Cardiac markers

M344

TWO TO THREE DAY BIOLOGIC VARIATION AND CONCENTRATION VARIATIONS DURING HEMODIALYSIS OF HIGH SENSITIVE TROPONIN T AND TROPONIN I IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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BACKGROUND-AIM

The aim of the study was to assess the two to three day analytical coefficient of variation (CVA), within - person biological variation (CVI), between – person biological variation (CVG), reference change value (RCV) and index of individuality (II) for two high sensitive troponin (hs-cTn) assays and to estimate the cTn concentration changes and variations in concentration changes in stable patients during hemodialysis (HD).

METHODS

Blood samples were collected before and after 10 concomitant HD treatments in 20 patients treated on a two to three day interval with high-flux HD. Serum samples were stored in -80 degrees and analyzed using the hs-cTnT assay from Roche Diagnostics and the hs-cTnI assay from Abbott Diagnostics. The two to three day CVA, CVI, CVG, RCV and II was estimated using nested ANOVA after ln transformation of the data. Estimates used after reverse transformation. Variation during HD was estimated using nested ANOVA and original data after correcting for volume changes during HD.

RESULTS

Mean hs-cTnT before HD was 71,1 ng/L (range 17,8-189,7). The CVA was 1,6% (95% confidence interval (CI) 1,4-1,9), the CVI was 7,3% (95%CI 6,6-8,4) and the CVG was 94,4% (95%CI 63,5-176,5). RCVpos was 23,0%, RCVneg was -18,7% and the II was 0,09. Mean hs-cTnI before HD was 35,7 ng/L (range 4,1-113,2). The CVA , CVI, CVG, was 5,3% (95%CI 4,6-6,4), 13,2% (95%CI 11,7-15,3) and 142,4% (95%CI 96,0-408,5), respectively. The RCVpos was 48,2%, the RCVneg was -32,5% and the II was 0,13. After HD quite similar results were shown, however the mean concentrations of cTn decreased by -7,8 ng/L (hs-cTnI) and -2,3 ng/L (hs-cTnT). The within-person and between-person variation in cTn concentration changes during HD was 81% and 120% for hs-cTnT and 134% and 111% for hs-cTnI.

CONCLUSION

The biological variation data is similar to earlier findings. Overall the cTn concentration decreases during high-flux HD, however there is a large variation in the magnitude of the changes. The within-person variation during HD was larger compared to the between-person variation. This means that an absolute cut off value (%) for pathological cTn changes during HD may be determined. cTnI show larger variation compared to cTnT for all investigated parameters.

Cardiac markers

M333

IDENTIFICATION OF PERI-PROCEDURAL MYOCARDIAL INFARCTION IN PATIENTS UNDERGOING TRANSCATHETER AORTIC VALVE IMPLANTATION BY USING A HIGH-SENSITIVITY TROPONIN I ASSAY

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BACKGROUND-AIM

The peri-procedural myocardial infarction (MI) in patients undergoing transcutaneous aortic valve implantation (TAVI) has been linked to worse prognosis. According to the current VARC-2 definition, peri-procedural MI is characterized by a pre-defined rise in myocardial biomarker levels, including cardiac troponin (cTn) and creatine kinase MB (CK-MB); however, many patients have elevated cTn concentrations prior to TAVI without clinical evidence of MI. The aim of the present study was to establish reference values for cTnI using a high-sensitivity assay (hs-cTnI) in patients scheduled for TAVI and to assess hs-cTnI and CK-MB concentrations up to 3 days after TAVI.

METHODS

Consecutive patients (n=505) with severe aortic stenosis undergoing elective transfemoral (TF) or transapical (TA) aortic valve implantation (AVI) were considered for the study. After exclusion of patients with peri-procedural cardiopulmonary resuscitation or annular/ventricular rupture, a total of 251 patients with TF-AVI and 227 patients with TA-AVI were analysed. Venous blood samples for the determination of hs-cTnI and CK-MB (Abbott Diagnostic) were collected prior to, 4 h after, and 1, 2, and 3 days after TAVI.

RESULTS

Nearly half (229, 47.9%) of all patients showed elevated hs-cTnI concentrations above the assay specific 99. Percentile prior to TAVI. In contrast, only 18 patients (3.8%) had elevated CK-MB concentrations. We calculated in our TAVI cohort a 99th percentile for hs-cTnI of 855.4 ng/L and for CK-MB 8.9 µg/L. According to the VARC-2 definition nearly all patients (211, 99.5%) undergoing TA-AVI showed a peri-procedural MI based on elevated hs-cTnI compared with only 10 patients based on elevated CK-MB (4.2%). In patients undergoing TF-AVI, 81.1% (193) were classified by VARC-2 as having a peri-procedural MI based on hs-cTnI compared with only 9.0% (19) based on CK-MB. A total of 10/478 (2.1%) patients underwent coronary angiography and showed a peri-procedural type 1 MI. The frequency of peri-procedural MI was significantly lower using the TAVI-specific 99th percentile of hs-cTnI levels compared with the VARC-2 definition (TF-AVI: 12 [5%] vs. 193 [81.1%]; P<0.001; TA-AVI: 47 [22.2%] vs. 211 [99.5%]; P<0.001).

CONCLUSION

The use of the VARC-2 definition leads to a significant overestimation of peri-procedural MI. The establishment of biomarker reference values for patients undergoing TAVI yields a more realistic estimation of the procedure-related myocardial ischemic risk.

Advanced technologies, new biomarker discovery, microparticles, exosomes...

M054

ANALYSIS OF MIRNA CARGO IN PLASMA MICROVESICLES IN HUMAN PLASMA

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BACKGROUND-AIM

Membrane derived microvesicles – microparticles (MPs) are important conveyors of secreted molecular mediators. Plasma MPs originate mainly from platelets, but also from leukocytes, erythrocytes and endothelial cells. MicroRNAs (miRNAs) can transferred via MPs into distant cells . We tested the hypothesis if plasma derived MPs have different miRNAs signature then MP-depleted plasma.

METHODS

Platelet poor plasma from 8 middle aged men was harvested. MPs were separated by centrifugation method (16 000g, 90 min at 4^{o}C). MiRNA was extracted following the miRNeasy (Qiagen) protocol. Reverse transcription was performed with the polyadenylation and cDNA synthesis kit (Exiqon). Levels of miRNAs were analyzed with serum/ plasma miRCURY LNA Universal panel (Exiqon) by the 7900HT Applied Biosystem system. Expression levels were globally normalized using $\Delta\Delta$Ct methods. Additionally AFM and CryoTEM analyses were performed.

RESULTS

The images of MPs in separated fractions were revealed by AFM and CryoTEM methods. Different signature of circulating miRNA were characterized in MP fraction and MP-depleted plasma: proangiogenic (miR-126, 21, 23a) and antiangiogenic (miR-15a, 16, 24) miRNAs were increased in MPs. The downexpression of some specific proangiogenic (miR-10b, 132, 210) miRNAs was also observed.

CONCLUSION

Circulating MPs have a specific miRNA signature which differs from plasma miRNA profile.

Patient management, biological sample management

W126

ARE PATIENTS WELL INFORMED ABOUT THE INFLUENCE OF OTC DRUGS, FOOD SUPPLEMENTS AND PREANALYTICAL FACTORS ON LABORATORY TESTS RESULTS?

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BACKGROUND-AIM

Consumption of some over the counter (OTC) drugs and food supplements can affect laboratory results. Therefore, the aim of this study was to assess the frequency of consumption of these preparations and the level of knowledge of their influence on the laboratory tests results in an outpatient hospital setting.

