both HILIC and RP chromatography. Electrospray ionization (ESI) was performed in the positive and negative mode. Using our in-house developed bioinformatics pipeline, the list of detected metabolites was searched against HMDB, KEGG, Reactome, and WikiPathways to identify specific pathways and molecule classes.
Both chromatographic methods profiled characteristic metabolic features of plasma and allowed the detection of up to 3800 features in the positive and 3200 features in the negative ESI mode. Plasma samples from healthy volunteers and disease patients were analyzed with both chromatography methods to discover disease-regulated metabolites. The combination of HILIC and RP procedures led to an in-depth characterization of molecule classes and pathways that could be differentially regulated in the compared sample groups. Interestingly, more regulated metabolic features were isolated by RP chromatography. This suggests that a careful selection of the chromatography is crucial for the success of non-targeted metabolic profiling by UHPLC-MS.

katrin.freiburghaus@insel.ch

SSCC2015 Abstract 41

Accelerating translational research – Evaluation of a new mass spectrometry-based metabolic profiling data analysis workflow for biomarker discovery

B Rindlisbacher (1); C Bovet (1); S Zahnd (2); M Hayoz (1); L Bally (3); C Stettler (3); C Trepp (4,5); V Enzmann (4,5); GM Fiedler (1); CR Largiadèr (1);

1. Clinical Metabolomics Facility, University Institute of Clinical Chemistry, Inselspital, Bern University Hospital, Bern, Switzerland; 2. Translational Research Unit, Institute of Pathology, University of Bern, Bern, Switzerland; 3. Division of Endocrinology, Diabetes, and Clinical Nutrition, University Hospital Inselspital, University of Bern, Bern, Switzerland; 4. Dept. of Ophthalmology, Inselspital, University of Bern, Bern, Switzerland; 5. Dept. of Clinical Research, University of Bern, Bern, Switzerland;

Mass spectrometry-based metabolic profiling has opened new prospects to discover potential candidate signatures and biomarkers in a wide range of clinical samples and materials (i.e., serum, plasma, tissues, or cell extracts). Although standardized assays have been established, the identification of clinically relevant metabolites and metabolic signatures remains challenging in complex mixtures.
Here we present an evaluation of a new non-targeted metabolic profiling data analysis workflow based on ultra-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-MS) for the identification of discriminatory metabolic profiles between experimental groups. Two test sets comprising human serum and cultured stem cell extract samples were analyzed on a quadrupole time-of-flight mass spectrometer (Synapt G2-S HDMS, Waters) coupled to an UPLC ACQUITY I-class system (Waters). After chromatographic alignment of the raw data, ion patterns were detected and deconvoluted with Progenesis QI (version 2.0, Nonlinear Dynamics, Newcastle, UK). Chemometric analyses were applied to identify discriminative metabolites between experimental groups using principal component analysis (PCA) and orthogonal projection to latent structures-discriminant analysis (OPLS-DA) with SIMCA (version 14, Umetrics, Umeå, Sweden). The list of discriminative metabolites – characterized by retention time and monoisotopic mass– was searched against metabolite databases to assess the identity of the features. An in-house established pathway analysis tool identified pathways or molecules classes that could be differentially regulated among experimental groups. The potential metabolite identities were then validated by comparing retention time and MS/MS spectra to those of reference standards that were analyzed with same analytical method. By combining new bioinformatics, chemometrics, and data processing tools, our non-targeted metabolomics analysis pipeline successfully discovered differentially regulated metabolites and validated their identities with reference standards for both serum and cell extract sample test sets.

barbara.rindlisbacher@insel.ch

SSCC2015 Abstract 42

Reference intervals for serum uromodulin, and estimated daily uromodulin excretion in young and healthy adults: a population based study

M. Risch (1); S.Aeschbacher (2); T. Schön (2); D. Hillmann (3); P. Medina Escobar (3); D. Conen (2); L. Risch (3)

1. Zentrallabor, Kantonsspital Graubünden, Chur; 2. Department of Medicine University Hospital Basel and Cardiovascular Research Institute Basel, University Hospital Basel; 3. labormedizinische zentren Dr. Risch, Schaan & Liebefeld