METHODS

The study included 200 outpatients who were referred to University Department of Chemistry for laboratory testing and voluntarily agreed to participate in the study. The survey was anonymous and performed in the form of interviews. It included questions about the frequency of consumption of various products, awareness of the importance of informing physicians and laboratory staff about it, and information about influence of preanalytical variables on the laboratory test results. Statistical analysis was performed using Microsoft Excel and chi-square test in MedCalc (Mariakerke, Belgium). Data are presented as numbers and percentages.

RESULTS

Out of total number of participants, 66% were female, and the most common age group is 46-65 years (38%). Results showed that 81% of patients take some preparations, mostly minerals (50%), vitamins (47%) and cranberry extract or tea (33%). Women were taking preparations more frequently than men (86% vs. 69%, P=0.008), while there was no difference between age groups (P=0.117). Majority of patients (52%) consider that it is not necessary to notify the laboratory staff about the consumption of preparations. However, 72% patients think that it is necessary to inform their physicians, even though only 53% of them did that. Patients recognized that alcohol (83%), physical activity (44%), grapefruit (23%) and broccoli (12%) can influence laboratory results. However, 47% think that coffee can affect laboratory results if taken the day before blood sampling. Also, 53% patients think that consumption of any of various products and food supplements doesn't affect result.

CONCLUSION

A large number of patients is taking food supplements and various OTC drugs and they are not sufficiently informed and aware about its potential impact on the laboratory tests results. Low level of knowledge and awareness about the influence of some preparations and preanalytical factors showed an urgent need for additional education.

Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

M116

TOWARDS A REFERENCE METHOD FOR ABSOLUTE QUANTIFICATION OF HEPCIDIN-25 IN SERUM BY MASS SPECTROMETRY

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BACKGROUND-AIM

The discovery of hepcidin and its role in iron metabolism has modified our understanding of the pathogenesis of iron-linked disorders. Diagnosis applications related to hepcidin measurement include iron-overload disorders or anemia in inflammatory context. Hepcidin quantification is challenging: indeed, the folded structure of this 25AA peptide results in a low immunogenicity, high aggregability and possibly in a poor analytical precision. We validated the hepcidin-25 analytically and clinically and for the first time we started the assessment of the standard purity with the objective to produce the first international standard for Hepcidin in conjunction with the IFCC Working group on clinical Mass Spectrometry Proteomics.

METHODS

A quantitative method with protein precipitation and LC-MRM was developed to quantify hepcidin-25 in human serum using isotope labeled synthetic refolded hepcidin as standard. The method was validated for an IVD use and its results were compared with those obtained with a reference ELISA test. For absolute quantification in the context of the development of a candidate reference method and the associated Certified Reference Materials, the purity of the calibration standard was evaluated by ion mobility and high resolution mass spectrometry.

RESULTS

The method allows quantifying hepcidin concentrations ranging from 0.179 nM to 62.7 nM in serum. The method needs small sample volumes, is inexpensive compared to ELISA tests and is relatively high throughput thanks to its fast deproteinization step and short LC-MRM analysis (13min). Results comparison with a reference ELISA test showed a good correlation. Purity assessment of the hepcidin standard by showed the presence of oxidized form and several hepcidin foldings. Ion mobility confirmed the existence of different mobiloforms. New experiments will be conducted to define the measurand more rigorously and determine what forms should be considered as impurities.

CONCLUSION

In conclusion, a LC-MRM method was developed for the quantification of hepcidin in human serum and was correlated with ELISA test. Significant efforts must still be made for the characterization of the calibration standards before MS absolute quantification and SI-traceable results can be claimed and propose a primary, higher order reference method. This will allow performing rigorous assessing trueness of field methods and standardizing results so as to improve comparability of results from different analytical platforms.

Endocrinology

T114

SWITCHING FROM RIA TO LC-MS/MS FOR PLASMA AND URINARY ALDOSTERONE

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BACKGROUND-AIM

Aldosterone measurement is critical for screening and diagnosis of primary aldosteronism, location of aldosterone producing tumors, and investigation of other disorders of the renin-angiotensin system. Liquid chromatography triple quadrupole mass spectrometry (LCMS2) has become an essential tool for small molecule quantitation due to its high sensitivity, specificity and its excellent reproducibility. We aimed to compare RIA and our new LCMS2 method for plasma and urinary aldosterone measurement.

METHODS

Until 2014 October we used Radio-Immunoassays (RIA) (Diasorin). From October 2014, we used a LCMS2 (TQ5500, ABSciex). The accuracy profile was determined in triplicate during 3 days with 5 plasma and 5 urine pool levels. A total of 68 plasma and 22 urine samples were assayed for method comparison. Slope and intercept were calculated using Passing and Bablok linear regression and we compared the methods with the Bland and Altman plots (Medcalc software).

RESULTS

CV intra-assay were 5.1% and 7.3%, total precision 5.1% and 8.6% (range:5-1000 ng/L for plasma and 7-110 µg/L for urine respectively). LOQ were at 20 ng/L for plasma and 2.7 µg/L for urine. Linearity was good between 5 and 1000 ng/L for plasma and between 2.7 and 112.5 µg/L for urine. Recovery is 100±4.7% (95%CI for the mean: 98.3-101.7%) for urine and 100±1.9% (95%CI for the mean: 98.9-101.1%) for plasma. For the comparison between RIA and LCMS2 in plasma, the regression equation was RIA=40.6+1.6 LCMS2 (95% CI of the intercept: (30.3; 52) and 95% CI of the slope: (1.5; 1.7)). In urine, the regression equation was RIA=2.4+0.8 LCMS2 (95% CI of the intercept: (1.2; 3) and 95% CI of the slope: (0.7; 0.9)). The Bland and Altman showed that results were in mean 59% higher in RIA than in LCMS2 for plasma and 26% lower in RIA than in LCMS2.

CONCLUSION

We noted a significant bias between results by RIA and LCMS2. Compared to LCMS2, RIA didn't differentiate aldosterone glucuronide (in CKD patients) from native aldosterone. After the comparison with 2 others laboratories using this method and results discussion with the clinicians, we switched from the RIA to LCMS2 for the aldosterone on the basis of its improved sensitivity and specificity.

Diabetes

T067

GLYCOLYSIS INHIBITION AND RELIABLE PLASMA GLUCOSE RESULTS: IS THE CLINICAL IMPACT CAREFULLY CONSIDERED?

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BACKGROUND-AIM

As in our daily practice rapid (<30 min) separation of plasma after blood drawing for glucose testing is impractical, we recently introduced blood collection tubes containing a fluoride-citrate mixture as effective antiglycolytic agent [Terumo Venosafe Glycemia (TVG)]. After 6 months from the introduction, we aimed to investigate the practical impact of the optimization of the preanalytical phase on glucose concentrations of our served population.

METHODS

We retrospectively retrieved fasting plasma glucose concentrations (FPG) from outpatients by comparing two periods, April-September 2014 (n=7192), using TVG tubes, vs. April-September 2013 (n=7120), in which blood was collected in sodium fluoride/oxalate tubes.

RESULTS

The use of TVG tubes determined a 'shift to the right' in the FPG distribution, with a significant increase (P<0.001) in the median FPG [5.44 mmol/L (2013) vs. 5.94 mmol/L (2014)]. Median HbA1c concentrations [49 mmol/mol (2013) vs. 45 mmol/mol (2014)] showed that the metabolic control of the population subjected to FPG measurements was not noticeably different in the two periods, confirming that the average increase in FPG was probably caused by the improved stabilizing effect of TVG. Considering FPG decision limits, this resulted in a different clinical classification for a significant number of subjects; particularly, using cut-off for desirable FPG (<5.60 mmol/L), the percentage of subjects with undesirable FPG increased from 26.8% to 45.2% (P<0.001) and, using the diagnostic cut-point for diabetes (≥7.00 mmol/L), the prevalence of abnormal FPG results increased from 17.8% to 23.3% (P<0.001).

CONCLUSION

Our experimental data emphasize that the use of TVG, although providing more reliable FPG, results in a significant change of clinical classification of evaluated individuals. These results highlight the need of an official position of diabetologist associations in stating if decisional limits for FPG should be redefined with the use of tubes that promptly inhibit the in vitro glycolysis or if current cut-offs should be maintained, so that the 'higher' FPG results could more effectively and early identify subjects at increased risk for diabetes.

Quality assessment, laboratory errors, patient safety, ethics

T431

STABILITY OF BIOCHEMICAL ANALYTES IN WHOLE BLOOD AND PLASMA DURING 6 HOURS STORAGE

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BACKGROUND-AIM

Stability of biochemical analytes has been previously assessed but published results differ depending on analytes, storage times and methodologies. We aimed to investigate the stability of twenty-four biochemical analytes in whole blood and plasma, after different storage times at room temperature, in order to define allowable pre- and post-centrifugation delays in hospital laboratory.

METHODS

1) Whole blood stability: five heparinized blood collection tubes were collected for 28 healthy volunteers. The first tube was kept in upright position during exactly 2 hours (baseline), and then centrifuged, following by plasma measurements of 24 parameters immediately performed on Modular® Roche analyzer. The second, third, fourth and fifth tubes were similarly treated but after being kept in upright position during 3h, 4h, 5h and 6h, respectively. 2) Plasma stability: all the analytes were quantified on heparinized tubes of 21 hospitalized patients centrifuged after a mean delay of 2 hours ± 18 min (baseline). These centrifuged tubes were kept in upright position and reassayed for all measurements after 2h, 4h and 6h of storage. Stability variations were expressed as mean biases from baseline, using the maximum analytical variation (1.96*√2*CVa) as acceptance limit.

RESULTS

In whole blood study, mean concentrations decreased after 3-4h for lactate dehydrogenase (−5.7% [95%CI: −7.4 to −4.1%]) and phosphorus (−6.1% [95%CI: −7.4 to −4.7%]), and after nearly 6h for potassium (−2.9% [95%CI: −5.3 to −0.5%]). In heparinized plasma study, mean concentrations decreased after 2-4h for bicarbonates (−13.3% [95%CI: −15.8 to −10.8%]), and increased after 2-4h for lactate dehydrogenase (+6.0% [95%CI: +4.3 to +7.6%]), and 4-6h for aspartate transaminase (+6.8% [95%CI: +4.1 to +9.5%]). All other analytes remained stable on whole blood and plasma for six hours.

CONCLUSION

This study proposes allowable delays for routine biochemical tests on tubes arriving to the laboratory or needing to be reanalyzed within six hours after centrifugation.

Cardiac markers

M323

FIBROBLAST GROWTH FACTOR 23 AND SOLUBLE KLOTHO IN CHRONIC SYSTOLIC HEART FAILURE

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BACKGROUND-AIM

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone regulating phosphate and vitamin D levels. FGF23 is associated with increased risk of cardiovascular mortality or heart failure (HF) development. Klotho, an FGF23 co-receptor, also has a direct effect on cardiovascular function. However, the mechanism of FGF23 increase and its prognostic value have not been thoroughly studied in HF. The aim of the present study was to assess the factors associated with FGF23 and to evaluate the prognostic value of FGF23 and Klotho in HF.

METHODS

FGF23 and soluble Klotho levels were measured in 369 patients (mean age 59±11 years, 84% male) with systolic HF (median duration 6.5 years, interquartile range (IQR) 2.4–12.3). Patients were followed for adverse events (death, urgent heart transplantation, ventricular assist device implantation).

RESULTS

Patients with CKD had significantly higher FGF23 levels than subjects without CKD [median 206 (IQR 123–434) vs. 120 (IQR 73–263) RU/ml, p<0.0001]. Tricuspid regurgitation severity, chronic kidney disease (CKD), alkaline phosphatase concentrations, inferior vena cava dilatation and absence of angiotensin-inhibitor therapy were independently associated with FGF23. Among patients with invasive hemodynamic data (n=174), the difference between mean arterial and right atrial pressure was the main determinant of FGF23. FGF23 was independently associated with outcome among patients without CKD (HR 1.43, 95% CI 1.14-1.78), but not in CKD patients (HR 1.12, 95% CI 0.87-1.45). There was no association between Klotho and FGF23 concentrations or between Klotho levels and outcomes. The addition of FGF23 to clinical variables and BNP led to an 8.0% net reclassification improvement.

CONCLUSION

Among patients with advanced systolic HF, FGF23 is a strong independent predictor of adverse events, particularly in those with preserved kidney function. The association of FGF23 with adverse events was independent of concentrations of soluble Klotho and likely reflected early changes in renal hemodynamics and an activation of the renin-angiotensin system. The prognostic values of BNP and FGF23 were additive; therefore, the simultaneous use of FGF23 and BNP further improved the risk stratification in our HF cohort.

Cardiac markers

M339

DOES NOVEL CARDIAC BIOMARKER CAN PREDICT CARDIAC-RELATED COMPLICATION AFTER OPEN HEART SURGERY IN PATIENTS WITH LOW EJECTION FRACTION?

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BACKGROUND-AIM

Our study focused on prognostic capacity of novel cardiac biomarker in patients with severe compromised ischemic and non-ischemic left ventricle (LV) to predict cardiac-related complication after open heart surgery.

METHODS

73 patients with severe depressed LV function (EF < 35%) were included in pilot prospective study. Cardiomyopathy developed due to coronary artery disease in 51 patients (mean age 62,2±4,9 years) or was confirmed as idiopathic in 22 patients (mean age 44,4±9,9 years). Patients underwent elective either combined coronary artery bypass grafting with mitral valve procedure (49 patients) or isolated mitral valve repair or chordal-sparing replacement (24 patients) consequently. Blood samples for measurements of cardiac biomarkers (sST2, NT-proBNP, hs-cTnI and CRP) were collected preoperatively, on 1st, 7th and 30th postoperative days. The primary end point was complicated postoperative period due to cardiac-related events (duration of isotopes more then 24 h, intra-aortic balloon pump using, temporary VAD application or hospital mortality).

RESULTS

Cardiac-related complications were observed in 27 patients (37 % of cases) during postoperative period. Preoperatively only level of sST2 was significantly higher in patients with cardiac-related complications during hospital stay (86,9 (49,4-113,1) vs. 25,3 (19,8-35,8) respectively, p = 0,001). While no difference were found in NT-proBNP (2000 (427-6577) vs. 1200 (870-2169), p = 0,422) and hs-cTnI (0,015 (0,005-0,035) vs.0,01 (0,005-0,019), p = 0,522) between patients with complicated or not postoperative period. AUC in ROC-analysis was also highest for preoperative sST2 level – 0,852 (95% CI 0,691-1,014, p = 0,02). The best cut-off value of the preoperative sST2 level was 45 ng/ml showed a sensitivity of 81,81% and specificity of 93,75% in predicting the complicated postoperative period. On logistic regression analysis, a sST2 level higher 45 ng/ml was identified as independent predictors for cardiac-related complication after open heart surgery (OR – 5,345 (95% CI 3,6-9,78, p = 0,01).