Background: Uromodulin is exclusively produced in the thick ascending limb (TAL) cells of the kidney and represents the most abundantly secreted protein in normal urine. Uromodulin concentrations in serum and urine become increasingly investigated in cardiovascular and nephrological research and may obtain a role in risk prediction for cardiovascular and nephrological diseases.
Aim: To determine reference intervals for serum uromodulin and daily uromodulin excretion in young and healthy adults.
Methods: We consecutively included a subset of the population-based GAPP study investigating determinants of blood pressure and other cardiovascular risk factors in young and healthy study participants. Concentrations of uromodulin in urine and serum were measured with a commercially available ELISA assay (EUROIMMUN, Luzern, Switzerland). Further, urinary creatinine was assessed by the Jaffe method. Urinary creatinine excretion per 24 hours was estimated with the Ellam-equation. Daily urinary uromodulin excretion was estimated by multiplying the estimated 24 hour excretion of creatinine with the urinary uromodulin/creatinine ratio. Double sided 95% reference intervals (RI) were calculated according to the CLSI EP28-A3c guideline.
Results: A total of 329 individuals (180 females, 149 males; mean age 39 + 9 years) were included into the study. All study participants had an eGFR >60 ml/min/1.73m2. Males had significantly lower serum uromodulin concentrations and estimated daily uromodulin excretion ($p<0.001$ for both). RI for serum uromodulin were 72 ng/mL 90% confidence interval CI [63-84] – 522 [446-601] ng/mL in males, and 101.3 [89.4-115.2] – 551 [485-626] ng/mL in females. The RI for daily uromodulin excretion was 1.9 [1.3-2.7] – 789 [538-1156] (mean 38 mg) mg per day in male, and 2.1 [1.4-3.1] – 2094 [1414-3101] (mean 66) mg per day in females.
Conclusion: Males have lower serum uromodulin concentrations and daily urinary uromodulin excretions of than females. This might reflect a gender difference in the number or function of thick ascending limb cells in the kidneys.

martin.risch@ksgr.ch

SSCC2015 Abstract 43

The cystatin C/creatinine ratio, a marker of glomerular filtration quality: associated factors, reference intervals, and risk prediction for morbidity and mortality in healthy seniors

M Risch (1), MT Purde (2), S Nock(3), P Medina Escobar (2), C Grebhardt (2) UE Nydegger (2), Z Stanga (4), L Risch (2)

1. Kantonsspital Graubünden, Zentrallabor, Chur 2. labormedizinische zentren Dr. Risch, Schaan & Liebefeld 3. Private University, Triesen 4. Department of Endocrinology, Diabetes and clinical nutrition, Bern University Hospital and University of Bern

The ratio of cystatin C and creatinine (cysC/crea) is regarded as a marker of glomerular filtration quality, which is associated with cardiovascular morbidities. We aimed to determine reference intervals for serum cysC/crea in seniors. Further, we aimed to determine whether other low-molecular-weight molecules exhibit a similar behavior in individuals with altered glomerular filtration quality. Finally, we investigated associations with adverse outcomes. A total of 1382 subjectively healthy Swiss volunteers 60 years of age or older were enrolled in the study. Reference intervals were calculated according to CLSI guideline EP28 A3c. After a baseline exam, a 4-year follow-up survey recorded information about overall morbidity and mortality. CysC/crea (mean 0.0124±0.0026 mg/µmol) was significantly higher in women and increased progressively with age. Other associated factors were hemoglobin A1c, HDL, eGFR, BNP, and CRP ($p < 0.05$ for all). Participants exhibiting shrunken pore syndrome had significantly higher ratios of molecules with a 3.5-66.5 kDa molecular weight (BNP, PTH, β2 microglobulin, cysC, retinol-binding protein, TSH, α1-acid glycoprotein, lipase, amylase, prealbumin, albumin) and creatinine. There was no such difference in ratios of very-low-molecular-weight molecules (urea, uric acid) and creatinine, and larger than 66.5 kDa molecules (transferrin, haptoglobin) and creatinine. CysC/crea was significantly predictive of mortality and subjective overall morbidity at follow-up in logistic regression models adjusting for several factors. CysC/crea exhibits age- and sex-specific reference intervals in seniors. In conclusion, the cystatin C/creatinine ratio may indicate relative retention of biologically active low-molecular-weight compounds and can independently predict risk for overall mortality and morbidity in the elderly.

martin.risch@ksgr.ch