CONCLUSION

These results demonstrated that preoperative level of sST2 compared with NT-proBNP and hs-cTnI can be used to identify patients with depressed LV function at increased risk of postoperative complicated period.

Vascular biology, endothelium, haemostasis, cardiovascular diseases

W201

PROTEOME ANALYSIS AND HISTOMORPHOMETRIC INVESTIGATION OF HUMAN ARTERIAL TISSUE REVEAL VASCULAR COLLAGEN ALTERATIONS AMONG ACTIVE SMOKERS

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BACKGROUND-AIM

Smoking affects the molecular composition of the arterial wall and increases the risk of cardiovascular disease. However, little is known of the pre-atherosclerotic changes in the arterial wall in relation to smoking. Collagen plays a crucial role in the arterial wall and our aim was to investigate the possible correlation between collagen levels and cigarette smoking in non-atherosclerotic arterial tissue.

METHODS

We studied the non-atherosclerotic arterial wall of the internal mammary artery used as repair artery in coronary artery by-pass surgery in 13 non-smokers and 11 active smokers. Using histomorphometric methods, the area fraction of collagen stainable material was determined in the tunica intima, media and the luminal 30 µm of adventitia. In addition to this, proteome analysis of matrix molecules and other proteins was performed.

RESULTS

The area fraction of collagen was significantly decreased in active smokers compared to non-smokers in all three layers of the arterial wall. All results are mean ± standard deviation. In tunica intima the area fraction of collagen was 43.3% ± 3.6% in non-smokers and 29.1% ± 3.8% (p=0.012) in active smokers. The area fraction of collagen in tunica media was 56.8% ± 5.6% in non-smokers and 39.7% ± 5.5% (p=0.042) in active smokers. In tunica adventitia we saw an area fraction of 61.0% ± 3.2% in non-smokers vs. 50.4% ± 3.9% (p=0.046) in active smokers.
Furthermore, we discovered through proteome analysis that there were significantly lower relative levels of collagen α1 I-chain in the smoking compared to the non-smoking group (0.68 ± 0.048 vs. 1.02 ± 0.112, p=0.013), as was the case with collagen α2 I-chain (0.81 ± 0.046 vs. 1.14 ± 0.118, p=0.038) and the collagen-adjacent protein decorin (0.64 ± 0.04 vs. 0.98 ± 0.11, p=0.009).

CONCLUSION

The internal mammary artery from active smokers contains lower area fractions of collagen stainable material in the tunica intima, media and adventitia. Furthermore, proteome analysis showed a decreased amount of two types of collagen and decorin in smokers. These findings shed new light on the effect of smoking on the arterial wall, which may explain some effects of smoking on the development of cardiovascular diseases.

Vascular biology, endothelium, haemostasis, cardiovascular diseases

W194

1H NMR-BASED LIPIDOMIC ANALYSIS OF RED BLOOD CELL MEMBRANES FOR THE IDENTIFICATION OF BIOMARKERS OF ISCHEMIC HEART DISEASE

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BACKGROUND-AIM

Alterations in composition of red blood cell membranes have been regarded as an important contributor to the initiation and progression of atherosclerosis leading to Ischemic Heart Disease (IHD).The aim of the present study is the investigation of the ability of the 1H NMR-based lipid profiling of red blood cell membranes to identify novel lipid biomarkers of the presence of Ischemic Heart Disease.

METHODS

Whole blood samples from 20 men with severe IHD (triple vessel disease, TVD), and 20 men with normal coronary arteries (NCA), age- and conventional lipid parameters-matched and all angiographically documented, were collected after an overnight fast. The total lipid content of the membranes of isolated red blood cells was extracted according to a standard procedure and pattern recognition analysis was applied on the 1H NMR lipidomic data recorded on a Bruker DRX-500 Spectrometer.

RESULTS

The 1H NMR-based lipidomic analysis showed that patients with severe IHD presented statistically significant altered lipid profile of the membranes of red blood cells compared to those with NCA. Patients with severe IHD presented higher levels of cholesterol and lower levels of omega-3 fatty acids, degree of unsaturation, phosphatidylcholine, the sum of eicosapentaenoic and arachidonic acids, unsaturated and diallylic fatty acids and sphingomyelin in the membranes of red blood cells compared to those with NCA.

CONCLUSION

1H NMR-based analysis reveals alterations in lipid composition of red blood cell membranes that possibly affect their shape, fluidity and functions. These lipid disturbances could constitute novel lipid biomarkers for the early evaluation of the presence of IHD and establishment of an appropriate therapeutic option.
This research project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program “THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013” of the National Strategic Reference Framework (NSRF 2007-2013)

Kidney diseases

W064

LEAN MASS AND AGE ARE STRONG DETERMINANTS OF GLOMERULAR FILTRATION RATE IN HEALTHY MEN

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BACKGROUND-AIM

Understanding determinants of glomerular filtration rate (GFR) is important in aiding prediction and interpretation of kidney function. Body composition is known to affect GFR, but is not included in current screening of kidney disease. We investigated the association between GFR and body composition in healthy young men with differing body mass but without known diabetes or kidney injury.

METHODS

Three age-matched groups were recruited: normal BMI (n = 22) < 25 kg/m2, muscular (n = 23) with BMI > 30 kg/m2 and a screened bioelectrical impedance (BIA) body fat < 20%, and obese (n = 22) with BMI > 30 kg/m2 and a screened BIA body fat > 30%. Dietary analyses, GFR by clearance of 99m Tc-DTPA, and body composition by dual-energy X-ray absorptiometry (DEXA) were measured in all participants.

RESULTS

Muscular men had higher GFR (mean 186.4 mL/min; 95% CI 171.7 to 201.1) than normal BMI and obese groups (P = 0.0007). Fat mass protein intake, and smoking status were not associated with GFR; whereas lean mass had the strongest association with GFR. In all subgroups, skeletal muscle mass correlated significantly with GFR (P = 0.04). In multi-variate models, variables with the strongest associations with GFR were age (P = 0.0009) and lean mass (P = 0.0001). A final derived multiple regression equation was; GFR = 38.3 – 0.997 (age) + 2.34 (total lean mass).

CONCLUSION

Age and lean mass were strong determinants of GFR in healthy men of various body compositions. We estimate that GFR decreases by 1 mL/min/year of age and increases 2.3 mL/min/kg of lean mass in healthy men.

Kidney diseases

W063

HOMOCITRULLINE: NEW BIOMARKER OF ACUTE RENAL FAILURE?

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BACKGROUND-AIM

Carbamylation is a nonenzymatic post-translational modification characterized by the irreversible addition of isocyanic acid to amino groups of proteins. Because isocyanic acid mainly originates from the spontaneous dissociation of urea, carbamylation rate is highly increased during renal failure. This reaction leads to the formation of carbamylation-derived products (CDPs), such as carbamylated albumin or carbamylated hemoglobin. The aim of the study was to evaluate homocitrulline (HCit), which results from the carbamylation of ε-amino groups of lysine (Lys) residues, in acute renal failure (ARF) and to determine if it could be useful for differentiating acute from chronic renal failure (CRF).

METHODS

213 patients with renal failure referred to the nephrology unit of the university hospital of Reims were included in this study. Patients were classified into three groups: patients with ARF (ARF group, n=39), patients with CRF complicated with ARF (A/CRF group, n=29) and patients with CRF (CRF group, n=145). Serum total HCit concentrations were determined by LC-MS/MS and expressed as μmol of HCit per mol of Lys. Kinetic profiles of HCit and urea concentrations were studied in patients suffering from ARF. An HCit threshold between ARF and CRF was investigated.

RESULTS

HCit concentrations increased in ARF patients reaching a peak generally delayed compared to the urea concentration peak. HCit concentrations were positively correlated with urea concentrations ($r=0.51$) and with the time elapsed since the estimated onset of ARF ($r=0.57$). Serum HCit were significantly higher in CRF ($p<0.05$) group compared to ARF group. The receiver operating characteristic curve analysis showed that HCit concentrations below 289 μmol/mol Lys were predictive of ARF with a sensitivity of 83 % and a specificity of 72 % and an area under the curve equal to 0.856.

CONCLUSION

Our results demonstrate that HCit is a promising biomarker for distinguishing between ARF and CRF patients.

Liver, pancreas, gastrointestinal diseases, microbiome

W242

SCREENING FOR THE IDENTIFICATION OF AUTOIMMUNE OR LYMPHOPROLIFERATIVE ONSET IN PATIENTS NAïVE TO HCV ANTIVIRAL TREATMENT.

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BACKGROUND-AIM

Hepatitis C virus(HCV) may be responsible of extra-hepatic manifestations. A chronic infection of immunocompetent cells is most likely at the origin of a benign mono-oligoclonal B lymphocyte proliferation, typically observed in mixed cryoglobulinemia(10% showing late B-NHL).The aim of this study is to identify early markers of autoimmune lymphoproliferative disease onset in a group of antiviral treatment-naïve patients infected by HCV that could identify the transition between a state of silent autoimmune and lymphoproliferative conditions and frank disease.

METHODS

Fourty patients were recruited. Antinuclear antibodies(ANA) were detected by indirect immunofluorescence. Autoantibody detection of IgG directed against M2,gp210,SP100,LKM1,LC1,SLA,Factin antigens were performed by Immunodot analysis. Free light chain(FLC) detection were carried out by turbidimetric assay. Cryoglobulin and cryofibrinogen analysis was carried out following the guidelines of the SIBIOC.

RESULTS

Our results show an 84% prevalence of cryoglobulinemia in samples collected from HCVinfected patients. Of these, 27% showed ANA positivity a negligible percentage of autoantibody liver disease and absence of positivity of cryofibrinogen. The most significant result concerns the finding of high doses of FLC in 73% of patients, of which 21% showing an abnormally elevated k/l ratio. Statistical analysis suggests that patients presenting cryoglobulinemia and FLCratio above 1.6 are also ANA positive.

CONCLUSION

ANA positivity is indicative of the presence of a persistent antigenic stimulus by the virus and the activation of any autoimmune clones.The presence of cryoglobulinemia suggests a continuous lymphocyte stimulation. Interestingly, our results suggest a possible role for the presence of high levels of FLCs and their use to identify the transition between a silent state of probable autoimmune lymphoproliferative disease or a frank illness, using k/l ratio as a cut-off value. The presence of a subpopulation of HCVpositive patients may open new scenarios to targeted therapeutic treatment strategies in subclinical phases. Our study is a contribution to presenting a panel of potential predictive markers of disease progression.

Autoimmune diseases, autoimmunity, allergy

M189

MONOCLONAL ANTIBODY THERAPEUTICS AS POTENTIAL INTERFERENCES ON PROTEIN ELECTROPHORESIS AND IMMUNOFIXATION

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BACKGROUND-AIM

The use of therapeutic recombinant monoclonal antibodies (mAbs) has triggered concerns of confusion and misdiagnosis of a monoclonal gammopathy in treated patients. The purpose of this study is to determine if infliximab, adalimumab, eculizumab, vedolizumab, and rituximab are detected as monoclonal proteins by serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE).

METHODS

Pooled normal sera were spiked with various concentrations (ranging from trough to peak) of infliximab, adalimumab, vedolizumab, eculizumab and rituximab. The peak concentration for each mAb was also added to samples (n=5) with known monoclonal gammopathies. All samples were analyzed by SPE (Helena Laboratories) and IFE (Sebia), and the ones with potential interferences were reflexed to electrospray-time-of-flight mass spectrometry (AbSciex Triple TOF 5600) for the intact light chain Monoclonal Immunoglobulin Rapid Accurate Mass Measurement (miRAMM). Intact light chains mass for these mAbs was calculated from the aminoacid sequence available at IMGT database and characterized using the pharmaceutical preparations.

RESULTS

For all mAbs tested, no quantifiable M-spikes were observed by PEL at any concentration used. Infliximab and adalimumab were not observed at 100 µg/mL, nor was eculizumab at 200µg/mL, on SPE or IFE. However, small gamma fraction abnormalities were noted in the SPE for vedolizumab at 300 µg/mL and rituximab at 400µg/mL, with identification of small IgG kappa proteins on IFE. The same small abnormalities were observed for the high concentrations of mAb therapeutics in sera with known IgG kappa M-spikes. All sera containing peak concentrations of mAbs, with and without M-spikes were reflexed to miRAMM. The therapeutic mAb light chain accurate masses were identified above the polyclonal background and distinct from any monoclonal gammopathy of each sample.

CONCLUSION

Biologics should not be easily confounded with monoclonal gammopathies in patients undergoing mAb therapy except when a SPE and IFE are performed within a couple of days from infusion (peak) for vedolizumab and rituximab. In ambiguous cases the use of the miRAMM technology will precisely identify the therapeutic mAb distinct from any endogenous monoclonal gammopathy.

Laboratory management, accreditation in laboratory medicine

W075

QUALITY IN LABORATORY MANAGEMENT IN INDIA - CHALLENGES WITH VOLUMES, COST AND OPERATION EXCELLENCE

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BACKGROUND-AIM

India houses more than 17% of the world population, 21% of the global diseases and the largest burden of communicable diseases in the world, yet our healthcare infrastructure is one of the weakest, spends only 1% of global healthcare expenditure. The out of pocket expenditure on healthcare is about 65% and only 10% of Indian population receives healthcare subsidies.
In India, the diagnostics and pathology laboratory industry comprises more than 100,000 labs. Test volumes serviced by them range from 3000 for major labs, to about 1000 samples/ day for regional and hospital labs. Labs located in smaller towns may even service 50-100 samples on a daily basis.

METHODS

Indian Dilemma
There are no legal regulations that specify rules for laboratories to follow. Therefore, quality could mean different things to different people. It could be equated with automation, quality controls, accreditation, etc., with different laboratories interpreting it in the way convenient to them. Thus, there is a wide variation in the performance of laboratories across the landscape.
Health insurance is a minor contributor in the healthcare and hardly covers routine diagnostics. Indian insurance has been limited to hospitalisation, critical illness and often one-time lump-sum payouts on a reimbursement basis.

RESULTS

Diagnostic providers have optimized business processes around product lines and focus has not always been patient centric. Patient expectations have increased along with a growing sense of entitlement of comprehensive diagnostics at a value for the money spent.

CONCLUSION

The key challenge for laboratories therefore, is to find innovative and cost-effective ways to improve testing quality and efficiency. Does high quality cost more? Will higher expenditures result in better care, or will better clinical outcomes help to contain costs?

Quality assessment, laboratory errors, patient safety, ethics

T424

MONITORING QUALITY IN THE PRE- AND POST-ANALYTICAL PHASES: A NEW UK NEQAS SERVICE

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BACKGROUND-AIM

The UK National External Quality Assessment Service (UK NEQAS) has developed an online system that allows participants to review and monitor the incidence of untoward events occurring in the pre and post analytical phases. This secure, online service extends quality surveillance beyond the analytical phase, providing a baseline of data against which users can benchmark their performance, with Sigma metrics.

METHODS

The service is entirely web based. Participants submit the number of failures or rejections, together with the total number of eligible patient requests, specimens or reports, for a range of up to 11 quality indicators. Participation may be at department, hospital or network level. Based on recommendations from the IFCC Working Group on Laboratory Errors and Patient Safety, the quality indicators were developed by UK NEQAS in conjunction with scheme advisors, and include patient identification, specimen labelling, specimen collection and reporting errors. The feasibility of and participant preferences for the service were tested in a pre-pilot distribution to 14 selected laboratories in the UK and the Republic of Ireland.

RESULTS

Initial feedback demonstrated a high level of interest in the service from laboratories and national quality oversight bodies. The challenges encountered centre on the practicability of data extraction from laboratory information management systems and the need for a glossary to ensure the standard description of terms used for data capture.

CONCLUSION

The full service will be available from mid-2015. It is flexible and will allow the addition or removal of indicators, including the collection of root cause analysis investigations of external quality assessment errors. The initial stages of the service are being offered to blood sciences and microbiology only, though the intention is eventually to cover all pathology disciplines.
This service has been developed in liaison with the Association for Clinical Biochemistry.

Pharmacogenetics, pharmacogenomics, personalized medicine

T170

CELL MODELS IN PHARMACOGENOMIC RESEARCH

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BACKGROUND-AIM

The potential of pharmacogenomic (PGx) research is to improve general health care by on one side reducing adverse drug reactions (ADRs) and on the other side by increasing the treatment efficacy. However, a lot of factors are affecting progress in PGx field for example: the need for large clinical populations of treated patients and control/placebo-treated cohorts; the difficulty in evaluating drug response; the interactions of underlying biochemical pathways (in either adverse or therapeutic drug effects) are often not fully understood etc. Therefore, the use of cell models would enormously decrease the time and costs of PGx research. Three steps where cell models could improve PGx research are: i) identification of PGx markers before clinical studies; ii) explanation of biochemical pathways of drug distribution, metabolism, elimination as well as therapeutic and adverse effects and iii) the pharmacokinetic evaluation of drug distribution, metabolism, elimination needed for development of dosage algorithms including PGx data.

METHODS

Methods such as genome wide association studies (GWAS) or sequencing have greatly facilitated the identification of gene loci and variations and have contributed to selection and rational introduction of genetic variation into clinical studies. In addition, the experiments on the cells or animals remain necessary in order to explain the function of such genes and variations. In cell models, usually plasmid like methods are used to investigate gene regulatory variations, while gene knock out, silencing or overexpression methods are used to investigate gene function and involvement in drug metabolism.

RESULTS

We have seen an example of OCT1, which was shown to be responsible for the cellular uptake of imatinib and therefore relevant for the success of the CML therapy, but imatinib was also shown not to be a substrate of OCT1 at all. Recently novel technology CRISPR/Cas9 allows for a relatively easy and quick disruption of genes and we are pursuing the implementation of this technology in elucidation of imatinib active transport mechanism which is responsible for the uptake of this drug in to the target cells and thus for its therapeutic efficiency.

CONCLUSION

The new and emerging methodology will provide ever more reliable and perhaps even quantitative information on the clinical relevance of particular genetic variants.

Haematology

T340

ENDOGENOUS THROMBIN POTENTIAL IN 3RD AND 4TH GENERATION ORAL CONTRACEPTIVE USERS

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BACKGROUND-AIM

Oral contraceptives (OC) have been recognized as a risk factor for venous thromboembolic disease (VTE) occurrence soon after their introduction. Their use induces hypercoagulable state, but the effect differs for various OC generations. The goal of the study was to investigate the effect of 3rd and 4th generation OC on endogenous thrombin potential (ETP), as global thrombin generation indicator, and its relation with some haemostasis related parameters.

METHODS

Case control study included 50 females, age range 20-25y, 25 of them 3rd and 4th generation OC users for at least 3 months, and 25 healthy controls. Following laboratory parameters have been analyzed: ETP, aPTT, PT, TT, von Wilebrand factgor Ag, fibrinogen, D-dimer, antithrombin activity and platelet function. ETP and coagulation parameters were determined using Siemens BCS XP automatic coagulometar, platelet function was assessed using Multiplate aggregometry. The difference between the groups was tested by T-test for parametric and Mann-Whitney test for nonparametric values. Pearson's correlation analysis was used to test correlation between obtained values. P-value <0.05 was considered to be statistically significant.

RESULTS

ETP-AUC was increased in the OC users (107±20.6 vs 96.2±21.2). Significantly shorter time to peak was found in OC users (69.85±9.7 vs 80.78±15, p=0.004). Significantly shorter aPTT was found in OC users (0.92±0.05 vs 0.98±0.09, p=0.007). Higher level of vWFAg (147.3±43.8 vs 89.9±24.3, p=0.008) was observed in long term OC users (24 vs 12 months) No difference was found in the level of platelet aggregation between two groups. No correlation was found between ETP parameters (AUC, lag time, time to peak) and investigated haemostasis parameters.

CONCLUSION

Our results indicate that the use of OC has significant effect on ETP, a global coagulation test, which might become important tool for identification of individuals with increased risk for VTE occurrence among OC users in the future.

Inherited disorders, metabolic disorders, rare diseases

M423

A CASE OF KEARN-SAYRE SYNDROME WITH SEVERE CEREBRAL FOLATE DEFICIENCY

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BACKGROUND-AIM

We report a case of a 43-year-old man with Kearn-Sayre syndrome (KSS). KSS is a mitochondrial DNA deletion syndrome. In some patients with KSS, an energetic defect associated with the accumulation of mutated mitochondrial DNA copies in the plexus choroid cells impairs its ability to transport 5-methyltetrahydrofolate (5MTHF) from the blood to the CSF, thus leading to a severe decrease of 5MTHF in the CSF.

METHODS

The patient has presented a deterioration of walking and cerebellar syndrome with dysathria for 6 years. He had an evolutive atrophic retinitis pigmentosa, bilateral ophtalomoplegia and ptosis since the age of 18, associated with presbycusis during last 2 years.

RESULTS

The vitaminic blood assessment found normal levels of acid folinic in the blood but a severe deficiency of 5MTHF in the CSF (5MTHF: 0 nmol/L - reference value: 200-1000 nmol/L) accompanied with a high CSF protein content (1447 mg/L – reference value: 150-450 mg/L). The electrocardiogram reveals a right bundle branch block with left anterior hemiblock. Magnetic resonance imaging (MRI) of the brain showed periventricular and cerebellar leukoencephalopathy.
Analysis of mtDNA on long PCR showed a unique band < 13kB (nucleotids 3214F-16146B) and a unique band <15 kB (nucleotids 15698F-14861B).

CONCLUSION

The patient was treated with folinic acid 90 mg per day (for one year) and slightly improved his walking performance. This strengthens the hypothesis that the treatement of KSS with (high-dose) folinic acid seems to be advisable for the therapy of KSS with decresed 5MTFHR CSF levels.

Inherited disorders, metabolic disorders, rare diseases

M420

QUANTITATIVE ANALYSIS OF PLASMA CHOLESTANE-3BETA,5ALPHA,6BETA-TRIOL AND 7-KETOCHOLESTEROL BY MASS SPECTROMETRY–LIQUID CHROMATOGRAPHY FOR THE DIAGNOSIS OF NIEMANN-PIC TYPE C

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BACKGROUND-AIM

Niemann-Pick type C (NPC) is a rare inherited error of metabolism (IEM) in which the intracellular trafficking of cholesterol, is altered, leading to the accumulation of unesterified cholesterol in the late endosome/lysosome. Until recently, the diagnosis still based on the filipin test requiring the invasive skin biopsy and cultured fibroblasts. Recently, two oxysterols, cholestane-3ß,5α,6ß-triol (3ß,5α,6ß-Triol) and 7-Ketocholesterol (7-KC), have been reported as a sensitive and specific markers for the diagnosis of NPC.

METHODS

In the present study we described a simple, sensitive, and specific liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS) method for the determination of 3ß,5α,6ß-Triol and 7-KC in human plasma. In order to enhance the spectrometric detection, 3ß,5α,6ß-Triol and 7-KC were first converted into the corresponding picolinyl-esters derivatives.

RESULTS

The percent recovery of spiked plasma was close to 99% and the method is linear in the range 15 to 2000 ng/ml, which is completely adequate to the patho-physiological interval of values. Intra-assay imprecision is 5.4% for 3ß,5α,6ß-Triol 3.2% for 7 KC. The inter-assay imprecision is 7.7% for 3ß,5α,6ß-Triol and 13.5% for 7KC. The method was used to measure unesterified 3ß,5α,6ß-Triol and 7-KC in plasma from 8 NPC and 18 controls subjects. The results confirms an increased 3ß,5α,6ß-Triol l and 7-KC in NPC subjects (3ß,5α,6ß-Triol = 447.9 + 235 nmol/, $p<0.0001$; and 7-KC = 554.2 + 365.8 nmol/l, $p<0.0001$) compared to control subjects (3ß,5α,6ß-Triol = 18.9 + 9.4 nomol/l, $p>0.0001$; 7-KC = 12.7 + 11.1 noml/l, $p<0.0001$).

CONCLUSION

In conclusion, LC-MS/MS is a simple and rapid technique for the quantification of triol and 7KC in human plasma and a sensitive and specific method for NPC screening.

Advanced technologies, new biomarker discovery, microparticles, exosomes...

M044

SERUM LEVELS OF CILP-2 AND COMP REFLECT DIFFERENTLY RADIOGRAPHIC SEVERITY OF KNEE OSTEOARTHRITIS IN MIDDLE-AGED SUBJECTS

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BACKGROUND-AIM

There is an increasing need for analytical assays for detection of molecular biomarkers in osteoarthritis (OA). The aims of the present study were (i) to develop an assay for detection of the biomarker Cartilage Intermediate Layer Protein (CILP-2) in human serum, and (ii) to compare the serum levels of CILP-2 and Cartilage Oligomeric Matrix Protein (COMP) in a well-defined group of knee OA patients

METHODS

A subset of 119 patients of the Estonian knee OA cohort and 17 relevant controls were investigated (36 – 62, mean age 49 years). Serum samples at baseline and after 3 years were analysed in 45 of them. Tibiofemoral (TF) and patellofemoral (PF) radiographs were graded for presence of osteophytes (OPH) and joint space narrowing (JSN). Radiographic progression was defined as: (i) emergence of changes in subjects with no previous OA or (ii) an increase in the grade and/or number of already existing OPH and/or JSN.
The CILP-2 levels were assessed with an in-house competitive immunoassay for CILP-2 (AnaMar AB), where a 60 amino acid long synthetic peptide (C-terminal part of CILP-2 domain 1) was used as coat peptide and a peroxidase-conjugated polyclonal goat anti-CILP2 was used for detection. The COMP levels were assayed with a commercially available COMP ELISA (AnaMar AB). Non-parametric methods were used for statistical evaluation.

RESULTS

We observed a significant decrease in the CILP-2 levels in the group with TF OA grade >2 versus patients without OA (TF and PF grade 0, p= 0.003). After 3-year follow-up, in comparison with controls, significantly lower levels of CILP-2 were observed in patients with TF0-PF 1 OA (p= 0.032) and in patients without radiographic OA (rOA) (TF0+PF0, p =0.019). At the same time, COMP levels were higher in the group of advanced OA (TF grades 2-3, PF2-3, p=0.036) and in TF OA "progressors" compared with patients without rOA (p= 0.011).

CONCLUSION

This was the first time to measure CILP-2 levels in a patient cohort with knee OA. Unlike other biomarkers, CILP-2 showed a significant decrease in patients with early grade of OA. At the same time, the values of COMP were increased in patients with advanced knee OA. Thus, our results confirm that biomarkers CILP-2 and COMP reflect different processes in early knee OA.

Biology of solid tumors

M256

CRIPTO -1: A NOVEL TUMOR MARKER FOR ORAL SQUMOUS CELL CARCINOMA (OSCC)

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BACKGROUND-AIM

Oral Squamous Cell Carcinoma (OSCC) is one of the commonest cancers, particularly in developing country like India. A high rate of mortality and morbidity reported due to OSCC is majorly attributed to late diagnosis of the disease mainly due to non-availability of a screening tool or tumor marker.
Cripto1 (CR1), a member of the EGF-CFC protein family differentially expresses during early embryogenesis. Expression of CR1 mRNA and/or immune-reactive protein, a key phenomenon in tumor dedifferentiation cancers, is associated with increased number of cancer stem cells, thus makes CR1 a potential target for a prospective tumor marker.
In this we elucidated the potential role of Human CR1 as a tumor marker in the cases of OSCC.

METHODS

Fifty consecutive biopsy proven OSCC cases and fifty age/sex-matched controls were recruited for the study. Serum CR1 level of controls as well as serum CR1 levels (soluble component of CR1) of the cases before and after standard therapy according to the stage of the disease were estimated by ELISA (R&D Systems^{TM}). Expression of CR1 were also checked at transcriptional mRNA level by Real time RT PCR and at protein level by IHC (Immuno-Histo Chemistry) in the cancer tissue. The data were analyzed by appropriate statistical tests for significance using GraphPad Prism v6.00.

RESULTS

There is significant (p=0.003) raise in the serum CR1 level in OSCC patients (mean 497pg/ml) with respect to controls (207pg/ml), which is significantly reduced (p=0.046) after completion of therapy. The difference was more significant in patients with well-differentiated tumors and in early stage disease. There is 4.32-fold increase in the mRNA expression of CR1 in cancer tissue with respect to the cancer free tissue and 68% of the cases showed 3+ cytoplasmic positivity for CR1 in tissue level in IHC. With a cut-off value of 200pg/ml the sensitivity and specificity of CR1 is calculated to be 77.4% and 86.7% respectively for diagnosing OSCC.

CONCLUSION

Human Serum CR1 is a potential tumor marker for Oral Squamous Cell Carcinoma. This study also suggests that CR1 may be useful in early diagnosis of OSCC and merits larger, prospective studies.

Toxicology, therapeutic drug monitoring, drug addiction

W419

MERCURY(I) CHLORIDE IN VIVO OXIDATION: THE CAUSE OF THE DEATH OF NAPOLEON?

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BACKGROUND-AIM

It has been reported that Napoleon in his last days once vomited a substance found to "consist of a black mass" understood to be a consequence of a massive gastric hemorrhage. It has also been stated that the witnesses had found Napoleon's stool in his last days "astonishingly black", which they understand to be because of his stomach bleeding as a result of his stomach walls corroded. A theory suggests that the death of Napoleon was a case of acute mercury (Hg) intoxication caused by administering calomel (Hg2Cl2). Hg has a long history of both medicinal uses and toxic effects. Hg chlorides used to be used as medicines; however, "corrosive sublimate" (HgCl2) was also used as a violent poison in the Middle Ages. The purpose of this investigation is to chemically examine the validity of the addressed theory concerning the death of Napoleon.

METHODS

Ingested aqueous Hg2Cl2 in the human stomach is in the presence of hydrochloric acid (HCl(aq)) and air, which is a source of oxygen gas (O2(g)). In this environment, the following oxidation-reduction (redox) reaction may be proposed:
$2\ Hg_2Cl_2(aq) + 4\ HCl(aq) + O_2(g) \rightarrow 4\ HgCl_2(aq) + 2\ H_2O(l)$
The following two half-reactions for the proposed redox reaction were used to determine the equilibrium constant for the above equation at 298 K:
Oxidation $Hg_2^{2+}(aq) \rightarrow Hg^{2+}(aq) + 2e^-$
Reduction $4\ H^+(aq) + O_2(g) + 4e^- \rightarrow 2\ H_2O(l)$

RESULTS

The equilibrium constant at 298 K, Keq = 9.4×1020, and the van't Hoff equation were then used to calculate the equilibrium constant for the proposed reaction at 37oC (the normal human body temperature). The equilibrium constant at 37oC, Keq' = 2.4×1019, is so large that we may say that the proposed reaction goes to completion at the normal human body temperature.

CONCLUSION

The fact that the proposed reaction goes to completion is toxicologically important because it means that ingested aqueous Hg2Cl2 in the human stomach is almost entirely converted to HgCl2, which is a violent poison. Hg2Cl2 in vivo oxidation to HgCl2 ("corrosive sublimate") can explain all the symptoms reported above concerning the death of Napoleon, and the theory of the death of Napoleon being a case of acute mercury (Hg) intoxication is strengthened.

Toxicology, therapeutic drug monitoring, drug addiction

W420

EVALUATION OF ELF INDEX AS NON-INVASIVE MARKER OF LIVER FIBROSIS IN PATIENTS WITH ALCOHOL LIVER DISEASE IN THE COMMUNITY.

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BACKGROUND-AIM

Chronic liver disease is a disease increasing. WHO estimates that more than a billion people worldwide are at risk because of this. Deaths from liver disease have doubled since 1993. The majority of these deaths have been from alcohol-related disease as a result of increasing alcohol intake. Unfortunately, liver disease develops silently and frequently presents with the late complications of cirrosis. The hospital mortality of cirrhosis has not changed for 30 years, suggesting a significant rethink is desperately needed. It is also necessary to detect liver disease before the development of cirrhosis, when lifestyle changes or specific treatment can prevent the progression of disease.
ELF is a diagnostic algorithm of liver fibrosis that combines three serum direct markers: hyaluronic acid, procollagen III amino terminal peptide and tissue inhibitor of metalloproteinase-1. The result becomes a score without units that indicates the level of fibrosis.
The approach to screening for hazardous drinking, detection of problems related to alcohol use and dependence are priorities in primary health care, to do so in recent years have been consolidated instruments such as the AUDIT and CAGE, (questionnaires to assist identification of excessive drinking).
Our aim is analyze the correlation between ELF, designed and used in specialized care, the CAGE test of extensive use in primary care, in order to apply for early detection and stratification of patients with alcoholic liver disease.

METHODS

85 primary care patients who underwent the CAGE test for suspected alcohol use and were classed according to their score. Group A: = 0-1; Group B: ≥2 (alcohol dependence). ELF test® (ADVIA Centaur, Siemens) was calculated in all patients.

RESULTS

69,4% were men, age=48,06(SD=15). The ELF values in all patients (mean±SD) were 9.113±1.07 (range: 6.5-12.6). Group A: 53 patients, ELF values=8.92±1.03, Group B: 32(37%) ELF values=9.44±1.08.
Significant differences in ELF results were found between the two groups (p=0,04).

CONCLUSION

The ELF test shows higher values in the population alcohol-dependent and therefore more liver pathology. Used in the community it could enhance the management of risk factors in primary care and rationalise secondary care referrals.

Reference ranges, standardization and decision levels

W137

THE FIRST MULTICENTRIC STUDY ON REFERENCE VALUES OF HEMATOLOGICAL PARAMETERS IN THE ADULT POPULATION IN TURKEY

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BACKGROUND-AIM

A multicenter study was organized to establish reference intervals (RIs) in the Turkish population for hematological parameters and to explore sources of variation in reference values, including regionality.

METHODS

K2 EDTA blood samples were collected nationwide in 12 laboratories (labs) from the seven regions (≥400 samples/region, 3486 in all). The sera were also collected for the measurement of iron, iron binding capacity and ferritin. The EDTA blood samples were analyzed within 2 hours in the participating labs using 4 different analyzers from 3 manufacturers: Cell Dyn and Ruby of Abbott (A); LH 780 of Beckman Coulter (BC); Sysmex XT-2000i of Roche (R). A panel composed of blood from 40 healthy volunteers was prepared in one center (Istanbul), distributed and measured on the same day, and used to align the results across all centers.

RESULTS

The correlation matrix of the panel test results revealed (1) generally good agreement of test results from all labs for hemoglobin, MCV, counts for WBC, neutrophil, lymphocyte, eosinophil, (2) variable degrees of between-lab differences for monocyte, basophil, and platelet counts, (3) a large beween-manufacturer difference in RBC count, hematocrit, MCH, MCHC, apparently due to a contrast of the R analyzer from others. Between-region differences, expressed as standard deviation ratio (SDR), of the test results all aligned to the values of Bursa were high (SDR>0.3) in hemoglobin, hematocrit, MCV, MCHC, and platelet counts.

CONCLUSION

The finding for erythrocyte was partly explained by the wide differences in the altitudes of the regions. The RIs for hematological parameters were determined from the merged results in consideration of between-manufacturer differences and after exclusion of individuals with latent anemia based on the serum iron study done simultaneously.

Cardiac markers

M331

TROPONIN HAS NO "UPPER LIMIT OF NORMAL"

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BACKGROUND-AIM

We set out to investigate the predictive value for cardiovascular disease (CVD) of troponin at low levels.

METHODS

Records of all patients who had troponin-I (Abbott Architect assay) measured over a five-year period from 1 Jan 2008 to 25 April 2014 in an acute general hospital were extracted in August 2014 to determine the patients' outcomes. During this period, the laboratory recommended a reference range <40ng/L and reported results below 20ng/L as "<20".
The patients' troponin assays were organised into "episodes of care", defined as one or more troponin assays with no intervening interval greater than 24 hours. Their survival curves were stratified by peak troponin within an episode, where "CVD free survival" was defined as absence of a record of death, or of subsequent readmission to hospital with a diagnosis of CVD.

RESULTS

During the study period the hospital laboratory performed 157,483 troponin assays comprising 100,819 episodes of care in 54,833 patients. These episodes had a single troponin assay in 48,793 cases, two assays in 32,454 cases, and 3 or more assays in 19,572 cases. During follow-up to a minimum of 3 months and a maximum of 5½ years, the patients had 8,533 subsequent admissions to hospital with a primary or secondary diagnosis of CVD, and 9,360 deaths.
The survival curves showed clear distinction in outcomes based on peak troponin for time periods from 3 months to 5 years, patients with lower peak troponin having a higher probability of CVD-free survival. This distinction extended down to troponin of 5ng/L and less, even although the clinicians had no knowledge of the actual values lower than 20ng/L.
Analysis of a restricted-age cohort, patients between age 40 and 60 at time of troponin measurement, showed similar stratification of survival to 90 days indicating that the prediction of outcome by troponin is unlikely to be an artefact of the patient's age.

CONCLUSION

CVD-free survival is predicted by troponin in a continuous fashion, with lower troponin indicating better outcome. There is no "upper limit of normal" for troponin.
Receiver operator characteristics curves from our dataset indicate that the optimal decision point is 28ng/L for males, 23ng/L for females, and 25ng/L for all patients irrespective of gender.